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S. Navarro, H. J. Banks, D.S. Jayas, C. H. Bell, R.T. Noyes, A.G. Ferizli, M. Emekci, A. A. Isikber, K. Alagusundaram [Eds.]

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SESSION 1

Biological responses of organisms to MA and fumigants

Chairpersons:
Jordi Riudavets, Spain
Maria Otilia Carvalho, Portugal
Ali A. Isikber, Turkey

SOME RESPONSES OF ARTHROPODS TO GAS EXPOSURES

C H Bell*

Fellow Emeritus, Food and Environment Research Agency, Sand Hutton
York YO1 1LZ UK
*Corresponding author's email: *chris.bell@fera.gsi.gov.uk*

ABSTRACT

For centuries various ways of limiting or modifying the atmosphere in an enclosure have been employed as a means of controlling insect infestation. This has evolved from hermetic storage of crops in underground pits and practices such as the burning of sulphur candles in mills to modern applications of fumigant gases and operation of sophisticated controlled atmosphere systems. Insects and mites still cause problems, however, and this paper examines some of the defences they operate through the range of responses available to them. These may be divided into behavioural and metabolic responses. Behavioural responses include avoidance by responding to concentration gradients, retreating into refuges and/or a shutdown of general activity. Metabolic responses include aspects of increased activity such as the active exclusion of gas or enhanced detoxification pathways, and aspects of reduced activity such as an induced delay in development prolonging a tolerant stage, or a switch to alternative biochemical pathways such as anaerobiosis. The link of these aspects with increased tolerance or resistance to control measures is discussed.

Key words: Stored-product insects, mites, phosphine, controlled atmospheres, stored grain, flour mills, infestation control, resistance.

INTRODUCTION

All living organisms have survived by adopting strategies for life in their ecosystem. For mankind the primary strategy has been one of seeking means of controlling the environment of the ecosystem; for insects and mites the primary strategy has been one of opportunist adaptation. To this end the evolutionary trend has been towards small size, a rapid breeding cycle and close links between environmental cues and behavioural responses. Economy in size leads to economy in the number of cells available to comprise vital organ systems and sensors. The insect nervous system is a wonder of creation in its simplicity and efficiency, enabling the most subtle of environmental stimuli received by a sensor system to elicit precise metabolic or behavioural responses that are of advantage to the individual.

Control intervention by any procedure attempts to render the environment unsuitable for survival of insects and mites that then respond by activating various defence mechanisms. Purging the atmosphere of an enclosure with gases offers a major challenge to the pests present as avoiding exposure by simply moving off a treated surface or rejecting a bait is not an option. However, as reflected by the need of ongoing research into the use of gases and indeed the very *raison d'etre* of these conferences, many individuals have, and still do,

survive these procedures. In this paper the behavioural and metabolic responses employed by insects that can increase the potential for survival in fumigant or controlled atmosphere are explored. Behavioural responses include movement in response to concentration or temperature gradients, seeking static locations such as crevices or food residue layers, shut-down of activity, activity responses linked to diurnal rhythms and aggregation responses. Metabolic responses include active exclusion of toxicant, switching to anaerobiosis, increased capacity of detoxification or elimination of toxicant, desensitisation of active sites and developmental aspects such as the prolonging of a tolerant stage or entry into diapause.

BEHAVIOURAL RESPONSES

Response to gradients
In each of the different storage or food processing environments that are encountered in practice, local microclimates exist and give rise to gradients, gradients of temperature, moisture or humidity, light intensity and even gradients of atmospheric gases where respiration of stored-products or pest populations is evident. When a building or silo is sealed, or a bag stack or cereal bulk sheeted, prior to a fumigation or controlled atmosphere treatment, some of these gradients may be buffered or modified but they will still be present.

The optimum requirements of insects and mites for active development are well known and much information has been gathered on their capacity to locate environments supplying the right conditions for breeding. In grain bulks insects have been shown to respond to gradients of temperature (Surtees, 1964; Jian et al., 2003), moisture content (Yinon and Shulov, 1969; Parde et al., 2004), oxygen (Navarro et al., 1981; Adler, 1992), carbon dioxide (Navarro et al., 1981; Parde et al., 2004) and light (Smereka and Hodson, 1959). In chamber tests they have also been shown to respond to moving gas fronts of the fumigants methyl bromide and phosphine (Bell, 1987). Table 1 lists some of the species for which some strains have been shown to respond to different gradients.

One of the problems associated with the assessment of insect responses to a gas concentration gradient is how to differentiate between what is simply an excitory response, whereby activity is increased causing non-directional random movement, and what is a true movement away or towards the stimulus. Where activity is increased, insects moving towards the gas front may become immobilised and create the impression of attractancy as numbers build up. Movements descending a concentration gradient are thus more reliably identified than movements in the opposite direction. Hence caution is required in identifying attractancy and that which has been ascribed to gases such as phosphine and high carbon dioxide concentrations (>30% in air) known to have a rapid knock-down effect may simply have been the result of an initial general stimulus of activity.

The response to a particular gradient in different insects may differ widely. For example in grain, *Trogoderma granarium* (Everts) will descend a moisture gradient while *Sitophilus granarius* (L.) will move towards zones of higher moisture (Yinon and Shulov, 1969; Smereka and Hodson, 1959), and whereas most insects are repelled by high carbon dioxide (CO_2) concentrations some beetles are attracted at least by concentrations up to 10-15% (Willis and Roth, 1954; Parde et al., 2004), *Tribolium confusum* Du Val is also attracted by much higher concentrations and *T. castaneum* (Herbst) even shows an increased level of productivity at a concentration of 10% CO_2 in air containing 5-10% oxygen (Spratt, 1984).

Many insects show a preference for moving up or down in grain bulks and the response to gradients might be overridden by the response to gravity. The positively geotropic movement of *Cryptolestes ferrugineus* (Stephens) in wheat required the combined effect of

high moisture and low CO_2 concentration gradients to be completely overcome (Parde et al., 2004). In contrast other factors may enhance the effect of movement in response to a gradient. The release of semiochemicals is an obvious example. When several insects responding to a stimulus arrive in one locality, sex or aggregation pheromones may be released that cause an increased level of aggregation, very possibly drawing in individuals that were not responding to the original gradient. A temperature gradient may then be set up by the local activity which will act as a further attractant or arrestant for other pests.

Table 1. Some storage insects that have been found to respond directionally to particular physical and chemical (other than pheromone) gradients

Species	Gradient or feature	Response
Cryptolestes ferrugineus	Low temperature gradient	Towards warmth (20°C+)
	High moisture/humidity	Towards high moisture
	Carbon dioxide	Attracted by up to 9% in air
	Gravity	Moved down in grain
	Light	Moves away
Oryzaephilus surinamensis	Methyl bromide	Attracted by a low (2 g m^{-3}) concentration at 25°C
	High carbon dioxide	Repelled
	Low oxygen	Repelled
Sitophilus granarius	Temperature	Towards warmth (20°C+)
	Humidity	Towards higher humidity
	Carbon dioxide gradients	Repelled by 19 or 95% CO_2
	Low oxygen	Attracted towards low O_2
	Methyl bromide	Repelled by a low (2 g m^{-3}) concentration (at 25°C)
	Phosphine	Repelled by high (>0.6 g m^{-3}) concentrations
Tribolium castaneum	Carbon dioxide gradients	Attracted by concentrations up to 15%, repelled by concentrations over 50%
Tribolium confusum	Carbon dioxide	Attracted by concentrations up to 90% in air
Trogoderma granarium	Humidity	Attracted to less than 40% r.h. Repelled by r.h. over 60%

From a practical viewpoint the question is whether the ability of pests within a treatment enclosure to move away from toxic gas fronts or low oxygen can enhance survival. If the seal on the enclosure is complete, the only benefit to the individual would be that the

time survived would be extended by the time taken for an even concentration of gas to be achieved throughout the enclosure. While this situation may apply to chamber or sealed bag stack treatments, it certainly will not apply to fumigation of a cereal bulk or food processing facility where it is exceedingly difficult to achieve an absolute seal. Here the potential will always be for insects to aggregate at leakage points where a local ingress of the external atmosphere will reduce the chances of a lethal atmosphere being maintained. The prospects for a successful fumigation treatment are reliant on calm weather, windy conditions necessitating addition of further gas and a prolongation of the fumigation period if adequate control is to be achieved. However, leakage points may also feature gradients of reduced temperature or increased moisture which may or may not counteract the effect a concentration gradient may have on the movement of insects. The survival of individual insects or mites in such situations will depend on the ability to respond to the life-threatening gradient amid these other stimuli.

Refuge seeking behaviour
Although the retreat of insects into refuges offers an obvious advantage for survival when surfaces are sprayed with insecticide, the advantage to insects within a fumigation enclosure may seem to be minimal unless the harbourage is at a site of leakage or, more importantly, ingress of the external atmosphere. Nevertheless some survival value must exist because bounce back of pest populations in flour mills and other food processing facilities is strongly linked with pockets of survival in the fabric of the building often well away from obvious leak sources. Very seldom is the residual population completely eradicated.

Refuge seeking behaviour is evident in many stored-product insects. Most prefer dark conditions for activity and seek a refuge in light, particularly bright light. In *C. ferrugineus* the response is enhanced by lowered temperature and the presence of food in the refuge (Cox et al., 1989; Cox and Parish, 1991). In *Oryzaephilus surinamensis* (L.) there is a diurnal rhythm of movement in and out of refuges that is entrained by the daily light cycle and dampened by the presence of food in the refuge (Bell and Kerslake, 1986). Occupancy of the refuge is naturally accompanied by a reduction or cessation of activity, particularly if food is absent, resulting in reduced respiration and hence reduced susceptibility to low oxygen or fumigant gases.

One aspect that is difficult to assess is the effect of microclimate on the rate of gas diffusion into a crack or crevice. The presence of insects and food residues in such harbourages create microclimates that are radically different to the external airspace and gradients may be set up that hamper entrance of gas into the recess. Pressures of as little as 10 mm water gauge have been demonstrated to greatly influence the movement of fumigant gases along an 8 mm diameter tube (Bell, 1987) and such positive pressures can be created by very small increases of temperature. Developing or feeding insects can produce marked temperature rises in commodities resulting in the creation of 'hot spots' in grain. The Mediterranean flour moth, *Ephestia kuehniella* Zeller, developing in 325 g cultures can raise the temperature from $25^{o}C$ to $32^{o}C$ at the peak of larval growth (Bell, 1976). A group of insects feeding on residues in a crevice may thus be protected from exposure to the treatment atmosphere for a considerable period.

With many fumigation applications in cool climates the addition of heat is necessary to increase the chance of a successful treatment. While this enhances gas diffusion and toxicity, for insects hiding and feeding in food residues a short term protective effect may be afforded by evaporative cooling, moisture being produced by the metabolism of carbohydrates as insects feed. In this situation the evaporation of water vapour from the food surface may

counteract the diffusion of gas and also delay the rate of heating in the vicinity of the pests (Bartlett et al., 2005).

Diurnal rhythms

Besides the diurnal rhythm associated with foraging behaviour mentioned above, there are many other instances of the response of insects to the daily light – dark cycle. Activities such as mating, oviposition, hatching of eggs, larval developmental rate, pupation and adult eclosion from the pupal case have all been found to run on entrained cycles in stored-product moths (Table 2), the most prominent trigger or zeitgeber being the onset of darkness. Photoperiodicity may also be an agent for the induction of resting stages in the life cycle. The receipt of a number of lengthening scotophases at the time of the last inter-instar larval moult gives rise to an overwintering diapause after completion of feeding in the warehouse moth, *Ephestia elutella* (Hübner) (Bell, 1977). Though this delayed response does not confer any advantage to the developing larvae at the time of receiving the photoperiodic signal, subsequently the susceptibility to a wide range of control measures is much reduced on progression to the inactive diapausing stage. Furthermore the challenge of a toxic gas on diapausing stages can result in an increased synchronisation of diapause termination after treatment, resulting in a flush of emergence shortly afterwards, thus improving prospects for the rapid establishment of fresh infestation.

Flight activity in pyralid moths is triggered at dusk with another response at dawn and here light intensity and ambient temperature are important additional stimuli. Mating and oviposition are closely linked with flight activity and generally follow a similar pattern, as in *Plodia interpunctella* (Hübner) (Lum and Flaherty, 1970; Lovitt and Soderstrom, 1973), *Corcyra cephalonica* (Stainton), *Ephestia elutella* (Bell, 1981) and *Ephestia cautella* (Walker) (Steele, 1970; Hagstrum and Tomblin, 1973).

Most of these entrained essential biological responses are associated with an increased level of activity and hence increased potential vulnerability to control measures based on respiratory action. As with other activity linked responses such as retreating to refuges or zones of reduced exposure to toxicant, any ability to modify behaviour patterns in response to the detection of a toxic atmosphere will enhance the prospect of survival. Such responses may be termed behavioural resistance. From the viewpoint of control, knowledge of the factors influencing activity can help to decide the time of starting a fumigation, particular when using fast acting fumigants such as methyl bromide or sulfuryl fluoride, to ensure that pests are most likely to be active soon after the application of gas.

METABOLIC RESPONSES

Developmental aspects

It is well known that large differences occur between the susceptibility levels of different stages of arthropods to fumigants and controlled atmospheres. The egg stage is more tolerant of a wide range of fumigants and modified atmospheres than other stages in mites, while in stored-product insects the most tolerant stage varies with species and fumigant. Treatment dosages and exposures are designed to kill all stages and often rely on tolerant stages to carry on development under gas so that the period of highest tolerance can be bridged. This has long been the strategy for the control of *Sitophilus* spp. with phosphine where tolerance peaks around the time of pupation within the grain and then declines (Howe, 1973). Further tests on this species revealed that a concentration of 280 ppm needed to be maintained for 16 days to achieve complete control in laboratory tests at 15°C (Hole et al., 1976).

Clearly the ability to survive a fumigant exposure in one insect stage can potentially be acquired in another, this being an obvious route to the development of resistance. Indeed an insight into the potential for resistance can be gained from looking at the natural tolerance spectrum which in the case of phosphine is very wide. Phosphine has been in widespread use since the 1960s and today resistant strains of many species are known. In Australia a strongly resistant strain of *C. ferrugineus* requires a 30-day exposure at 360 ppm for control at $20^{o}C$, displaying a resistance factor of 875 compared to non-resistant strains (Nayak et al., 2010).

Exposure to a toxic gas may slow the rate of development in an insect and an effective survival mechanism would be for an individual to remain at a stage of relative tolerance to the hostile atmosphere. Such a response could be described as 'developmental' resistance and has been observed in stored-product mite species exposed to high CO_2 levels (60-99% in air) or

Table 2. Responses of some storage insects to daily cycles of photoperiod or temperature

Species	Stimulus	Response
Corcyra cephalonica	The daily onset of darkness	Oviposition
Ephestia cautella	The daily onset of darkness	Adult emergence Flight, mating Oviposition
	Night-time falling temperature	Oviposition
	Lengthening scotophases	Diapause in mature larvae
	Lengthening photophases	Termination of diapause
Ephestia elutella	The daily onset of darkness	Flight Oviposition
	Lengthening scotophases	Diapause in mature larva
Ephestia kuehniella	The daily onset of darkness	Adult emergence Oviposition
Oryzaephilus surinamensis	The daily onset of darkness	An increase in foraging behaviour
Plodia interpunctella	The daily onset of darkness	Flight, mating Oviposition Pupation
	Daily temperature peak	Oviposition
	Lengthening scotophases	Diapause in mature larva
	Lengthening photophases	Termination of diapause

low oxygen atmospheres (0.5-2%) at $15^{o}C$ where delays of hatch in excess of 20 days were recorded after exposure in *Tyrophagus longior* (Gervais), *Acarus siro* L., *A. farris*

(Oudemans) and *Lepidoglyphus destructor* (Schrank) (Conyers and Bell, 2003). Similar delays in the hatch of the first two species have also been observed following exposure to phosphine (Bowley and Bell, 1981).

Resistance mechanisms
Apart from the reduced susceptibility arising from a shutdown of activity or development, there are many other mechanisms available to organisms to help survival. The uptake of the toxicant can be reduced or even actively excluded, the sensitivity of active sites can be reduced, the rate of excretion of toxicant can be increased and the toxicant itself can be metabolised to non-toxic derivatives. Insects and mites show a capacity to tolerate far lower oxygen levels than mammals, being able to breed down to oxygen levels of 4% in air (Conyers and Bell, 2007), their small size enhancing oxygen uptake. There may also be a capacity to survive anaerobically for long periods, particularly during periods of the egg stage, and this can enable extended periods under low oxygen levels to be tolerated. This ability may be the reason why eggs of many species are so tolerant of the fumigant phosphine when compared to other stages, the presence of oxygen being required for phosphine to act.

Resistance to phosphine by insects has become a world-wide problem. In some species an active exclusion mechanism seems to operate in resistant strains. Price (1984) observed that in *Rhyzopertha dominica* (F.) the rate of uptake of phosphine by a phosphine resistant strain was very low, and greatly increased when insects died. In *T. castaneum* concentrations between 0.5 and 1 g/m^3 have been found to induce a kind of narcosis whereby some insects become inactive and reduce their uptake of gas, actually surviving longer than at higher or lower concentration levels (Winks, 1984). When removed from the exposure chamber while in this narcotised state the insects recovered, though prolonged exposure resulted in death (Winks, 1985). A narcotic response has since been observed in several other stored-product insects, the concentration threshold stimulating the response varying widely according to the species (Zhang, 1999; Cao and Wang, 2001). The phenomenon was first observed in scale insects exposed to HCN when it was termed protective stupefaction (Pratt et al., 1931).

The genetics of phosphine resistance has long been a matter of study to shed further light on how the gas interacts with the oxidative metabolic cycle to produce lethality. In several pests resistance has been shown to be the result of two incompletely recessive genes or gene complexes that when expressed fully produce the very high resistance levels seen in *R. dominica*, *Sitophilus oryzae* (L.) and *C. ferrugineus* (Schlipalius et al., 2002; Collins et al., 2005; Thorne et al., 2010; Wang et al., 2010; Nayak et al., 2010). The resistance includes mechanisms for reducing the uptake of phosphine as well as reduced sensitivity of the active sites and an increased capacity for metabolism. Nevertheless it is still possible to control all resistant populations by increases of concentration and exposure time though of course there are limits on the range of circumstances this can be achieved in practice and the possibility remains for still further increases in the level of resistance. Compounds do exist, however, that are specifically active against resistant strains. One such compound is methyl phosphine (Chaudhry et al., 1997).

Phosphine is not the only fumigant or gas that has been implicated with the development of resistance. Reports of resistance in the field towards hydrogen cyanide date back to early last century and laboratory selection studies have produced strains resistant at least to some extent to fumigants such as methyl bromide, and even carbon dioxide (Navarro et al., 1985), though whether resistance is the right term for the increases in tolerance produced is a matter of debate. With such more generally-acting compounds, the levels of resistance or increased tolerance do not approach those obtained with phosphine and so far no

cases of control failure attending their use in practice have been reliably attributed to the resistance of pests.

CONCLUSION

Resistance is a potential problem for any control process and can arise in many different ways. Plants have produced many defences against attack by insects and insects have responded by specialising to be able to utilise the resources provided by specific plants. Hence many bruchid beetle species have specialised to be able to develop on host plants that are poisonous to other animals. In the stored product field the most successful species are generalist feeders with life cycles that can be prolonged to bridge periods where food supplies are not readily available, as for example between harvests, and which can develop rapidly when conditions are favourable. Hence most stored-product beetles have long-lived adult stages and a diapausing larval stage is common among the major moth pests. As this paper has briefly shown, there are many other ways in which insects and mites can respond to changing circumstances and as man intervenes to protect food stocks the measures taken produce changes in the behaviour and biochemistry of pests in response to the challenge.

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MORTALITY OF FOUR STAGES OF *LASIODERMA SERRICORNE* (FABRICIUS) EXPOSED TO LOW OXYGEN

Zhang Tao[1], Liu Shiwei[2], Li Yanyu[1], Cao Yang[1] *, Li Guangtao[1], Cui Yongkui[2], Zhang Zihai[2]

[1] Academy of State Administration of Grain, №11 Baiwanzhuang Street, Beijng, 100037, P.R. China, Tel: 86-10-68623665. Fax: 86-10-58523599
[2] Hunan Hua Wang Fumigation Technology Co., Ltd, №523, East Laodong Road, Yuhua District, Changsha 410014, P.R. China
*Corresponding author's e-mail: *cy@chinagrain.org*

ABSTRACT

Eggs, lava, pupa, and adults of *Lasioderma serricorne* (Fabricius) were exposed at different temperatures in 2% oxygen, 98% nitrogen, and 75% r.h. using the FAO recommended assay method. Probit analysis was used to determine the LT_{99} values. The results indicate that the 99% lethal time (LT_{99}), (95% Fiducial Limits) of the eggs were for 12.65 d (7.98~73.27), 9.40 d (5.70~41.82), 7.58 d (3.74~8180.93), 5.08 d (4.48~5.97), 2.86 d (2.39~3.81) at 15°C., 20°C., 25°C., 30°C., and 35°C, respectively in 2% oxygen, 98% nitrogen, 75% r.h.. The LT_{99} for eggs was increased by 0.48 d with the reduction of temperature by each 1°C. At 30°C and the same conditions, the LT_{99} of the lavae and the pupae were 9.62 d (8.86~10.74), and 8.07 d (7.22~9.58), respectively. At 30°C. and 35°C., the LT_{99} for adults was 21.93 d (14.28~83.47), and 13.43 d (8.32~40.34), respectively. Based on the comparison of the LT_{50} values of the four development stages of *L. serricorne* the sensitivity level to low oxygen in decreasing order was: eggs, adults, pupa, and lava. Based on the comparison of the LT_{99} values the sequence of sensitivity in decreasing order was: eggs, pupa, lava, and adults.

Key words: *Lasioderma serricorne*, low oxygen, lethal time

INTRODUCTION

Lasioderma serricorne (Fabricius) belongs to the family of Coleoptera; Anobiidae and is harmful to the tobacco, cigarettes and cigar products in storage (Jizhen et al., 2006), causing remarkable value loss to tobacco industry. *L. serricorne* is known as the world's number one pests of stored tobacco (Jianhua et al., 2010). *L. serricorne* has a wide range of diets like food grains, teas, beans, dried jujube, oil products, plant and animal specimen, cocoa bean, leather, vine and bamboo products (Ryan , 1995; Guangjun et al., 2000). *L. serricorne* can be rolled

into the cigarette and feed on tobacco, damage the outside paper, meanwhile, the fragment of its corpses and faeces also pollute the tobacco products. (Arbogast et al.,2002; Xinwen et al., 1995; Fangxiao et al., 2000).

The growth and development impact of harmful pests to stored bulk products at low oxygen have been of common interest in China and other countries. The adult lethal time of *Oryzaephilus surinamensis* L. is above 1 d at <1% oxygen and that of *Rhizopertha dominica* (Fabricius) above 4 d, *Sitophilus oryzae* (L.) above 14 d, more than 7 d for both *Tribolium castaneum* (Herbst) and *Tribolium confusum* Jacquelin du Val (Burton, 1998). Resistance in different low oxygen environments of adult internal feeders including *R. dominica*, *Sitophilus granarius* L. and *S. oryzae* is generally higher than that of *Cryptolestes ferrugineus* (Stephens), *O. surinamensis* and *T. castaneum* which are external feeders (Conyers et al.,1997; Krishnamurthy et al., 1986). Since studies on lethal time of four stages *L. serricorne* in low oxygen scarce, the present work aimed at investigating the sensitivity of eggs, larva, pupa, and adults of *L. serricorne* exposed to low oxygen.

MATERIALS AND METHODS

1.1 Test insect:

L. serricorne was provided by the Stored Grain and Oil Research Laboratory of the Academy of State Administration of Grain. Insects were incubated at $30\pm1^{\circ}$C, 75%$\pm$5 % r.h., and reared on whole wheat flour containing 5% yeast.

1.2 Test gas

High purified nitrogen, 99.999%, produced by Beijing Bei Temperature Gas Factory. High purified oxygen, 99.99%, produced by Beijing Bei Temperature Gas Factory was used in the present experiments.

1.3 Equipments

1.3.1 Main equipment and apparatus:

Fume cupboard, constant temperature incubator (Binder), Orsat gas analyzer, gas flow-meter, rapid oxygen indicator, U-tube pressure meter, electronic drying oven (Shanghai Sumsung Experimental Instrument Co., Ltd), stereomicroscope (Beijing Fuka Keyi Science and Technology Co., LTD), special vacuum dryer, and common dryer were used for the experiments.

1.3.2 Equipment combination:

Equipment combination shown in Fig. 1.

1.3.3 Testing desiccator gas tightness:

The special desiccator was first washed with deionized water and dried, then vaseline was applied to all connections to ensure gastightness. An U-tube pressure meter was used to measure the vacuum desiccator to maintain about 1000 Pa at constant temperature and pressure environment. The pressure value was recorded after it was stabilized in the desiccator. If the pressure remained the same after 24 h, the special desiccator was used in the experiments.
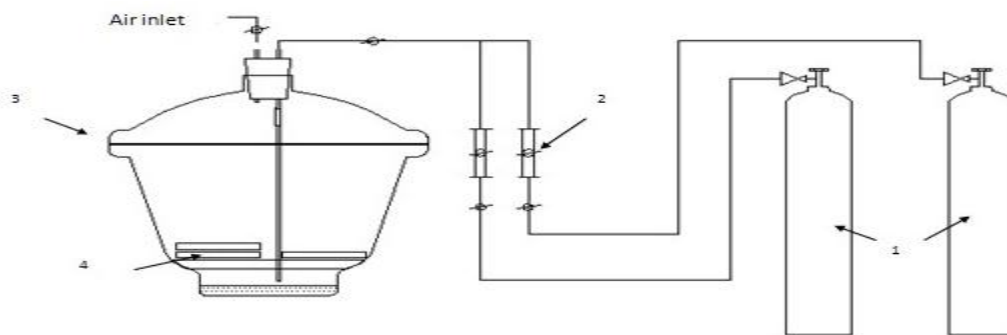
Fig. 1- Diagram of equipment used for exposure of *Lasioderma serricorne* (Fabricius) to low oxygen atmospheres (1= nitrogen and oxygen source; 2= gas flow control gauge; 3= desiccator; 4= insects).

1.3.4 Oxygen content adjustment:

The desired oxygen concentration was obtained by purging the desiccator with nitrogen, and measuring the gas mixture using the Orsat gas analyzer.

1.3.5 Temperature and humidity control:

A temperature controlled incubator (German Binder) was used in which the equilibrium relative humidity was adjusted using saturated saline solutions placed at the bottom of the incubator.

1.4 Test method

1.4.1 Treatment of eggs

An egg holding board was used to hold 1 d old 20 eggs which were evenly spread on a slide facing with double-sided adhesive 20 mm×20 mm.. Three in a row of such egg holding boards were glued on a slide for exposure to low oxygen environment; 3 egg holding boards were kept untreated served as control. After treatment the treated egg holding boards were incubated until larva emergence or mortality..

1.4.2 Treatment of larvae

On the wall of a transparent plastic pipe which is 15 mm in diameter and 50 mm long, 50 small holes were pierced. *L. serricorne* culture media was placed in a cage to contain 30 larvae of same age and size and exposed to low oxygen, 3 of such cages were left untreated as control. After treatment, the treated larvae in the cages were incubated and daily observations were made until larvae were dead or turn to pupa.

1.4.3 Treatment of pupae

Culture media of *L. serricorne* together with 30 larva of same age and size were placed in the larva containing cages and kept at 30°C and 75% r.h. for about a week for pupation. Three cages containing the pupae were exposed in a group to low oxygen, 3 untreated cages served as control. After treatment, the treated pupae in the cages were incubated and daily observations were made until pupae were dead or adult emergence.

1.4.4 Treatment of adults

A small piece of tobacco leaf of 14% moisture content together with 30 of 2 d old adults after

emergence were placed in a cage for exposure to low oxygen, 3 untreated cages were kept as control. After treatment, the treated cages were incubated for 24 h to observe their survival under the stereomicroscope. Those adults that did not show ant response to touching using a brush were considered as dead.

1.5 Statistics

PROC PROBIT method was used to compute the regression line and calculate half lethal time LT_{50} and 99% lethal time LT_{99} at 95% Fiducial Limits, b value and its standard deviation. Test of μ and t test were carried out on different four development stages to compare LT_{50} and b values for each stage.

RESULTS AND DISCUSSION

2.1 Effect of low oxygen on *L. serricorne* eggs

Egg mortality of *L. serricorne* exposed to 2% oxygen at 75% r.h. was significantly different at the tested temperatures of 15-35°C. Table 1 shows that temperature plays a significant role on the killing effect of *L. serricorne* eggs exposed to 2% oxygen. The higher was the temperature, the higher was the mortality of eggs. *L. serricorne* eggs were more resistant at the lower temperature region, 99% mortality could be achieved after 10 days of exposure to 2% oxygen at 20°C. and above. Therefore, for the control of *L. serricorne* eggs exposed to 2% oxygen, the temperature should be above 25°C, response of *L. serricorne* eggs 2% oxygen and 75% r.h. was linear to temperature decrease by each 1°C the LT_{99} increased by 0.48 d (Fig. 2).

Table 1. Mortality of *L. serricorne* eggs exposed to 2% oxygen at 75% r.h. and 15-35°C

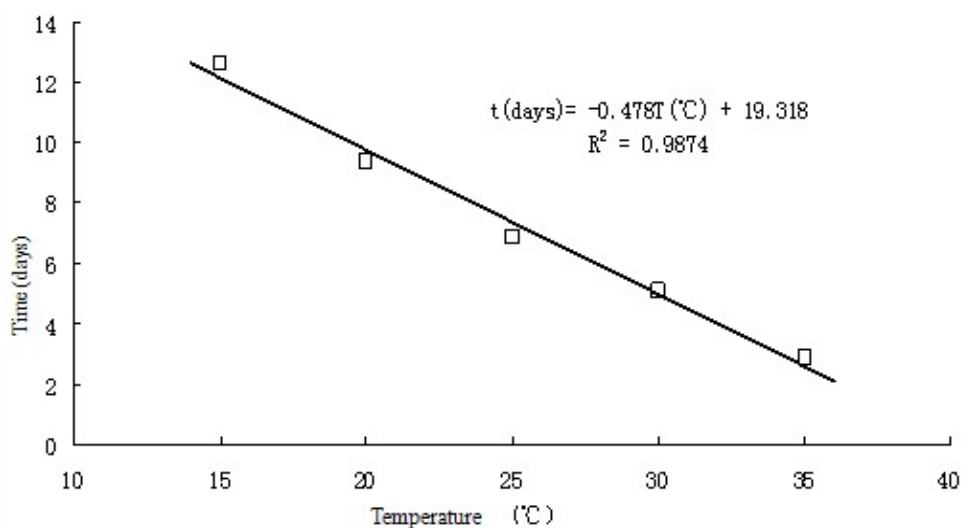| time (d)	temperature (°C)				
	15	20	25	30	35
0	5.00±2.89	16.67±1.67	6.10±1.10	11.67±4.41	13.33±1.67
1	\	25.00±5.78	46.67±9.28	21.67±1.67	58.33±13.02
2	20.00±5.00	55.00±12.58	66.67±3.33	56.67±16.91	93.33±1.67
3	\	86.67±4.41	88.33±6.01	90.00±2.89	100.00±0.00
4	40.00±5.78	85.00±0.00	96.67±1.67	96.67±3.33	100.00±0.00
5	\	88.33±3.33	\	100.00±0.00	100.00±0.00
6	76.67±3.33	\	\	\	\
8	96.67±1.67	\	\	\	\
10	100.00±0.00	\	\	\	\
Reg. equation	Y=4.60 lg(x) -2.74	Y=3.56 lg(x) -1.14	Y=2.96 lg(x) -0.28	Y=5.24 lg(x) -1.37	Y=5.04 lg(x) +0.03
LT_{50} (95% F. Limits)	3.95 (2.33~5.47)	2.09 (1.36~2.75)	1.24 (0.12~1.90)	1.83 (1.69~1.96)	0.97 (0.86~1.09)
LT_{99} (95% F. Limits)	12.65 (7.98~73.27)	9.40 (5.70~41.82)	7.58 (3.74~8180.93)	5.08 (4.48~5.97)	2.86 (2.39~3.81)

Fig. 2- The effect of temperature and exposure time to obtain LT_{99} values for *Lasioderma serricorne* (Fabricius) exposed to 2% oxygen at 75% r.h.

2.2 Effect of low oxygen on *L. serricorne* larvae

At $30^{\circ}C$ and 75% r.h. 2% oxygen 10 d exposure caused 100% mortality to *L. serricorne* larvae. Table 2 shows that a good equation fitness is obtained: Y=9.33 lg(x) -6.85, LT_{50} is 5.42 d, 95% Fiducial Limit is 5.18~5.66 d, LT_{99} is 9.62 d, 95% Fiducial Limit 8.86~10.74 d, the b value of toxicity regression curve is 9.33.

Table 2. Mortality of *L. serricorne* larvae and pupae exposed to 2% oxygen at 75% r.h. and $30^{\circ}C$

time(d)	stages	
	larva	pupa
0	5.56± 4.01	7.78± 2.94
4	17.78±2.22	54.44±12.81
6	63.33±1.92	88.89±2.94
8	95.56±2.22	100.00±0.00
10	100.00±0.00	100.00±0.00
Reg. equation	Y=9.33 lg(x) -6.85	Y=7.71 lg(x)-4.67
LT_{50}(95% F. Limits)	5.42(5.18~5.66)	4.03(3.71~4.29)
LT_{99}(95% F. Limits)	9.62(8.86~10.74)	8.07(7.22~9.58)

2.3 Effect of low oxygen on *L. serricorne* pupae

At $30^{\circ}C$ and 75% r.h. 2% oxygen 8 d exposure caused 100% mortality to *L. serricorne* pupae. Table 2 shows that a good equation fitness is obtained: Y=7.71 1g(x)-4.67, LT_{50} is 4.03 d.

95% Fiducial Limit is 3.71～4.29 d, LT_{99} is 8.07 d, 95% Fiducial Limit is 7.22～9.58 d.

2.4 Effect of low oxygen on *L. serricorne* adults

Table 3 shows that the biological assay equation is: Y=3.12 lg(x)-1.1966, when the *L. serricorne* adult was treated at 35°C, 75% r.h. and 2% oxygen, the LT_{50} is 2.42 d, 95% Fiducial Limit is 1.76-3.02 d, at LT_{99} is 13.43 d, 95% Fiducial Limit is 8.32-40.34 d.

Tests on *L. serricorne* adults were repeated 7 times at 30°C, 75% r.h. and 2% oxygen (Table 3). However, the reproducibility was with high variation. Probit method resulting that LT_{99} was from 5.08 d to 35 d. These results may be related to the sexual distinction and longevity difference on adults, which needs further investigation.

Results on LT values of *L. serricorne* adults at 2% oxygen, based on bioassays repeated twice under the same condition of oxygen and r.h. at 25°C are shown in Table 3. The results analyzed by Probit method could not generate biological assay equation.

In the above experiments of exposure of adults, no eggs were found in the cages, which mean there was no F1 *L. serricorne* after 2% oxygen treatment.

At the lower range of temperatures, mortality results were lower, which were not reported here.

Table 3. Mortality of *L. serricorne* adults exposed to 2% oxygen at 75% r.h. and 25-35°C

time（days）	temperature（°C.）					
	25	25	30	30	30	35
0	4.44±1.11	2.22±2.22	28.89±20.58	8.89±4.01	5.56±2.94	6.67±1.76
1	\	15.56±2.94	\	\	8.89±2.22	13.33±1.76
2	\	34.44±4.44	\	\	8.89 ±2.22	53.33±8.35
3	\	11.11±4.84	70.00±1.92	\	25.56±9.09	58.00±13.61
4	\	33.33±5.09	\	\	44.44±4.84	85.33±5.46
5	46.67±3.85	45.56±5.88	98.89 ±1.11	68.89±8.01	45.56±2.94	81.33±2.67
6	\	\	\	\	51.11±6.17	87.33±5.92
7	86.67±3.85	\	100.00±0.00	82.22±2.94	62.22±1.11	\
8	\	\	\	\	72.22±7.78	\
9	86.67±3.85	\	\	88.89±6.17	\	\
Reg. Equation	Y=5.32 lg(x) -3.76	Y=1.02 lg(x) -1.08	Y=8.23 lg(x) -3.48	Y=2.98 lg(x) -1.67	Y=2.92 lg(x) -2.19	Y=3.12 lg(x) -1.20
LT_{50}(95% F. Limits)	5.09 (…~…)	11.30 (…~…)	2.65 (2.29~2.87)	3.64 (1.88~4.55)	5.63 (4.92~6.66)	2.42 (1.76~3.02)
LT_{99}(95% F. Limits)	13.93 (…~…)	2115 (…~…)	5.08 (4.41~6.84)	21.93 (14.28~83.47)	35.25 (22.23~79.56)	13.43 (8.32~40.34)

2.5 Comparative effects of treatment on different development stages of *L. serricorne*

Bioassay equation shown in Fig. 3 is ideally representing the four development stages of *L. serricorne* treated at 30°C, 75% r.h. and 2% oxygen.

The regression equations of LT_{50} data of four stages are shown in Table 4 which shows that 2% oxygen has no significant difference on the LT_{50} for pupae and adults.

Table 4. Comparison of LT_{50} values expressed by regression equations ($\alpha=0.05$) on four stages of *L. serricorne*

P\μ	egg	larva	pupa	adult
egg	\	<0.0001	<0.0001	0.0084
larva	25.54	\	<0.0001	0.0103
pupa	7.72	4.58	\	0.5943
adult	2.63	2.57	0.53	\

Whereas, differences on the sensitivity of the other two stages is remarkable (Fig. 3). Based on LT_{50} values, the sensitivity of four stages of *L. serricorne* exposed to 2% oxygen in increasing order were: eggs, adults, pupae, and larvae; and based on LT_{99} values, the sensitivity of four stages of *L. serricorne* to 2% oxygen in increasing order were: eggs, pupae, larvae, and adults.

Table 5. Comparison of slope (b) of toxicity regression equation ($\alpha=0.05$)

P\t	egg	larva	pupa	Adult
egg	\	<0.0001	0.0134	0.0106
larva	4.7642	\	0.1790	<0.0001
pupa	2.4723	1.3439	\	0.0001
adult	2.5569	5.7487	3.8833	\

Comparison of slopes (b) of regression equations for four stages is shown in Table 5, indicating that there were no significant differences on slopes between larvae and pupae. The pupae as a transitional stage from larvae to adults, influenced the sensitivity of adults. The sensitivity variation range inside the strain is more apt to larvae. However, the tendency of adults to influence other stages is significantly lower than that of other stages. This means that the adult species bears wider variation range of resistance to low oxygen, probably because the large difference in longevity between male and female adults. *L. serricorne* adult can live about 14~50 d (Jisheng et al, 2006), therefore, it low oxygen treatment to attain LT_{99} should be targeted in pest control strategies.
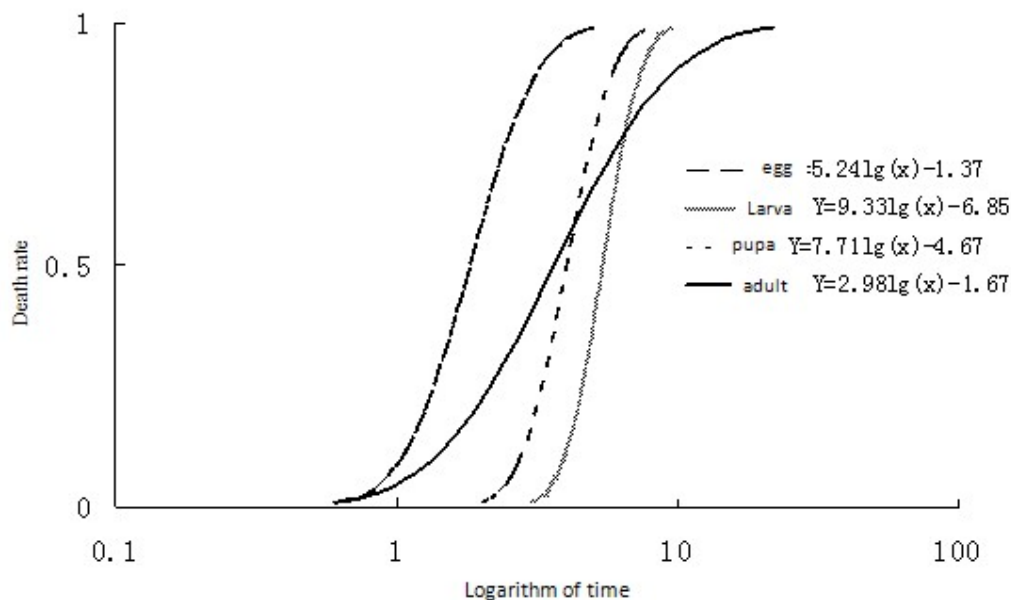
Fig. 3- Mortality of the four development stages of *Lasioderma serricorne* (Fabricius) exposed to 2% oxygen at 75% r.h. and 30°C.

CONCLUSIONS

Low oxygen is a very effective way for control of *L. serricorne* in all four stages since 99% mortality can be achieved within 10 d of treatment of eggs, larvae, and pupae at 30°C, 75% r.h. and 2% oxygen. While for adults about 22 d of exposure same conditions produce 99% mortality. Since multiple adult tests resulted in a wide variation range of mortality, such data will need further investigation.

Each stages of *L. serricorne* bear different sensitivity to low oxygen. Based on LT$_{50}$, the sensitivity of four stages of *L. serricorne* to 2% oxygen in increasing order was: eggs, adults, pupae, and larvae. Based on LT$_{99}$, the sensitivity of four stages of *L. serricorne* to 2% oxygen in increasing order was: eggs, pupae, larvae, and adults.

Temperature plays an important role in the control of *L. serricorne* exposed to 2% oxygen. Low temperature reduces effectiveness of the treatment. For the control of the egg stage at 75% r.h., for each temperature decrease by 1°C, the treatment time increased by 0.48 d. The influence of temperature on the other stages of *L. serricorne* needs further investigation.

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LETHAL EFFECT OF CO_2 MODIFIED ATMOSPHERES ON EGGS OF DIFFERENT AGE OF TWO BRUCHID SPECIES

Francisco Wong[1], Cristina Castañé[2], Jordi Riudavets[2*]

[1]Departamento de Investigación y Posgrado en Alimentos, Universidad de Sonora, Hermosillo, México
[2]IRTA, Entomology, Ctra. Cabrils km 2, E-08348 Cabrils, Barcelona, Spain,
*Corresponding author's e-mail: *jordi.riudavets@irta.es*

ABSTRACT

Stored legumes are attacked by a diversity of insect pest species of the family of Bruchidae. The most economically important and widespread species are the cowpea seed beetle, *Callosobruchus maculatus*, and the bean weevil, *Acanthoscelides obtectus*. These species laid their eggs loose (*A. obtectus*) or glued (*C. maculatus*) to the grain legume, and the emerging larvae burrow directly into the legume. The control of these pests relies mainly on the use of chemicals which are mainly directed to kill the egg stage. However, the need for repeated fumigations when eggs are present (at intervals of 25 to 30 days), that promotes the development of resistant populations, the low residue levels allowed in the final food products and the need to be environmentally friendly is making necessary to look for alternatives. Modified atmospheres (MA) with high carbon dioxide (CO_2) content are safe and environmentally friendly pest control methods for stored products. They are effective in controlling a wide range of species, and they can be applied in a variety of food products without leaving any toxic residues. The present study aimed to evaluate the efficacy of MA with high CO_2 to control eggs of different developmental age from the two bruchid pest species mentioned. Three concentrations of CO_2 MAs (50%, 70% and 90%) were tested at 28ºC, to identify the egg stage more susceptible to the treatments. Eggs sensitivity to the MA varied according to their age and species, with the 4 days old eggs of *A. obtectus* being the most tolerant of all eggs tested. A high level of control was achieved with 70% and 90% CO_2 during 3 days of exposure for all egg stages of both species.

Key words: modified atmospheres, carbon dioxide, *Acanthoscelides obtectus*, *Callosobruchus maculatus*, legumes, storage.

INTRODUCTION

Among the dry grain legumes, chickpeas are the second largest commodity in the world after dry beans. *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is the major pest of chickpeas. The infestation can start in the field, when the pods are dry, and continue in the storage, where it causes extensive damage to healthy or whole grains (Durón et al., 2005). The females attach their eggs to the surface of the seeds using a gelatinous glue-liked material. The eggs are milky white, oval, and dome-shaped with a flat floor. From the eggs hatch a larva that burrows into the grain where it develops to adulthood (Cope and Fox, 2003; Shazali et al, 2004).

Acanthoscelides obtectus (Say) (Coleoptera: Bruchidae) is one the main pest of dry beans and it also spend most of its development time inside the grain legume. The female lays their eggs inside the pods in the field or on the beans when in storage. Eggs are laid randomly under seeds and batches of eggs can be attached between them only slightly, if at all. First-instar larvae have legs and move around to find a suitable site to enter the seed (Papachristos and Stamopoulos, 2002).

The chemical control of *C. maculatus* and *A. obtectus* is currently based mainly in the use of fumigants in a preventive manner. Quality control is very strict as the legumes must not have eggs or insect-damaged kernels. For example, in Mexico for the export market, legumes are fumigated with hydrogen phosphide (PH_3) usually at intervals of 25 to 30 days. The number of applications of gas depends on the storage time, which sometimes may extend to a year or more depending on the international market prices. Modified atmospheres (MAs) based on high carbon dioxide (CO_2) are effective alternatives to the use of traditional chemical control (methyl bromide, phosphine and other insecticides) as pest control strategy. This is a cleaner environmental technology and toxicologically safe. Its cost is affordable in comparison to other control techniques to treat bags stored in different types of flexible airtight structures.

The objective of this study was to assess the susceptibility of eggs of this two pest species to three high CO_2 MAs. We evaluated the effect on eggs of different developmental status because they can show a different susceptibility to toxics, and it is important to determine at which age the eggs are more resistant.

MATERIAL AND METHODS

Insect colonies and experiments were performed in a climatic chamber at 28±2ºC, 70±15% r.h., and with a photoperiod of 16:8 hours of Light:Dark.

Stock colonies of the two bruchid species were reared on standard diets (chickpeas for *C. maculatus* and kidney beans for *A. obtectus*). A subsample of 50 individuals was reared in 200 g of standard food diet in ventilated plastic cages, in order to daily collect newly deposited eggs. For *A. obtectus* three eggs were deposited inside a gelatin capsule and 5 capsules were deposited in a small ventilated cage, containing also 50 g kidney beans. For *C. maculatus* five chickpeas with a minimum of three eggs that are attached to the pulse were deposited in a small ventilated cage, containing also 10 extra chickpeas. Eggs of one to five days old were treated with the different gas mixtures.

The small ventilated cages were placed inside plastic bags (Cryovac BB4L) of 300 x 210 mm of size and 59 micrometer-thick. The plastic bags had barrier properties to O_2 and CO_2. The plastic bags were filled with the desired atmosphere, which was previously prepared using a gas mixer (Witt KM 100-3M/MEM), using a vacuum packaging machine (Multivac A 300/16). A gas analyzer (Abiss model TOM 12) was used to verify the CO_2 and O_2 contents inside the plastic bags. Gas levels were determined at the start and at the end of the exposure.

After exposure to the MAPs, the plastic bags were subsequently opened to release the modified atmospheres and the cages were removed from the bags. To check the effect of the treatments on eggs, the cages were kept in the climatic chamber for up to 10 days to allow the hatching of eggs.

Three different MAPs were tested: i) 50% CO_2, with a residual of 10% O_2 and a 40% balance of N_2; ii) 70% CO_2, with a residual of 6% O_2 and a 24% balance of N_2; and iii) 90% CO_2, with a residual of 3% O_2 and a 7% balance of N_2. Eggs of all ages from *A. obtectus* were exposed during 3 days and also, eggs of all ages from *C. maculates* during 2 and 3 days to the

treatments. Five replicates with 15 eggs each were prepared for all treatments and species. Sets of control cages with the same number of eggs were tested for the different treatments applied in order to determine the percentages of natural mortality.

RESULTS AND DISCUSSION

ANALYSIS OF GASES

The CO_2 contents within the sealed plastic bags during exposure to modified atmospheres remained quite constant throughout the period of exposure. For all three gas mixtures, CO_2 decreased less than 3% to 8% of the initial content. The O_2 levels in the sealed bags increased less than 1% to 3% of the initial content.

EGG MORTALITY

Comparison between species.
Figure 1 shows the mortality from the eggs of different age of both species exposed to the three MAs during 3 days. All eggs from the different age stage of *C. maculatus* were killed at these conditions. In comparison, while very few eggs of *A. obtectus* survive at 90% CO_2, more eggs survive at 70% CO_2 and even more at 50% CO_2. Therefore, the eggs of *C. maculatus* were more sensitive to the MAs tested.

Comparison among egg developmental stages.
In *A. obtectus*, 4 days old eggs were the most tolerant to 50% and 90% CO_2 MAs. However, at 70% CO_2, eggs of 2, 3 and 4 days old were more tolerant than eggs of one and five days old (Fig. 1). Since at three days exposure 100% mortality was recorded for *C. maculatus* we tested a shorter exposure time of two days in order to assess the sensibility of the different egg ages (Fig. 2). For all three MAs, the 2 days old eggs were the most tolerant. Therefore, the susceptibility of the eggs according to their developmental stage seems to be different in each of the species tested. While for *A. obtectus* mature eggs (4 days) were more resistant, for *C. maculatus* younger eggs (2 days) were more resistant.

As expected, an increase in CO_2 concentration from 50% to 90% produced an increase in egg mortality. This was more evident observed in the case of *A. obtectus* than in *C. maculatus*.

These results indicate that the developmental stage of the eggs has to be considered when testing different strategies for controlling them, because the sensitivity varies according to their age and this also may change for different species. That is, not always mature eggs are more tolerant than young eggs or the opposite. In the present work we have tested two species from the same Bruchidae family, showing an opposite susceptibility pattern. These two species have also different strategies for laying their eggs; while *A. obtectus* located them freely near the pulses, *C. maculatus* glued them onto the surface of the pulse. These differences in egg laying strategies might be related with a difference in the permeability to gas exchanges, and then to susceptibility to be killed by CO_2.
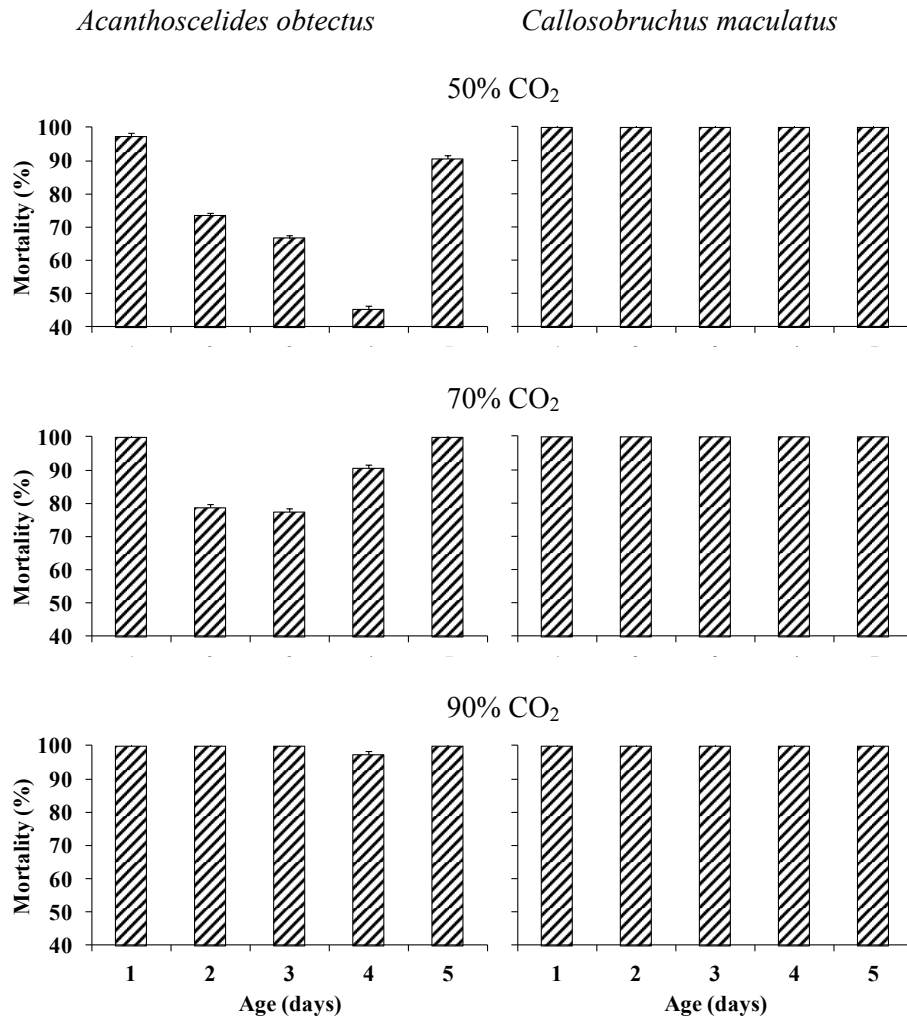
Fig. 1- Percent mortalities of *A. obtectus* (left) and *C. maculatus* (right) eggs of different age after exposure to various concentrations of carbon dioxide (CO_2) for 3 days at 28°C. n=5 of 15 eggs each.
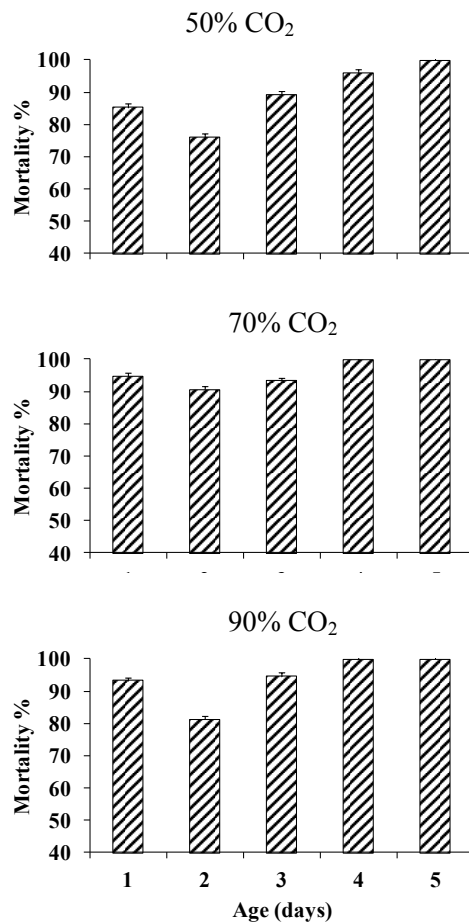
Fig. 2- Percent mortalities of *C. maculatus* eggs of different age after exposure to various concentrations of carbon dioxide (CO_2) for 2 days at 28°C. n=5 of 15 eggs each.

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SUSCEPTIBILITY OF STORED PRODUCT MOTH EGGS OF DIFFERENT AGE TOWARDS FUMIGATION WITH SULFURYL FLUORIDE – A REVIEW

Gabriele Flingelli*, Matthias Schöller, Dagmar W. Klementz, Christoph Reichmuth

Julius Kühn-Institut, Federal Research Centre for Cultivated Plants,
Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection,
Königin-Luise-Straße 19, D 14195 Berlin, Germany
*Corresponding author's e-mail: *Gabriele.Flingelli@jki.bund.de*

ABSTRACT

Eggs of insects require much higher dosages for pest control with sulfuryl fluoride (SF) than any other developmental stage. On the other hand, this fumigant is the most important gas replacing the phased out methyl bromide for control of insect pests in food processing premises. The pyralid moths *Ephestia kuehniella*, *E. elutella*, *Cadra (Ephestia) cautella*, *Corcyra cephalonica* and *Plodia interpunctella* are among the economically most significant pests in storage. In large mills, the shut down time for fumigation has to be short due to cost reasons. Additional heating instead of increasing the SF concentration is an alternative to ensure control while minimising the treatment time. Surviving individuals, especially eggs, are not acceptable for the food and feed industry. Eggs of *C. cautella* were found to be most tolerant. The available literature data concerning the control of eggs of different pest moths with SF is reviewed. A comparison of efficacies towards eggs of different age is carried out. Following the results as presented, will ensure effective fumigation with SF and complete kill of the moths.

Key words: sulfuryl fluoride, pyralid moths, control, eggs

INTRODUCTION

Pest moths cause significant damage in food and feed factories and the following chain of packaged products (Reichmuth et al., 2007; Rees, 2004). Layers of webbings on the top of stored grain in silo bins and granaries reduce the ventilation of the bulk with fresh air and favour mould growth. The webbings of the larvae, often mixed with frass and faeces, lead to blockage of the product flow in the factories, contamination of the processed raw materials and later claims by customers. Eggs surviving a control measure will lead again to the problems described above within a few weeks, and must therefore be fully controlled with the other stages.

Sulfuryl fluoride (SF) has been established as one of the main fumigants for pest control when methyl bromide (MeBr) was phased out in industrialized countries in 2005 due to its

ozone depleting potential (Anonymous, 2011a). Apart from other advantages compared to other fumigants (quick release, good penetration, not being corrosive to electronic equipment), SF has been suggested as a means of managing phosphine-resistant strains of pest insects (Williamson et al., 2011). A disadvantage of SF, the comparatively low susceptibility of egg stages of pest insects, has been discussed in comparison with other developmental stages (Bell et al., 1999, 2003; Reichmuth and Klementz, 2008; Anonymous, 2011b). This contribution summarizes the available mortality data for application of SF against eggs of moth pests and provides graphs and tables that allow the choice of an appropriate dosage for complete control for the given species.

LITERATURE SURVEY

For *Ephestia kuehniella* (Zeller), Bell and Savvidou (1999) observed the 1-2 day old eggs to be the most tolerant age group followed by 2-3, 0-1 and 3-4 day old eggs. According to the authors, a ct-product of about 800 g h m^{-3} at 25°C and about 3000 g h m^{-3} at 15°C were necessary for complete control of eggs. Efficacy was assessed by recording adult emergence reduction. As an additional result, no hatch occurred after treatment with 1000 g h m^{-3} at 25°C. About 4000 g h m^{-3} were necessary at 15°C. Reichmuth et al. (1999) found for *E. kuehniella* the 1 day old eggs to be more susceptible than 2, 3 and 4 day old eggs. In their study, complete control of all eggs was achieved with 1440 g h m^{-3} at 20°C. Ct-products for 24-h exposures achieving a high degree of control are summarized in the Table 1. The temperature dependency of the concentration is described in Fig. 1.
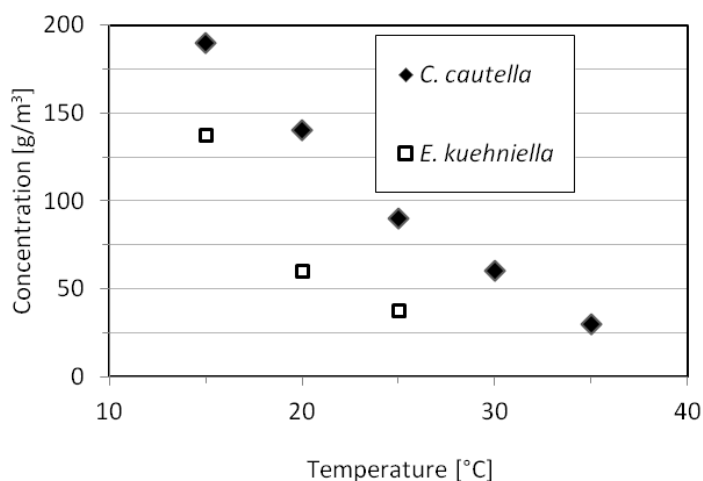

Fig. 1- Sulfuryl fluoride concentrations for complete control within one day at different temperatures data from Bell and Savvidou (1999) and Reichmuth et al. (1999) for *E. kuehniella* and data from Akan and Ferizli (2010) for *C. cautella*.

Akan and Ferizli (2010) found evidence for 0-1 day old eggs of *Cadra* (*Ephestia*) *cautella* (Walker) to be generally more susceptible than the older eggs of 1-2 or 2-3 days. According to the authors, this difference disappeared at temperatures above 30°C. Complete kill within 24 h fumigation with SF of eggs of all ages was achieved at 15°C, 20°C, 25°C,

30°C and 35°C with 190, 140, 90, 60 and 30 g m^{-3}, respectively (see Table 1 for the ct-product at 25°C). The eggs of this species were more tolerant than eggs of all other described pest moths. Fig. 1 offers information on the temperature dependence of the efficacy.

Table 1. Concentration time products for control with sulfuryl fluoride of different moth eggs of different ages; most data for 24 h fumigation at 25°C

Species	Tempe-rature [°C]	SF ct-product [gh/m^3]; (egg age in days)		Reference
Ephestia kuehniella	25	347	(0-1)	Bell and Savvidou (1999)
	25	667	(2-3)	
	25	912	(1-2)	
	25	240	(0-1)[a]	
	25	611	(2-3)[a]	
	25	764	(1-2)[a]	
	20	480	(1)(2)	Reichmuth et al. (1999)
	20	720	(3)[b](4)[c]	
Plodia interpunctella	27	175	(4)	Barakat et al. (2011)
	27	191	(2)	
	27	207	(1)	
	27	236	(3)	
Ephestia elutella	25	278	(0-1)[a]	Baltaci et al. (2006)
	25	511	(0-4)[a]	
Cadra cautella	25	1440	(0-1)	Akan and Ferizli (2010)
	25	1920	(2-3)	
	25	2160	(1-2)	

[a]data based on assessment of adult emergence, [b]95% kill, [c]89% kill

Baltaci et al. (2006) reported for eggs of ages between 1 to 4 days of *Ephestia elutella* (Hübner) that one day old eggs were most susceptible. A concentration of 21 g m^{-3} with at least 24 h exposure at 25°C led to complete kill of all stages.

Barakat et al. (2011) found with *Plodia interpunctella* (Hübner) a strong variation of egg susceptibility towards SF in tests at 27°C. With few exceptions, the 1-day-old eggs were most tolerant. This tendency was confirmed by Reichmuth et al. (1999) in tests at 20°C. In their study, 1-day-old eggs were slightly more tolerant than the 2-4 day-old eggs in a mixed age group. Contrary to these results, Schneider and Hartsell (1999) found at various concentrations 3-day-old eggs to be less susceptible than the other tested age groups. Walse (cited in Anonymous, 2011b) determined a LD$_{99}$ of 559 g h m^{-3} (concentration = 23.3 g m^{-3}) for a 24-h treatment with SF of a mixture of 1-3 day-old eggs at 26.7°C.

One to 4 day-old eggs of *Corcyra cephalonica* (Stainton) were tested by Barakat and Reichmuth (2009) at 27°C. The youngest eggs were slightly more tolerant. A 72-h fumigation led to 100% mortality of all exposed eggs with a ct-product of 450 g h m^{-3}. A 48-h fumigation of eggs of all ages resulted in similar mortalities. Comparing all moths investigated, the eggs of *C. cephalonica* proved to be the most susceptible.

Table 1 summarizes the data of SF time concentration products for high mortality of moth eggs according to the cited references for about 25°C. Fig. 2 compares the efficacy of

SF against eggs of different age and moth species at almost comparable conditions of treatment and the corresponding lethal concentrations.
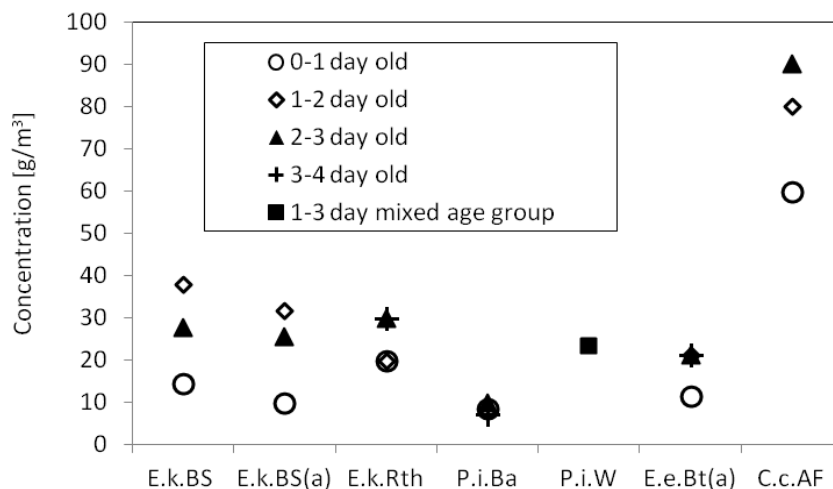

Fig. 2- Concentration of sulfuryl fluoride leading to control of eggs of different age and moth species within 24h fumigation at about 25°C. Data from table according to references: E.k.- *E. kuehniella*, P.i.- *P. interpunctella*, E.e.- *E. elutella*, C.c.- *C. cautella*, BS - Bell and Savvidou, (1999), Rth – Reichmuth et al. (1999), Ba -Barakat et al. (2011), Bt - Baltaci et al. (2009), AF - Akan and Ferizli (2010), W – Anonymus (2011b), (a)- data based on assessment of adult emergence.

DISCUSSION

According to data from the literature, there is contradicting information as to which age group of moth eggs is most tolerant towards SF. The results presented here were obtained from different laboratories with different experimental set ups for fumigation. Fig. 2 indicates the tendency of youngest eggs to be most susceptible when fumigated with SF at 25°C for 24 h.

Tests at higher temperatures (above 25°C) caused higher mortality at otherwise similar fumigation conditions. Presumably, increased speed of development during fumigation into more susceptible age stages of eggs or into susceptible young larvae may be the reason for this effect at higher temperature. According to Weidner (1983), hatch of eggs of *P. interpunctella* occurs within about 1.5 d, 2.5 d and 6 d at 30°C, 26°C and 23°C, respectively. In the case of *E. elutella*, the eggs require 5 days at 25°C to hatch. Since there is a minimum time required to allow for the developmental processes in the egg until hatch, a minimum exposure time at a given concentration should be ensured for efficacious fumigation (Barakat et al., 2011). With the exception of the data for *C. cautella,* a concentration of ca. 35 g m^{-3} seems to be adequate for 24 h fumigation at 25°C leading to high level of control of eggs of all moth species – if *C. cautella* occurs, 90 g m^{-3}. On the other hand, the determination of the moth species prior to fumigation offers the chance to determine the amount of SF really necessary for control following the principle of good fumigation practice: as little as possible but as much as necessary. Also longer exposure times could offer the opportunity to achieve control using less gas.

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POSTHARVEST FUMIGATION OF CHINESE YA PEAR WITH CARBONYL SULFIDE FOR THE CONTROL OF BLACK SPOT DISEASE

Tao Liu[1], Lili[1], Yuejin Wang[1*], Siqin Lei[2], and Xin Wang[1]

[1]Chinese Academy of Inspection and Quarantine, No. 241, Huixinxijie, Chaoyang District, Beijing, 100029, P.R. China; [2]Shuangliu District Village Development Bureau, No. 125, North West Street, Dongsheng Town, Shuangliu District, Chengdu, 610200, P.R. China
*Corresponding author's e-mail: *wangyuejin@263.net.cn.*

ABSTRACT

The black spot disease caused by *Alternaria alternata* (Fr.) is the main postharvest disease on Chinese Ya pear. Here we report the effect of temperature and exposure time on fungitoxicity of carbonyl sulfide fumigation against *A. alternata* and the response of Chinese Ya pear to the postharvest fumigation. Test results *in vitro* showed that *A. alternata* was susceptible to carbonyl sulfide fumigation at all the tested temperatures. The toxicity potency of carbonyl sulfide increased linearly and the LC99 decreased from 2,457.6 mg·l^{-1} to 174.5 mg·l^{-1} with temperature increasing from 4°C to 25°C. Exposure time extended from 4 h to 8 h at 25°C decreased linearly the concentration of LC99 from 199.2 to 104 mg·l^{-1}. In confirmation and phytotoxicity tests, carbonyl sulfide fumigation of Chinese Ya pear artificially infected with *A. alternata*, with a schedule of 200 mg·l^{-1} dosage and 4 h exposure time at 25°C, inhibited completely the further growth of the fungus in the fruit pulp. However, when dosage was higher than 90 mg·l^{-1}, the surface injury was not acceptable by market, although the fruit pulp quality parameters were not significantly changed.

Key words: Carbonyl sulfide, fumigation, black spot disease, Chinese Ya pear

INTRODUCTION

Black spot disease caused by *Alternaria alternata* (Fr.) Keissler is one of the most harmful diseases in Chinese Ya pears (*Pyrus pyrifolia*), particularly during storage stage (Zhang et al., 2003). Current control measures in the field mainly include cultivating resistant pear, placing the fruit in a special paper bag during growth, and spraying fungicides (He et al., 1995; Tetsuo et al., 1999; Terakami et al., 2007). These measures can effectively decrease the infection of Ya pear in the orchards, but cannot prevent infection after harvest. Moreover, some importing countries may consider *A. alternata* as a pest of quarantine importance, which directly influences the exportation of this fruit. Therefore, development of an economic and effective postharvest disinfection measure becomes necessity.

Carbonyl sulfide (COS) is a potential new fumigant that is present in nature (Fields and White, 2002). Laboratory and field studies have shown that COS is effective against a wide range of pests at all life stages, without any adverse effects on grains and stored products (Desmarchelier, 1994; Zettler et al, 1997; Weller, 1999; Xianchang et al., 1999). Quality studies on lemon, nectarine, papaya, and avocado indicated that COS fumigation did not

cause significant skin or flesh injury at reasonable concentrations (<80 mg·l^{-1}) and exposure times (1–24 h) (Chen and Paull, 1998; Weller et al., 1998; Aung et al., 2001). However, little is known about the fungitoxicity of COS against fungal plant pathogens. We report herein the effects of COS fumigation against *A. alternata* and the tolerance of Chinese Ya pears to COS fumigation.

MATERIALS AND METHODS

Fumigation of *A. alternata* in vitro
A.alternata L-3 was isolated from Ya pears infected with black spot disease in several orchards of Hebei Province, China. COS was purchased from Yanglilai Company (Beijing, China) as a compressed gas with 99% purity. COS fumigation tests with an exposure time of 4 h were performed separately at 4°C, 10°C, 15°C, 20°C, and 25°C to investigate the influence of treatment temperature on COS fungitoxicity. Fumigation with different exposure times of 3, 4, 5, 6, and 8 h at 25°C were also conducted. Three replicates were fumigated at each temperature and exposure time.

Fumigation of infected pears
Chinese Ya pears were purchased from a local orchard that did not receive any fungicide spray for 1 month before harvest. After storing at 25°C for 1 d, the fruit were surface-disinfested with 70% ethanol for 30 s. Eight small wounds (2 mm in depth and 5 mm in diameter) were made on each pear using a sterile pin. The wounded pear was inoculated with *A. alternata* by covering the fruit with small pieces of sterile filter paper that have been dipped in the previously prepared fungi suspension.

Fumigation containers were modified from 6-l vacuum containers. Eight Ya pears were placed in each container with about 40% load by volume. At the end of fumigation, the containers were quickly unsealed and forcibly aerated for 12 h at ambient temperature before the pears were again removed to store at 25°C for 7 d for efficacy evaluation. After 7 d storage at 25°C, the fungal spots on the infected pears were counted and the disease incidence rate was calculated.

COS phytotoxcity tests
Healthy Ya pears were fumigated at 25°C to evaluate phytotoxicity. Different dosages of 30, 60, 90 and 120 mg·l^{-1} COS were applied respectively with a same exposure time of 4 h. The possible phytotoxic response(surface injury) and the effect on fruit quality parameters (weight loss, firmness, soluble solids, total acidity) were examined after 7 d of storage at 25°C. Surface injury was classified as none (0), very slight ($\leq$5%), slight (5%–15%), moderate (15%–25%), severe (25%–50%), and very severe (>50%). Fruits with moderate, severe, and very severe surface injury were considered to be unmarketable.

Data analysis
Probit analysis was performed by PoloPlus (Leora Software 2003, USA), and the slope, LC50 value, and LC99 value of each test were calculated. Mean comparisons were performed using Duncan's multiple range test. All analyses were performed with SPSS software package v.11.0 for Windows.

RESULTS AND DISCUSSION

COS fungitoxicity against *A. alternate* in vitro
The influence of temperature on efficacy of COS fumigation was distinctive. The LC50 values decreased by about 21% as the temperature increased every 5 degrees from 4°C to 25°C, while the LC99 values decreased by about 24%. However, when temperature was at 10°C, the 99% inhibition rate required a concentration of 1,807.8 mg·l^{-1} with a 95% confidence limit from 1,503.9 to 2,311.2 mg·l^{-1}, the upper limit of which almost equals to pure COS gas, indicating complete inhibition (100%) could only be achievable when temperatures were higher than 15°C (Table 1).

Table 1. Probit analysis of COS fungitoxicity against *A. alternata* at different temperatures

Temperature (°C)	Slope[a]	Duncan test (0.95 CI)	Hetero.	LC50 (mg·l^{-1}) (0.95 CI)	LC99 (mg·l^{-1}) (0.95 CI)
4	3.12 ± 0.25	c	3.45	442.7 (302.1–563.1)	2,457.6 (1,584.7–6,476.2)
10	3.28 ± 0.27	c	0.75	353.0 (309.0–393.6)	1,807.8 (1,503.9–2,311.2)
15	5.19 ± 0.36	b	1.78	294.1 (255.8–330.4)	825.1 (672.7–1,141.0)
20	4.94 ± 0.29	b	3.18	116.0 (102.9–129.6)	343.0 (274.3–488.2)
25	6.12 ± 0.32	a	4.22	72.7 (62.0–82.6)	174.5 (146.6–228.3)

[a]Mean ± SE, CI means confidence interval

Parallelism comparisons of the probit regression lines showed that the probit regression lines were parallel between 4°C and 10°C, and also between 15°C and 20°C, which indicated that COS fumigation had the same fungitoxical potency within these two temperature ranges. Therefore, despite the linear decline of LC50 and LC99 values among all the temperatures tested, the temperature influence on fungitoxicity could be divided into 3 temperature groups. They were cold condition (<10°C), where the fungitoxicity of COS was low; cool condition (10°C–20°C), where the fungitoxicity of COS was medium; and warm condition (above 20°C), where the fungitoxicity of COS was high.

Exposure time also influenced COS fungitoxicity. The results demonstrated that COS dosage required to achieve a certain inhibition rate decreased when the exposure time increased, but not in a directly proportional manner. The LC99 value decreased from 199.2 to 104.0 mg·l^{-1} at a percentage of nearly 50%, and the slope increased from 6.4 to 12.0 when

Parallelism comparisons of the probit regression lines relative to different exposure times were also conducted. The probit regression lines of 3, 4, and 5 h exposure time seemed parallel (Table 2). The fact that the fiducial limits for all the inhibition levels with exposure time from 3 to 6 h overlapped at a large range further showed that COS fungitoxicity within 6 h exposure did not have substantial difference.

As described by Haber's (1924) rule, for a specific response level, the product of concentration (C) and exposure time (t) is constant (i.e., $Ct = k$), a famous relationship that has provided a good guide for methyl bromide fumigation. However, when calculating the CT products at a specific inhibition level in the tests with exposure time from 3 to 8 h (Table 2), we found the CT products increased linearly along with the extension of exposure time, which does not satisfy the relationship Ct=K.

Table 2. Probit analysis of COS fungitoxicity against *A. alternata* during different exposure times at 25°C

Time (h)	Slope[a]	Duncan test (0.95 CI)	Hetero.	LC50 (mg·l^{-1}) (0.95 CI)	LC99 (mg·l^{-1}) (0.95 CI)	$C_{LC50}t$ value (g·h·m^{-3})	$C_{LC99}t$ value (g·h·m^{-3})
3	6.37 ± 0.51	C	0.39	86.5 (81.6–92.1)	199.2 (173.6–239.5)	257.8	597.6
4	6.12 ± 0.32	C	4.22	72.7 (62.0–82.6)	174.5 (146.6–228.3)	291.0	697.6
5	6.30 ± 0.72	C	3.10	70.1 (58.6–84.6)	143.1 (105.4–226.0)	350.5	715.5
6	8.64 ± 0.74	B	2.82	68.6 (60.0–76.2)	127.4 (106.4–188.2)	411.4	764.4
8	12.01 ± 0.99	A	1.10	66.6 (63.2–70.3)	104.0 (94.0–122.0)	532.7	832.0

[a]Mean ± SE.

A more conventional form of $C^n t = k$ was further applied, where n represents the toxicity index. The values for n of 1.459 or $C^{1.459}t=7063$ was found to be comprehensively describing the relationship between the COS concentration and exposure time at an inhibition rate of 99% in all the tested exposure times. In this relationship, COS concentration played a more important role.

Efficacy of COS fumigation for infected pears

The infected pears were fumigated at 25°C with 4 h exposure time and different dosages of 30, 60, 90, 120, 160, and 200 mg·l^{-1}. Concentrations of COS were measured at 0.5 h after introduction of the gas, and at 0.5 h before aeration. The initial concentrations of COS were nearly 1.5 times of the applied dosages because of the almost 40% load factor, which further demonstrated the correct dosing. COS concentration decreased during exposure, and the final concentrations were almost equal to the applied dosages, indicating about 35% adsorption. The incidence of black spot disease on the infected pears after COS fumigation revealed that the efficacy of COS fumigation is dose dependent: a dose of 200 mg·l^{-1} resulted in 99% inhibition, which was comparable to the LD99 value (174.5 mg·l^{-1}) in the *in vitro* test (Fig. 1F).
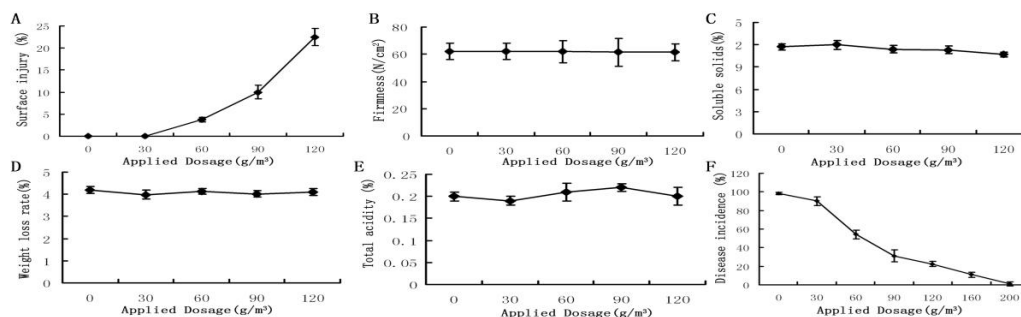
Fig. 1- Effect of COS fumigation on pear black spot disease and quality of Ya pears. (A) The surface injury of pears fumigated with COS (B) The firmness of pears fumigated with COS. (C) The soluble solids of pears fumigated with COS. (D) The weight loss rate of pears fumigated with COS. (E) The total acidity of pears fumigated with COS. (F) The disease incidence of infected pears. All data were averaged from 3 replicates after 7 d of storage. Error bars indicate SE.

Quality index of ya pears after COS fumigation

In our experiments, although there were no significant changes in internal quality parameters (Fig. 1B-E), large surface injuries occurred when the dose was higher than 90 mg·l^{-1}, indicating it is unacceptable for the control of black spot disease in Chinese Ya pears with COS fumigation.

Different kinds of fruits differ in their response to COS fumigation, for example, avocado and mango could only tolerate 45 and 23 mg·l^{-1} COS for less than 4 h fumigation (Weller, 1999). On the contrary, lemons could tolerate COS fumigation with a dosage of 70 mg l^{-1} and exposure time of 8 h (Weller, 1999). Therefore, COS can be considered as a postharvest disinfestation or disinfection measure on some kinds of fruits.

In conclusion, although COS could control *A. alternata* at reasonable temperatures (>15°C) and exposure times (4–8 h), the obvious surface injuries occurred with dosages higher than 90 mg·l^{-1} in 4-h exposures restricted its application as an effective fumigant for control of the black spot disease in Chinese Ya pears.

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MONOTERPENOIDS AS FUMIGANTS IN THE MANAGEMENT OF *CALLOSOBRUCHUS MACULATUS* (F.) (COLEOPTERA: BRUCHIDAE): OVIPOSITION DETERRENCE AND MORTALITY OF DEVELOPMENTAL STAGES

George N. Mbata[1*], Dana Smith[1], and Kendra Mcneal[1]

[1] Department of Biology, Fort Valley State University, Fort Valley, Georgia 31030, USA.
*Corresponding author's e-mail: *gn1185mbata@aol.com* or *mbatag@fvsu.edu*

ABSTRACT

Monoterpeniods have been demonstrated to cause mortality in certain stored-product insect pests. The current report investigated the prospects of using monoterpenoids in the management of all stages of the cowpea beetle, *Callosobruchus maculatus* (Fabricius). Newly emerged males and females (36 – 48 h), that were observed mating were exposed to cowpea seeds treated with the following monoterpenoids; *E*-anethole, estragole, *S*-carvone, linalool, L-fenchone, geraniol, γ-terpinene and *DL*-camphor at the following concentrations 66.7, 33.3, 16.7, 8.33 and 0 µL/L to determine the effect of the fumigants on egg laying. Treated *C. maculatus* females did not lay eggs even when exposed to sublethal doses of the monoterpenoids, while control adult beetles exposed to 0 µL/L laid several eggs. However, mated *C. maculatus* females laid eggs on cowpea seeds treated with monoterpenoids if the treated seeds were aerated for one week. The monoterpenoids did not exhibit residual toxicity to the cowpea beetles. Exposure of the developmental stages of the beetle, which include eggs, young larvae (first instar), 4th instar, pupae and adults to different concentrations of the monoterpenoids over 24 h period generated varying levels of mortality. The developmental stages of the beetle that were most tolerant to the monoterpenoids were the 4th instar, and the pupae. All the monoterpenoid evaluated were effective in causing mortality to the stages of *C. maculatus*. These monoterpenoids could be further investigated for the postharvest management of seed beetles of grain legumes.

Key words: pest management, dried beans, monoterpenoids, methyl bromide alternative, *Callosobruchus maculatus*

INTRODUCTION

The cowpea weevil, Callosobruchus maculatus (F.) (Coleoptera: Bruchidae), is a worldwide pest of cowpea, *Vigna unguiculata* (L.) Walpers (Fabales: Fabaceae). Infestation of cowpea by this bruchid commences in the field before mature seeds are harvested (Huignard et al., 1985). Infestation level of cowpea is very low at harvest and may sometimes be undetectable (Huignard et al. 1985). The cowpea weevil multiplies very fast in storage, giving rise to a new generation every month, and losses up to 30 percent in three months (Ouedraogo et al., 1996). Barring containment of the pest, complete loss of cowpea could occur within six months of storage (Caswell 1961). The most effective pest management tool used in the

disinfestation of commercial quantities of cowpea is fumigation with synthetic insecticides such as methyl bromide or phosphine gas (Mbata 2004). Use and production of the fumigant methyl bromide was scheduled to end in developed countries by January 2005 and worldwide by 2015 under the terms of the Montreal Protocol (United Nations Environment Programme 1998). Uses of other insecticides in stored products are facing restriction, and pest populations are evolving resistance to chemical insecticides (Phillips et al., 2000). Several traditional measures for protecting harvested cowpea are in use in subsistence agriculture, but their efficacy is often unverified (Alabeek 1996). Monoterpenoids, which are volatiles from plants, are being proposed here for the management of *C. maculatus* populations in post harvest storage of cowpeas.

Monoterpenoids are 10-carbon, secondary plant chemicals that are major components of essential oils extracted from leaves or fruits of herbs such as *Eucalyptus*, *Ocimum* spp., *Carum carvi* L. (caraway), *Coriandrum sativum* L., and many others (Rice and Coats 1994; López et al., 2008). Most monoterpenoids are volatile and have distinct aromas or flavors which may be pleasant to humans. The monoterpenoids are believed to aid plants in chemical defense against phytophagous insects and are now being exploited as insecticides. Monoterpenoids that have been investigated for insecticidal actions include *E*-anethole, estragole, *S*-carvone, linalool, *L*-fenchone, geraniol, γ-terpinene and *DL*-camphor (Lopez et al., 2008; Pascual-Villalobos et al., 2004; Pascual-Villalobos and Ballesta-Acosta 2003). Many monoterpenoids have been found to be effective against several postharvest insects (López et al., 2008). The authors hypothesize that some or most of these monoterpenoids will deter oviposition in exposed mated females of *C. maculatus*, cause mortality of exposed developmental stages and adults of *C. maculatus*.

MATERIALS AND METHODS

Insects
The cowpea weevil colony used in this study was obtained from laboratory rearing facility of Grain Marketing and Production Research Center, USDA-ARS, Manhattan, Kansas, and has been maintained for in the rearing facility for ten years at the Department of Biology, Fort Valley State University, Georgia. The beetles were reared on cowpea seeds in 1-liter wide-mouth glass jars at 30 ± 0.5°C, 70 ± 5% r.h., and a photoperiod of 12:12h (L:D) as described by Shu et al., (1996).

Eggs, 6 –24 h old (except where otherwise specified), first and last (fourth) instars, 24 h old pupae and adults were used in these experiments. Females of *C. maculatus* glue their eggs on cowpea seeds and the eggs are easily discernable. Seeds bearing 1 to 3 eggs were sorted to obtain a total of thirty seeds with a collective number of sixty eggs per jar and were transferred to 1000 ml rearing jars. The larvae of *C. maculatus* feed and develop internally, and could not be discerned externally by observing the surface of seeds. Radiography was used previously to follow larval and pupal development in this beetle at 30 ± 0.5°C, 70 ± 5% r.h. (Mbata and Reichmuth 1996, Mbata et al., 2000), and these developmental schedules were used to estimate the life stages present in infested seeds tested in this study. Eggs hatch into first instar after 2 d. Following hatching, the color of egg changes from clear to cream-white because of frass deposition in the eggshell. The last instar (4th) is attained between 14 – 17 d while the pupal stage is attained between 18 - 21 d from the day eggs were laid. Thus, in these experiments, 3 d old and 16 d old developing individuals were assumed to be first and last instars, respectively. The number of eggshells with the appearance of successful hatching was used to estimate the number of larvae in seeds used in experiments requiring larvae.

Seeds from cultures of desired ages bearing first and last instars were sorted as described for the eggs and placed in ventilated glass vials with each vial containing 50 larvae. The pupae used in the experiments were from infested seeds that were 19 -20 d old, and could be seen through an opaque pre-emergence "window" in the cotyledons of the seed. Seeds bearing 30 pupae were placed in ventilated vials as described above for eggs. Mated adults used were 36 h old and 6 adults comprising 3 males and 3 females were placed in 1000 ml along with either treated or control pristine uninfested seeds in rearing jars as described for the eggs. Filter papers held in place with a metal lid having hollow-core were used as covers for jars and treatments with monoterpenoids were conducted by injecting the monoterpenoids with 100 µL syringes through the filter papers onto test seeds, which were either uninfested or bore developmental stages. The monoterpenoids investigated are *E*-anethole, estragole, *S*-carvone, Linalool, *L*-fenchone, geraniol, γ-terpinene and DL-camphor. The monoterpenoids were obtained from Sigma-Aldrich Co. LLC, St. Louis, MO 63178, USA. The concentration of monoterpenoid used was 66.7 µL L^{-1}, except when investigating mortality of adults, and oviposition by treated adults that 66.7, 33.3, 16.6 and 8.3 µL L^{-1} concentrations of monoterpenoids were used. As soon as the monoterpenoid was introduced into the system, filter papers used in covering the jars were replaced with metal plates. The jars used were air-tight canning jars. Eighteen jars were set up for each of seven trials and each trial consisted of 2 jars for each monoterpenoid and 2 for the control which had untreated infested seeds. The treatment jars were placed in a chamber maintained at 30.0 ± 0.5°C and 70 ± 5% r.h. The metal lids of the jars were replaced with filter papers after 24h and the jars replaced in the cooled chamber for up to 4 weeks or until the complete emergence of all surviving beetles. Seeds bearing eggs, larvae, and pupae were observed daily for adult emergence. The individuals that failed to emerge 2 wk following emergence of the last adult from control are deemed to have died. Adults treated in these studies were considered dead if they were immobile 24 h after treatment. Mortality values were recorded and analyzed.

Data analysis
Treatment effects were determined by using analysis of variance (ANOVA; Proc GLM), and differences in treatments were elucidated through Turkey's test and LSD ($\propto = 0.05$) (SAS, 2001). Percentage data were arcsine of square root transformed.

<div align="center">RESULTS AND DISCUSSION</div>

Mortality data resulting from treatments of cowpea seeds infested by life stages of *C maculatus* with monoterpenoids are shown in Tables 1 -3.

Exposure of adults beetles to cowpea seeds treated with concentrations (8.3 – 66.7 µL L^{-1}) of monoterpenoids showed that minute quantities of the different monoterpenoids had effect on the mortality of the beetles. The critical concentration of the monoterpenoids investigated that generated 100% mortality in adult beetles was 16.7 µL L^{-1}. Exposure of seeds infested with life stages, eggs, 1st instar, 4th instar, pupae and adults of *C. maculatus* with 66.7 µL L^{-1} of monoterpenoids generated mortality in the life stages that ranged between 85.3 and 100%. These mortality values were significantly higher than those of the control (Table 2).

The eggs and adults of the beetle were more susceptible to the monoterpenoids than the 4th instar and pupae since individuals from these late developmental stages survived to adulthood. All the eight monoterpenoids investigated in this study were effective against *C. maculatus*. In an earlier observation, DL-camphor and estragole were the only

monoterpenoids effective against diapausing larvae of *Plodia interpunctella* (Mbata et al., 2012 in press).

Table 1. Mortality (% ± SE) of adult *C. maculatus* exposed to different concentrations of monoterpenoids

Monoterpenoids	Concentration(μg L^{-1})			
	8.3	16.7	33.3	66.7
E-anethole	78 ± 14.5B	100 ± 0A	100 ± 0A	100 ± 0A
Estragole	62 ± 18.5BC	100 ± 0A	100 ± 0A	100 ± 0A
S-carvone	74 ± 21.3B	100 ± 0A	100 ± 0A	100 ± 0A
Linalool	72 ± 16.7B	100 ± 0A	100 ± 0A	100 ± 0A
L-fenchone	85 ± 18.5AB	100 ± 0A	100 ± 0A	100 ± 0A
Geraniol	77 ± 18.5B	100 ± 0A	100 ± 0A	100 ± 0A
γ-terpinene	67 ± 13.7BC	94 ± 18.9A	100 ± 0A	100 ± 0A
DL-camphor	50 ± 16.7C	91 ± 13.5A	91 ± 14.8A	100 ± 0A
Control	7 ± 1.8D	7 ± 1.8D	7 ± 1.8D	7 ± 1.8D

Values in rows or columns having different uppercase letters are significantly different ($P < 0.05$)

Table 2. Mortality (% ± SE) of life stages of *C. maculatus* exposed to monoterpenoids (66.7 μL)

	Eggs	1st instar	Fourth instar	Pupae	Adults
E-anethole	100 ± 0A	100 ± 0A	100 ± 0A	85.6 ±17.5A	100 ± 0A
Estragole	100 ± 0A	100 ± 0A	100 ± 0A	91.3 ± 9.2A	100 ± 0A
S-carvone	100 ± 0A	100 ± 0A	99.2 ± 2.7A	99.3 ± 2.2A	100 ± 0A
Linalool	100 ± 0A	100 ± 0A	98.3± 5.6A	85.3± 16.9A	100 ± 0A
L-fenchone	100 ± 0A	100 ± 0A	98.4 ± 5.4A	98.7 ± 3.3A	100 ± 0A
Geraniol	100 ± 0A	100 ± 0A	95.1 ± 9.2A	86.7 ± 13.6A	100 ± 0A
γ-terpinene	100 ± 0A	100 ± 0A	96.7 ± 10.5A	92.0 ± 15.4A	100 ± 0A
DL-camphor	100 ± 0A	100 ± 0A	97.5 ± 4.9A	88.7 ± 8.9A	100 ± 0A
Control	9.0 ± 3.7C	17.3 ± 5.2C	7.4 ± 1.8C	2.0 ± 0.6D	7.0 ± 1.8C

Values in rows or columns having different uppercase letters are significantly different ($P < 0.05$)

Mated adult females provided with seeds exposed to 8.3 μg L^{-1} of the monoterpenoids did not deposit eggs on the seeds. However, when seeds treated with monoterpenoids were aerated for 21 d following treatment mated females laid eggs on them (Table 3). It is probable that the monoterpenoids inhibited oviposition by the female beetles. In addition, it appears that the monoterpenoids did not exhibit residual toxicity to the beetles.

Table 3. Eggs (No. ± SE) laid by *C. maculatus* females on seeds treated with monoterpenoids (8.3 μL)

	1 hr Post treatment	21 d Post treatment
E-anethole	0C	27.5 ± 4.3A
Estragole	0.4 ± 0.2C	29.3 ± 6.9A
S-carvone	0C	25.3 ± 3.5A
Linalool	0.6 ± 0.2C	24.3 ± 7.4A
L-fenchone	0C	31.8 ± 4.7A
Geraniol	0C	24.5 ± 3.4A
γ-terpinene	0C	25.8 ± 5.1A
DL-camphor	3 ± 1.8B	27.0 ± 3.3A
Control	28.7 ± 2.7A	27.0 ± 3.1A

Values in rows or columns having different uppercase letters are significantly different ($P < 0.05$)

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CHEMICAL COMPOSITION AND FUMIGANT TOXICITY OF ESSENTIAL OILS ISOLATED FROM EGYPTIAN PLANTS AGAINST STORED PRODUCT INSECTS *SITOPHILUS ORYZAE* (L.) AND *TRIBOLIUM CASTANEUM* (HERBST)

Samir A.M. Abdelgaleil[1*] Mohamed E.I. Badawy[1], Magdy I.E. Mohamed[2], Mohamed S. Shawir[1]

[1]Department of Pesticide Chemistry and Technology, Faculty of Agriculture, 21545-El-Shatby, Alexandria University, Alexandria, Egypt
[2]Department of Stored Product Pests, Plant Protection Research Institute, Agricultural Research Center, Sabahia, Alexandria, Egypt
*Corresponding author's e-mail: *samir1969us@yahoo.com*

ABSTRACT

The essential oils from six Egyptian plants, namely *Citrus paradisi*, *Artemisia judaica*, *Astoma seselifolium*, *Citrus limon*, *Pituranthos tortuosus* and *Citrus sinensis* were isolated by hydrodistillation. The chemical composition of the isolated oils was identified by gas chromatography/mass spectrometry (GC-MS). The major constituents of the isolated oils were *dl*-limonene (74.29%) in *C. paradise*, *β*–thujone (49.83%) and chrysanthenone (10.88%) in *A. judaica*, sabinene (23.02%) and 4-terpineol (17.83%) in *A. seselifolium*, *dl*-limonene (56.30%) in *C. limon*, sabinene (32.09%) and 4-terpineol (20.31%) in *P. tortuosus* and *dl*-limonene (89.23%) in *C. sinensis*. The isolated oils were tested for their fumigant toxicity against two of the most destructive stored product insects *Sitophilus oryzae* and *Tribolium castaneum*. The oil of *C. limon* showed the highest toxicity against *S. oryzae* followed by the oil of *C. sinensis* with LC_{50} values of 9.89 and 19.67 mg/L respectively, while the oil of *A. seselifolium* revealed the lowest toxicity. When tested against *T. castaneum*, the oils *C. sinensis* and *C. limon* were again the most potent toxicants, whereas the oil of *A. judaica* showed the weakest insecticidal activity. In general, the isolated oils were more toxic against *S. oryzae* than *T. castaneum*. The results of the present study suggested that the isolated oils, particularly of *C. limon* and *C. sinensis* could be used as potential natural products for control of *S. oryzae* and *T. castaneum*.

Key Words: Egyptian plants, essential oils, fumigant toxicity, *Sitophilus oryzae*, *Tribolium castaneum*

INTRODUCTION

The stored grain losses caused by insect damage and other organisms vary from 10% to 40% in countries where modern storage technologies are yet to be fully adopted (Raja et al., 2001; Ogendo et al., 2003). The quantity of loss is dependent upon the insect species involved, storage duration and pest control methods among other factors. The rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and the rust red flour beetle, *Tribolium castaneum*

(Herbst) (Coleoptera:Tenebrionidae), are among the most widespread and destructive stored product insects throughout the world. They cause significant losses of stored products, particularly in tropical and warm temperate regions (Hill, 1990).

Control of stored product insects around the world relies heavily on use of organophosphorus and pyrethroid insecticides, and fumigants (i.e. methyl bromide and phosphine). However, continuous and heavy use of these synthetic pesticides has created serious problems such as ozone depletion and environmental pollution (World Meteorological Organization, 1995), toxicity to non-target organisms such as parasitoids, predators, pollinators and fish, pest resistance (Mohan and Fields, 2002) and pesticide residues (Ogendo et al., 2003). Therefore, there is an urgent need to develop new, convenient and safer alternatives to synthetic pesticides. The extracts and secondary metabolites of plants are among the most promising alternatives. These botanical pesticides have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for the design of target-specific molecules (Isman, 2008).

Essential oils and their major constituents, mainly monoterpenoids, attracted research attention in recent years as potential alternatives to synthetic insecticides (Aslan et al., 2004). The present study describes the isolation and chemical analysis of essential oils of six Egyptian plants. The fumigant toxicity of the isolated oils was evaluated against two major stored product insects *S. oryzae* and *T. castaneum*.

MATERIALS AND METHODS

Plant materials

The fruits of the three *Citrus* plants: *Citrus paradisi* Macfad., *Citrus limon* (L.) Burm. f. and *Citrus sinensis* (L.) Osbeck were purchased from Alexandria Main Market for Vegetables and Fruits in February, 2011. The fruit peels were used as the source for essential oils. The aerial parts of three other plants: *Artemisia judaica* L., *Pituranthos tortuosus* (Desf.) Benth and *Astoma seselifolium* DC. were collected from Alhamam (west Alexandria) and Edko (east Alexandria) regions, Egypt, in April and May 2011. The plant materials were identified with guidance from the Student's Flora of Egypt by Tackholm (1974) and confirmed by Prof. FathAllah Zaitoon of the Faculty of Agriculture, Alexandria University. Voucher specimens have been deposited in the Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University.

Test insects

Colonies of the rice weevil, *Sitophilus oryzae* (L.), and the rust red flour beetle, *Tribolium castaneum* (Herbst), were reared in our laboratory over 10 years without exposure to insecticides on sterilized whole wheat and wheat flour mixed with yeast (10 : 1, w/w, respectively). Insect rearing and all experimental procedures were carried out at 26±1°C and 65±5% rh and in a 12: 12 light: dark photoperiod. Adults used in fumigant toxicity studies were 2 weeks post-eclosion.

Isolation of essential oils

The aerial plant parts were partially dried at room temperature (26±1°C) for five days and the fruit peels were used fresh. Essential oils were extracted by hydrodistillation in a Clevenger-type apparatus for 3 h. The oils were dried over anhydrous sodium sulfate, and stored at 4°C until used for biological activity tests and GC-MS analysis.

Analysis of essential oils

Essential oils were diluted in diethyl ether and 0.5 µl was injected into the gas chromatography (Hewlwett Packard 5890)/mass spectrometry (Hewlwett Packard 5989B) (GC-MS) apparatus. The GC column was a 30 m (0.25 mm i.d., film thickness 0.25 µm) HP-5MS (5% diphenyl) dimethylpolysiloxane capillary column. The GC conditions were as follows: injector temperature, 240°C; column temperature, isothermal at 70°C for 2 min, then programmed to 280°C at 6°C/ min and held at this temperature for 2 min; ion source temperature, 200°C; detector temperature, 300°C. Helium was used as the carrier gas at the rate of 1 ml/min. The effluent of the GC column was introduced directly into the ion source of the MS. Spectra were obtained in the EI mode with 70 eV ionization energy. The sector mass analyzer was set to scan from 40 to 400 amu for 5 s.

Fumigant toxicity assay

The toxicity of essential oil vapors was tested against *S. oryzae* and *T. castaneum* adults by using a modified fumigant toxicity assay as described by Huang et al. (2000). Glass jars (1 L) were used as fumigation chambers. Essential oils were applied to filter paper pieces (2×3 cm) attached to the undersurface of the screw caps of jars at: 1, 2.5, 5, 10, 20, 30, 40, 60, 80 and 100 mg. The inner side of the jar's neck was painted with vaseline to prevent direct contact of insects with essential oils. Caps were screwed tightly on the jars after adding 20 adults of *S. oryzae* or *T. castaneum* to each. Control insects were kept under the same conditions without application of essential oils. Three replicates of each treatment and control were set up. The number of dead insects was counted after 24 h of treatment, and the mortality percentages and median lethal concentrations (LC_{50} values) were calculated according to Finney (1971).

RESULTS AND DISCUSSION

Chemical composition of the isolated essential oils

The major components of essential oils identified from the 6 plants are given in Table 1 and Fig. 1. The major constituents of the essential oils were β- thujone (49.83%), chrysanthenone (10.88%) and α-thujone (8.21%) in *Artemisia judaica*, sabinene (23.02%), 4-terpineol (17.83%), γ-terpinene (8.97%) and germacrene D (8.27%) in *Astoma seselifolium*, dl-limonene (56.30%), β-pinene (8.81%) and γ-terpinene (6.42%) in *Citrus limon*, dl-limonene (74.29%), L-linalool (4.61%) and linalool oxide (4.18%) in *C. paradisi*, dl-limonene (89.23%) and linalool (2.98%) in *C. sinensis*, sabinene (32.09%) and 4-terpineol (20.31%) in *Pituranthos tortuosus*. Some major components were found in more than one plant, such as dl-limonene, sabinene, 4-terpineol and γ-terpinene but others were specific to the plant species. The major constituents of the essential oils mainly belonged to three groups: oxygenated monoterpenes (α-thujone, chrysanthenone, 4-terpineol, L-linalool and linalool oxide), monoterpene hydrocarbons (dl-limonene, sabinene, γ-terpinene, β-pinene) and sesquiterpene hydrocarbons (germacrene D).

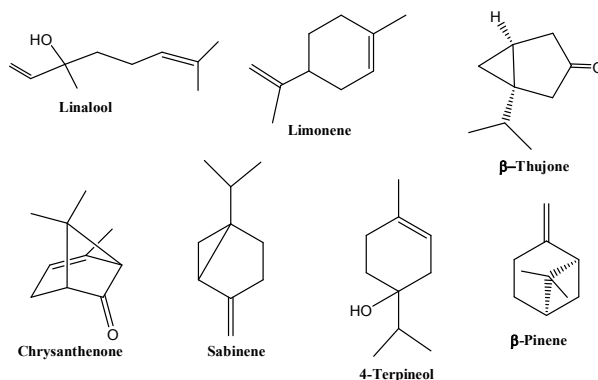Fig. 1- Chemical structures of major compounds of the isolated oils.

Table 1. Major constituents of the essential oils isolated from Egyptian plants

Plant oil	Major constituents (%)
Artemisia judaica	*β* –Thujone (49.83)
	Chrysanthenone (10.88)
	α- Thujone (8.21)
	1,8-Cineole (4.91)
	L-Camphor (3.0)
Astoma seselifolium	Sabinene (23.02)
	4-Terpineol (17.83)
	γ-Terpinene (8.97)
	Germacrene D (8.27)
	α-Pinene (6.20)
	β-Myrcene (3.64)
Citrus limon	dl-Limonene (56.30)
	β-Pinene (8.81)
	γ-Terpinene (6.42)
	α-Citral (4.96)
	β-Citral (3.83)
	α-Terpineol (3.38)
Citrus paradisi	dl-Limonene (74.29)
	L-Linalool (4.61)
	Linalool oxide (4.18)
	β-Citral (2.66)
Citrus sinensis	dl-Limonene (89.23)
	Linalool (2.98)
	β-Myrcene (1.77)
	Octanal (1.28)
Pituranthos tortuosus	Sabinene (32.09)
	4-Terpineol (20.31)
	Myristicine (6.84)
	Dillapiole (5.72)
	γ-Terpinene (4.16)
	α-Pinene (3.25)

The oil composition of *A. judaica* from Alhamam in this study differed from that isolated from *A. judaica* growing in Sinai Peninsula, Egypt (Mohamed and Abdelgaleil, 2008) and the oil isolated from *A. judaica* growing in Algeria (Charchari, 2002). Interestingly, piperitone, a major constituent of many *A. judaica* oil samples, was absent in the isolated oil while *β*-thujone, the major compound isolated, had not been previously reported.

The oil of *A. seselifolium* was analysed for the first time in this study. The chemical compositions of the isolated essential oils from the three *Citrus* species are in accordance with those previously reported (Lota et al., 2001; Ahmed et al., 2006; Viuda-Martos et al., 2009). Some of the major constituents of the essential oil of *P. tortuosus* were similar to those previously reported for the oil isolated from plants growing in Egypt (Singab, 2003). However, the percentages of constituents differed. The differences in essential oil compositions could be due to several factors, such as geographical location, season, environmental conditions, nutritional status of the plants and other factors (Perry et al., 1999).

Insecticidal activity of essential oils against *Sitophilus oryzae*
The six isolated oils showed pronounced fumigant toxicity against the adults of *S. oryzae*. The values of LC_{50}, 95% confidence limits and other parameters generated from the concentration-mortality regression lines are shown in Table 2. The oil of *C. limon* revealed the strongest fumigant toxicity, followed by *C. sinensis* and C. *paradise*. The LC_{50} values of these oils were 9.89, 19.67 and 24.13 mg/L, respectively. In contrast, the oils of *A. seselifolium* and *P. tortuosus* were the less effective among the tested oils.

Many essential oils have been reported to possess fumigant toxicity against *S. oryzae*. Of the many oils tested only those of *Mentha microphylla*, *Asiasarum sieboldi* and *Carum copticum* showed higher activity than those tested here (Sahaf, et al., 2007; Mohamed and Abdelgaleil, 2008; Kim and Park, 2008; Chaubey, 2011).

Table 2. Fumigant toxicity of essential oils against the adults of *Sitophilus oryzae*

Compound	LC_{50}[a] (mg/L)	95% confidence limits (mg/L)		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper			
Artemisia judaica	29.97	25.21	36.14	1.72±0.25	-2.54±0.37	3.34
Astoma seselifolium	44.43	36.36	59.77	1.99±0.30	-3.29±0.45	1.26
Citrus limon	9.89	7.23	13.85	3.74±0.28	-3.72±0.28	9.57
Citrus paradisi	24.13	19.23	29.59	7.17±0.59	-9.92±0.83	13.97
Citrus sinensis	19.67	13.60	33.93	5.39±0.44	-6.97±0.57	26.31
Pituranthos tortuosus	41.01	38.49	44.36	4.97±0.60	-8.01±0.94	1.97

[a]The concentration causing 50% mortality.
[b]Slope of the concentration-mortality regression line ± standard error.
[c]Intercept of the regression line ± standard error.
[d]Chi square value.

Insecticidal activity of essential oils against *Tribolium castaneum*
Fumigant toxicity of the isolated oils against the adults of *T. castaneum* in terms of LC_{50} values is summarized in Table 3. The essential oils of *C. sinensis* (LC_{50} = 24.57 mg/L) and *C. paradisi* (LC_{50} = 25.52 mg/L) were the most potent toxicants. The oil of *A. judaica* showed the lowest toxicity. The value of LC_{50} for this oil was greater than 50 mg/L. The oils of *P. tortuosus* and *A. seselifolium* revealed similar insecticidal activity against *T. castaneum*.

All of the tested oils were more effective fumigants against *S. oryzae* than *T. castaneum*. This finding is in agreement with our previous studies on the fumigant toxicity of some essential oils and monoterpenes in which *S. oryzae* was more susceptible than *T. castaneum* (Mohamed and Abdelgaleil, 2008; Abdelgaleil et al., 2009).

Table 3. Fumigant toxicity of essential oils against the adults of *Tribolium castaneum*

Compound	LC_{50}[a] (mg/L)	95% confidence limits (mg/L)		Slope[b] ± SE	Intercept[c] ± SE	(χ^2)[d]
		Lower	Upper			
Artemisia judaica	>50	-	-	-	-	-
Astoma seselifolium	46.55	42.17	53.95	4.29±0.63	-7.15±0.97	0.11
Citrus limon	25.52	20.17	35.64	3.55±0.31	-4.99±0.42	9.33
Citrus paradisi	30.06	20.16	63.70	5.38±0.43	-7.95±0.63	29.63
Citrus sinensis	24.57	14.76	97.93	4.84±0.37	-6.73±0.50	39.01
Pituranthos tortuosus	45.31	34.02	158.70	3.50±0.42	-5.80±0.64	13.16

[a]The concentration causing 50% mortality.
[b]Slope of the concentration-mortality regression line ± standard error.
[c]Intercept of the regression line ± standard error.
[d]Chi square value.

The essential oils of the three *Citrus* species showed strong insecticidal activity against the adults of *S. oryzae* and *T. castaneum*. This potent toxicity could be attributed to the major constituent, *dl*-limonene. In fact, limonene showed relatively less insecticidal activity than these three oils. The LC_{50} values of limonine on *S. oryzae* and *T. castaneum* were 29.92 and 33.37 mg/L, respectively (Abdelgaleil et al., 2009). It has often been found that some essential oils have a greater insecticidal activity than their isolated major constituents as observed with the essential oil of *Asiasarum sieboldi* against *S. oryzae* (Kim and Park, 2008), indicating the beneficial effect of combined action of the different components.

It is well recognized that the insecticidal activity of essential oils are mainly attributed to their monoterpenoidal contents (Huang et al., 1998; Garcìa et al., 2005; Abdelgaleil et al., 2009). Monoterpenes act as neurotoxicants against different insect species (Coats et al., 1991). They have been shown to inhibit acetylcholinesterase (AChE) isolated from different insect species (Ryan and Byrne, 1988; Abdelgaleil et al., 2009).

In conclusion, this study shows that the essential oils tested, especially those obtained from *Citrus* species, have remarkable fumigant toxicity against the adults of *S. oryzae* and *T. castaneum*. The huge consumption of the *Citrus* fruits generates tons of waste peels. Converting these waste products to safer natural insecticides is highly recommended. Based on the insecticidal activity of the *Citrus* oils demonstrated in this study, these oils could be used in integrated pest management (IPM) programs of *S. oryzae* and *T. castaneum*.

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CONTROL OF THE CAROB MOTH *ECTOMYELOIS CERATONIAE* WITH ESSENTIAL OIL FUMIGATION

Jouda Mediouni Ben Jemâa[1*], Soumaya Haouel[1], Mohamed Larbi Khouja[2]

[1]Laboratoire de Biotechnologies Appliquée à l'Agriculture, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage.
[2]Laboratoire d'Ecologie et d'Amélioration Sylvo-pastoral, Institut National de Recherche en Génie Rural, Eaux et Foret, Université de Carthage.
*Corresponding author's e-mail: *j_mediouni@hotmail.fr*

ABSTRACT

In Tunisia, date palm is of great socio-economic importance. The carob moth, *Ectomyelois ceratoniae* is among the most important and destructive insects attacking dates in storage. Currently, chemical control using fumigation is the most used tool for managing this pest in postharvest treatment. However, the use of fumigants is controversial due to human health and environmental concerns. Therefore, this work was undertaken to investigate chemical composition and fumigant activity of essential oils from *Eucalyptus camaldulensis* (Dehnh) collected at summer and winter seasons.

Oils were tested for their toxicities against adults and last instar larvae of *E. ceratoniae*. Amounts were 0.5, 1, 3 and 5 µl in each of the Plexiglas bottle with 38 ml capacity, corresponding to concentrations 13.16, 26.31, 78.95 and 131.58 µl/l air. Chemical composition was assessed by GC-MS analyses.

Results showed that chemical composition varied between summer and winter seasons. 1,8 cineole (20.62%) and α-pinene (16.49%) were the major components during the summer season whereas, O-cymene (18.12%), spathulenol (13.35%), aromadendrene (9.55%) and α-pinene (8.7%) were major for plants collected in winter. As expected, insect mortality increased as the doses of essential oils and exposure period increased. Although desirable fumigant activities against the pest were achieved with essential oils from both seasons, *E. camaldulensis* summer oil was found to be more effective against larvae and adults with respective LC_{50} values of 34.08 and 73.80 µl/l air against 56.39 and 110.23 µl/l air respectively for winter oil.

Key words: Fumigation, Essential oil, Stored dates, Carob moth, Tunisia, *Eucalyptus*

INTRODUCTION

In Tunisia, date palm is of great socio-economic importance. Dates and their secondary products are the main agricultural products of the oases having a major role in local economy (Mediouni-Ben Jemâa, 2008). Tunisia is currently the tenth largest world producer and the foremost exporter of dates in terms of value (Besbes et al., 2009). The carob moth, *Ectomyelois ceratoniae* Zeller 1881 (Lepidoptera: Pyralidae) is the most important and destructive insect attacking dates in storage in Tunisia (Mediouni et al., 2004). The use of fumigants is the most economical tool for managing these stored-date pests (Azelmat et al.,

2006). Methyl bromide is still the primary insecticide used in post-harvest insect control for dates in Tunisia and in several other countries (Zare et al., 2002). However, the use of this pesticide is to be phased out due to human health and environmental concerns (Bell, 2000). Recently, adoption of natural pest control methods including essential oils is becoming a promising prospect (Batish et al., 2008). Essential oils exhibit various and variable insecticidal properties (Prabuseenivasan et al., 2006). The interest in essential oils has regained momentum during the last decade due to their fumigant and insecticidal activities (Isman, 2006).

MATERIALS AND METHODS

Insect rearing
A laboratory rearing colony was established from infested field-collected dates. The moth was reared on an artificial diet based on wheat bran (Mediouni and Dhouibi, 2007). Rearing was conducted in plastic boxes (20×15×10 cm) placed in a rearing room. The rearing conditions were: temperature of 28 ± 1°C, photoperiod of 16: 8 (L: D) and 65 ± 5% relative humidity.

Plant material
Leaves from *E. camaldulensis* were collected from the arboretum of Korbous (north Tunisia) during summer and winter seasons (2010). The harvested material was air-dried at room temperature (20-25°C) for one week and then stored in cloth bags.

Essential oil extraction & analysis
The essential oils were extracted by hydrodistillation of dried plant material using a Clevenger-type apparatus for 4 h. The oils were dried over anhydrous sodium sulphate and stored in sealed glass vials at 4-5°C prior to analysis. Yield based on dry weight of the sample was calculated. Chemical analyses were performed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 220 and 290°C, respectively. The column temperature was programmed from 80 to 220°C at a rate of 4°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. The flow rate of the carrier gas (Helium) was 1.0 ml/min. A sample of 1.0 μl was injected, using split mode (split ratio, 1:100). All quantifications were carried out using a built-in data-handling program provided by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The identification of the essential oils constituents was based on a comparison of their retention times to n-alkanes, compared to published data and spectra of authentic compounds. Compounds were further identified and authenticated using their mass spectra compared to the Wiley version 7.0 library.

Toxicity bioassays
To assess fumigant toxicity of *E. camaldulensis* essential oils, 2 cm diameter filter papers (Whatman No.1) were impregnated with a range of oil doses. Amounts were 0.5, 1, 3 and 5 μl in each of the Plexiglas bottles which were of 38 ml capacity, corresponding to concentrations of 13.16, 26.31, 78.95 and 131.58 μl/l air. The impregnated filter paper was then attached to the screw caps of bottles. Caps were screwed tightly on the vials, each of which contained either 10 unsexed adults (0-24 hours old) or 10 unsexed five instar larvae. Each treatment and

control was replicated five times. Mortality was calculated using Abbott's correction formula (Abbott, 1925). Probit analysis (Finney, 1971) was used to estimate LC_{50} values.

RESULTS AND DISCUSSION

Essential oils yield and chemical composition
Results showed that essential oil yields strongly varied according to the season of collection. High yield was obtained from leaves collected at the summer season (1.32%) compared to winter season (0.76%). Table 1 reports major components of *E. camaldulensis* essential oils collected during summer and winter seasons.

Table 1. Major components (%) from the two essential oils

E. camaldulensis essential oil	
Summer collect	Winter collect
1,8 cineole (20.62%)	1,8 cineole (0.5%)
α-pinene (16.49%)	α-pinene (8.7%)
Spathulenol (2.36%)	Spathulenol (13.35%)
Aromadendrene (3.93%)	Aromadendrene (9.55%)
O-cymene (0%)	O-cymene (18.12%)

Results showed that chemical composition varied between summer and winter seasons. 1,8 cineole is predominant during the summer season whereas winter season oil was characterized by O-cymene as the principal component. Results also showed that α-pinene is a major common component for the two oils with respective percentages of 16.49 and 8.7% for summer and winter seasons.

Fumigant toxicity
Results revealed that *E. camaldulensis* summer oil was more toxic to *E. ceratoniae* adults and larvae compared to the winter oil. Moreover, bioassay results showed that adults of the carob moth were more sensitive to the essential oils than were last instar larvae (Table 2).

Table 2. Median Lethal Concentration LC_{50} (µl/l air) values calculated for mortality within 24 h of exposure of *E. ceratoniae* adults and larvae to *E. camaldulensis* summer and winter oils

Summer collect		Winter collect	
Adults	Larvae	Adults	Larvae
34.08	73.80	56.39	110.23

Our study showed some similarity regarding chemical composition of the essential oils. Indeed, quantitative rather than qualitative variations in the composition were observed. The chemical variation of the essential oil could be due to many factors besides harvest time (Perry et al., 1999). On the other hand, few previous data reported the insecticidal and

fumigant toxicity of *E. camaldulensis* essential oil against adults and larvae of the carob moth *E. ceratoniae*. In this respect, Haouel et al., (2010) reported that essential oils from *E. rudis* and *E. camaldulensis* exhibited high fumigant toxicity against adults of *E. ceratoniae*. Similarly, Mediouni-Ben Jemâa et al., (2012) indicated the toxicity of five *Eucalyptus* essential oils against adults of *E. ceratoniae*.

To summarize, we can propose that variations in insecticidal toxicity of the two essential oils against *E. ceratoniae* could be related to changes in the active component amounts from one oil to another, depending on seasonal variations. This work provides data to support the use of *E. camaldulensis* essential oil as an alternative to the fumigant methyl bromide for treating stored-date commodities in Tunisia.

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NANO-INSECTICIDAL ACTIVITY OF ESSENTIAL OIL FROM *CUMINUM CYMINUM* ON *TRIBOLIUM CASTANEUM*

Maryam Negahban[1*], Saeid Moharramipour[1], Mojgan Zandi [2], Seyed Ali Hashemi[3] and Fariborz Ziayee[4]

[1]Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
[2]Department of Biomaterials, Iran Polymer Institute, Tehran, Iran
[3]Department of Dug Delivery, Paints and Coatings, Iran Polymer Institute, Tehran, Iran
[4]Department of Chemistry, University - Central Tehran , Tehran, Iran
Corresponding author's e-mail: *mnegahban2009@gmail.com*

ABSTRACT

In recent decades, use of botanical insecticides has been developed as alternative to synthetic pesticides. Nowadays, use of new technologies such as nano-encapsulated formulation can overcome the constraints of plant essential oils. In this study, nano-capsules of essential oil from *Cuminum cyminum* L. was prepared by in situ polymerization (O/W) emulation using poly urea-formaldehyde as wall forming material and oil as a core material. The toxic effects of nano-capsules were assessed against *Tribolium castaneum* (Herbst), as one of the most serious pest of stored products. The fumigant toxicity from nano-capsules (LC_{50}= 16.25 ppm) were highly effective than pure essential oil (LC_{50}= 32.12 ppm) with 7 days of exposure. The influences of surface morphology, wall thickness and diameter on the thermal stability of nano-capsules were investigated. In this study, the nano-capsules basically exhibited good storage stability at room temperature. Our findings show that the nano-encapsulation of *C. cyminum* oil might provide a new method for management of *T. castaneum*.

Key words: Essential oil, nano-capsule, nano-insecticide, *Cuminum cyminum, Tribolium castaneum,* medicinal plants, formulation, bioassay, mortality.

INTRODUCTION

The application of various synthetic insecticides and fumigants to grain storage over the years has led to a number of problems, including the development of insecticide resistance in stored grain insect pests (Suthisut, 2011). Potential of plant essential oils as source of insecticides has been worked out and reported with references to various pests (Rajendran and Sriranjini, 2008; Zapata and Smagghe, 2010). Additionally, many plant essential oils show a broad spectrum of activity against pest insects and plant pathogenic fungi ranging from toxic, antifeedant, repellent and oviposition deterrent (Negahban et al., 2006; Sahaf and Moharramipour, 2008; Negahban et al., 2007b). These oils have also a long tradition of use in the protection of stored products (Negahban et al., 2007a; Arabi et al., 2008). Natural products are an outstanding alternative to synthetic pesticides as a means to reduce the negative impacts on human health and the environment (Vanichpakorn et al., 2010). The shift to green

chemistry processes and the continuing need for developing new crop protection tools with novel function makes discovery and commercialization of natural products as green pesticides an attractive and cost-effective search that deserve attention (Koul et al., 2008). Green pesticides are more compatible with the environmental components than synthetic pesticides.

The nano-encapsulated essential oil has the advantage of overcoming the restrictions of plant essential oils usage in storage through the control release of active ingredients (Negahban et al., in press a, b). The nano-encapsulated essential oils have therefore the advantages of solubility of hydrophobic pesticides (hence no need for toxic solvents), no precipitation (therefore no need for constant mixing), increased stability (protect against oxidation), and improved uptake. However, it should be recognized that at this stage the industrialization opportunities are limited, as the precise mechanisms by which nano-encapsulated essential oils perform are still the subject of intense basic research. The present study has ascertained the potential of nano-capsule formulations of *C. cyminum* essential oil on *T. castaneum*, the widespread and critical stored-product pest in cereals and cereal products. Also chemical structure, surface morphology and thermal stability of the nano-capsules were characterized.

MATERIALS AND METHODS

Plant materials and preparation of essential oil formulation
Seeds of *C. cyminum* were obtained from Ferdowsi University, Mashhad, Iran. Essential oil was extracted from the seed samples using a Clevenger-type apparatus where the seeds were subjected to hydrodistillation. Conditions of extraction were: 50 g of air-dried sample; 1:10 seed material/water volume ratio, 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (4.16% w/w) was calculated on a dry weight basis. Extracted oil was stored in a refrigerator at 4ºC.

The nano-encapsulation procedure was conducted by polymerization technology. Essential oil was used as a core material, and Urea (U) and formaldehyde (F) as shell materials. Sulphuric acid solution (10% w/w) was used to control the pH of emulsion and tween 80 (Polysorbate 80), used as emulsifier (Merck Germany). After the UF pre-polymer solution was obtained, aqueous solution of tween 80 was added to the prepared pre-polymer solution. Then the prepared oil was added to form oil in water (O/W) emulsion. The pH of the emulsion was adjusted slowly to 3 while the solution was slowly heated to the target temperature of 60-65ºC. After 4 h, the reaction was stopped. The obtained suspension of nano-capsules was cooled down to ambient temperature, rinsed with deionized water, filtered and finally dehydrated by freeze-drying. Chemical structure of samples was identified using Fourier transform infrared spectroscopy (FTIR) (BRUKER EQUINOX 55). Transmission electron microscope (TEM, Philips CM120) were used to observe surface morphology of the nano-capsules. The thermal properties were analyzed by differential scanning calorimetry (NETZSCH DSC, 200 F3) at a heating rate of 10°C/min from 25 up to 400°C in nitrogen atmosphere. Thermal stability and overall quality of the prepared capsules was assessed by thermogravimetric analysis (TGA) at a heating rate of 10°C/min.

Test insects
T. castaneum was reared on wheat flour mixed with yeast (10:1 w/w), Adult insects, 1–3 days old, were used for toxicity tests. The cultures were maintained in dark in a growth chamber set at 27±1°C and 65±5% r.h. All experiments were carried out under the same environmental conditions.

Bioassay

Fumigant toxicity of the essential oil was investigated to determine lethal concentration for 50% mortality (LC_{50}) (Negahban *et al.*, 2006). A series of concentrations ranging from 6 to 28 ppm for nano-capsules and 13-15 ppm for pure oil were used with logarithmic distance. Then, 20 adults (1–3 days old) were placed into 280 mL glass bottles with screw lids. The experimental apparatus was designed in order to obtain *T. castaneum* kept 10 cm away from the oil formulation. Control insects were kept under the same conditions without any oil. Each concentration was replicated five times. The number of dead and live insects in each bottle was counted 7 days after initial exposure to the essential oil. Probit analysis (Finney, 1971) was used to estimate LC_{50} values.

RESULTS
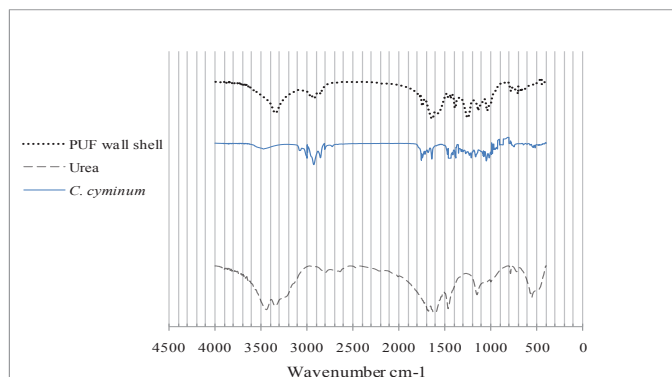
Fig. 1- Fourier transform infrared spectroscopy (FTIR) of urea, *Cuminum cyminum* oil and nano-capsules containing oil with PUF shell wall material.

Fig. 1 indicates FTIR spectra of urea, *C. cyminum* and nano-capsules containing disperse *C. cyminum* oil. N-H and C-H stretching vibration at 3348, 2923 cm^{-1} are presented by the FTIR spectrum of urea and formaldehyde, respectively. As it can be seen, poly condensation reaction between urea and formaldehyde were proved by the absence of absorption band owing to urea at 3450, 2640, 1490 and 580 cm^{-1} and manifestation of absorption peak of poly (urea formaldehyde), which is assigned at 3507-3050 (NH and OH), 1635 (—NH–C—NH—), 1568 (—C—NH—) and 1037 (—CH–O—CH—) cm^{-1}. The absorption peaks of 1568, 1037, and 646 cm^{-1} of poly urea and the absorption peaks of 2850, 2905, 1751, 1248 and 1105 of *C. cyminum* appeared in nano-capsules containing *C. cyminum* spectra which indicate that the core content has been embraced with poly urea formaldehyde.
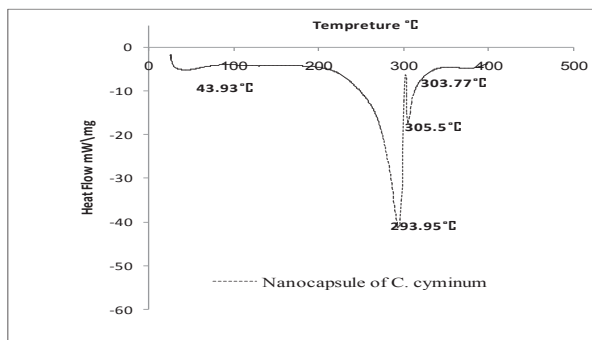
Fig. 2- The differential scanning calorimetry (DSC) of nano-capsules containing *Cuminum cyminum* with PUF shell wall material.

The DSC thermogram is shown in Fig. 2 two endothermic peaks at 43.69 and 295°C appear in the DSC thermogram of nano-capsules. The weak endothermic peak below 43°C is related to the evaporation of free formaldehyde. The second one at temperatures about 262-295°C is due to the decomposition of poly urea formaldehyde as shell materials and the weak exothermic peak at about 303°C on DSC curves may be due to the continuous polymerization reaction of core material and the weak endothermic peak at approximately 305°C is due to the further decomposition of the residue. TG curves for nano-capsules of *C. cyminum* is shown in Fig. 3 indicates that the weight loss near 100-127ºC is mainly due to the removal of entrapped residual water and the elimination of free formaldehyde and the weight loss at temperatures between 235 and 351ºC is mainly due to the decomposition of the PUF wall shell.
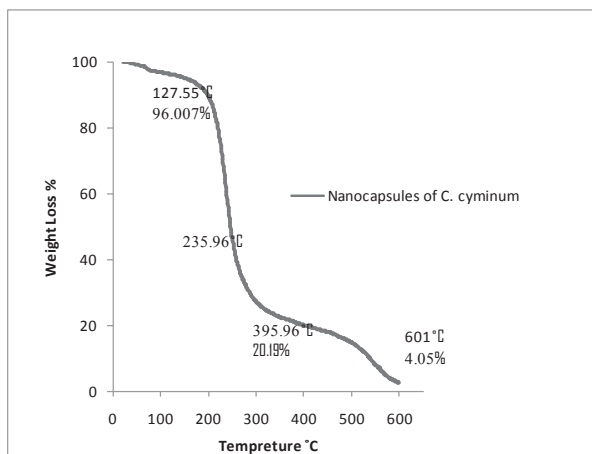
Fig. 3- Thermogravimetric analysis (TGA) of nano-capsules containing *Cuminum cyminum* with PUF shell wall material.

The weight loss of nano-capsules was in the range of 351– 600ºC. In Fig. 4, transmission electron microscopy (TEM) shows that the nano-particles are composed of a core phase entrapped in a shell material of a fairly constant thickness. Nano-capsules appear to be made up of spherical particles of about 30 nm in diameter. The external surface of each

particle is almost regular and smooth, showing that poly urea-formaldehyde forms a continuous film surrounding the essential oil droplets.
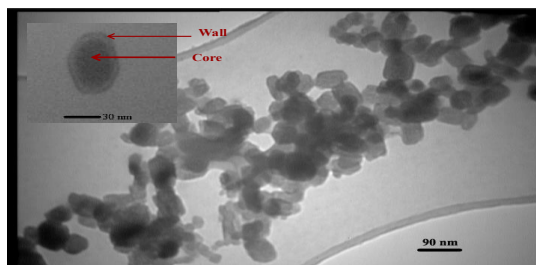

Fig. 4- Transmission electron microscopy (TEM) image of nano-capsules of *Cuminum cyminum* oil shows the core–shell-structured poly urea formaldehyde nano-capsules.

Lower LC_{50} value of nano-capsule (16.25 ppm) indicates higher toxicity than pure essential oil (32.12 ppm) (Table 1).

Table 1. Fumigant toxicity of nano-encapsuled essential oil and pure essential oil of *Cuminum cyminum* against *Tribolium castaneum* after 7 days exposure at 27°C and 65% r.h.

Type	n	LC_{50} (ppm) (95% fiducial limits)	Slope ± SE	df	P-value	Chi square (χ^2)
Nano-capsule	600	16.25 (14.84 - 17.63)	2.83 ± 1.10	4	0.86	1.05
Pure oil	600	32.12 (30.34 - 35.18)	6. 7 ± 0.43	4	0.96	0.87

DISCUSSION

Several studies have been undertaken to explore the potential use of essential oils and their constituents as insect fumigants (Nikooei et al., 2011; Ghasemi et al, 2011). For taking into account the limitations and the physicochemical characteristics of the essential oils, nano-encapsulated formulations seem to be the best choice. In this study, high fumigant toxicity of nano-encapsulated *C. cyminum* essential oil have been demonstrated as a new formulation against *T. castaneum* as a result of controlled-release formulations allowing smaller quantities of essential oil over a given time interval. Also, the cross-linked polymer yielded by the core material and the weight loss of nano-capsules in the range of 351– 600ºC indicate higher thermal stability. Moreover, the thermal degradation of PUF nano-capsule containing oil is complicated and indicating that the prepared nano-capsules with PUF wall shell material has a good thermal stability. Therefore, it is time to focus the consideration of the researchers on the way to the expansion and application of known essential oils and their constituents by highly developed formulation technologies.

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SESSION 1

POSTERS

USE OF *EUCALYPTUS* ESSENTIAL OILS AS FUMIGANT FOR THE CONTROL OF THREE STORED FOOD LEGUME WEEVILS

Soumaya Haouel[1,3], Mohamed Larbi Khouja[2], Abdellatif Boudabous[3] Jouda Mediouni Ben Jemâa[1*]

[1]Laboratoire de Biotechnologies Appliquée à l'Agriculture, Institut National de la Recherche Agronomique de Tunisie, Université de Carthage
[2]Laboratoire d'Ecologie et d'Amélioration Sylvo-pastoral, Institut National de Recherche en Génie Rural, Eaux et Foret, Université de Carthage
[3]Laboratoire Microorganismes et Biomoléculaires actives, Faculté des sciences de Tunis, Université de Tunis El Manar
*Corresponding author's e-mail: *j_mediouni@hotmail.fr*

ABSTRACT

The cowpea weevil *Callosobruchus maculatus* (Fabricius, 1775), the lentil weevil *Bruchus lentis* (Frolich, 1799) and the broad bean weevil *Bruchus rufimanus* (Boheman, 1833) are the most devastating and destructive primary insect pests of stored food legumes in Tunisia.

Control of these pests around the world primarily depends upon applications of organophosphorus, pyrethroid insecticides and the fumigants (methyl bromide and phosphine). These still the most effective treatments for stored food protection from insect infestation. Nevertheless, undesirable effects on non-target organisms, and fostered environmental and human health concerns were evoked. Thus, biological control using plant extracts mainly essential oils were investigated as an alternative to these chemicals. In this respect, the genus *Eucalyptus* is well known to possess various insecticidal activities including its fumigant action. The present work was carried out to investigate chemical composition and fumigant toxicity of two *Eucalyptus* essential oils namely *Eucalyptus camaldulensis* and *Eucalyptus leucoxylon* against adult of *C. maculatus, B. lentis* and *B. rufimanus*.

The GC-MS analyses showed that chemical composition varied with Eucalyptus species. The three essential oils contained α-pinene, α-terpineole and 1,8 cineole as major common compounds. Results demonstrated that fumigant toxicity varied with insect species, essential oil concentration and exposure time. At the lowest tested dose 26.31µl/l air, *B. lentis* is more sensitive than *C. maculatus* and *B. rufimanus*. *E. camaldulensis* and *E. leucoxylon* oils exhibited 100% mortality after 30 hours of exposure. At the highest dose 131.51µl/l air, *E. camaldulensis* oils achieved 100% mortality respectively after 6h for *B. lentis,* 30 h for *C. maculatus* and 24h for *B. rufimanus*. *E. camaldulensis* essential oil was more toxic against *B. lentis, C. maculatus* and *B. rufimanus*. LC_{50} values were respectively 19.87, 24.83 and 36.13µl/l air. The results suggested that *Eucalyptus* essential oils may have potential as a control agent against these stored product weevils.

Key words: Food legume, Fumigation, weevil, Bruchidae, *Eucalyptus*

INTRODUCTION

Recently, there has been a growing interest in research concerning the possible use of plant extracts as alternatives to synthetic insecticides. Essential oils are among the best-known substances tested against insects. These compounds may act as fumigants (Risha et al., 1990; Rice and Coats, 1994; Renault-Roger and Hamraoui, 1995; Shaaya et al., 1997) and may affect some biological parameters such as growth rate, life span and reproduction (Gunderson et al., 1985; Stamopoulos, 1991; Saxena et al., 1992; Renault-Roger and Hamraoui, 1995). Most of these substances were tested against insects attacking stored products in order to establish new control practices with lower toxicity and low persistence in the environment. In fact, management of stored product pests, using substances of natural origin, is nowadays a major research issue. Among the insects attacking stored products, Bruchidae and especially *Callosobruchus maculatus* (F.), *Bruchus lentis* Froelich and *Bruchus rufimanus* Boheman have attracted the attention of many scientists not only because they can easily be manipulated but also because of economic importance they have. The present work was carried out to investigate chemical composition and fumigant toxicity of two *Eucalyptus* essential oils namely *E. camaldulensis* and *E. leucoxylon* against adult of *C. maculatus, B. lentis* and *B. rufimanus*.

MATERIALS AND METHODS

Insect rearing
C. maculatus and *B. lentis* were reared in the laboratory respectively on chickpea and lentil grains. Insects were maintained at a temperature of 30.0 ± 2ºC and a relative humidity of 75.0± 2%. *Bruchus rufimanus* adults were obtained from bean fields.

Essential oil extraction
The essential oils were extracted by water steam distillation using a Clevenger apparatus from leaves of *E. camaldulensis* and *E. leucoxylon* collected from natural populations at the flowering stage on November 2009 from the arboretum of Korbous (North Tunisia).

Chemical analysis
The essential oils composition was analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm; Agilent-Technologies, Little Falls, CA, USA).

Fumigant toxicity
To assess fumigant toxicity, 2 cm diameter filter papers (Whatman No. 1) were impregnated with three oil doses: 1, 3 and 5 μl giving 26.31, 78.95 and 131.51 μl/l air in 38 ml Plexiglas bottles. The impregnated filter paper was then attached to the screw caps of the bottles. Ten adult insects one day old were used in each replicate. Each treatment and control was replicated five times. Mortality was recorded hourly until death. The mortality was calculated using Abbott's correction formula (Abbott, 1925) and probit analysis was used to calculate LC_{50} values (Finney, 1971).

RESULTS AND DISCUSSION

Essential oils composition
Oil yields based on dry matter weight were respectively 1.42% for *E. camaldulensis* and 0.61% for *E. leucoxylon*. GC-MS analysis of *E. camaldulensis* and *E. leucoxylon* essential oils is reported in Table 1.

A total of 90.96% from the constituents of *E. camaldulensis* essential oil were identified (Table 1). Among them 23.38% were monoterpene hydrocarbons, 27.93% oxygenated monoterpenes, 2.14% oxygenated sesquiterpenes and 16.54% sesquiterpene hydrocarbons. The major compounds were α-pinene (17.75%), 1,8-cineole (15.52%), Spathulenol (12.55%) terpinene-4-ol (6.84%) and γ-terpinene (5.63%). Regarding *E. leucoxylon* essential oil, results showed that 91.95% of the constituents were identified (Table 2). Among them 21.73% were monoterpene hydrocarbons, 31.23% oxygenated monoterpenes, 20.22% oxygenated sesquiterpenes and 4.04% sesquiterpene hydrocarbons. Major compounds were α-pinene (25.51%), camphene (5.22%), 1,8-cineole (15.64%), α-terpineol (6.08%) and globulol (14.38%). 1,8- cineole which is the major compound of the two essential oils, is recognized to be toxic to several insect species (Batish et al., 2008).

Table 1. Chemical fractions, others constituents and total identified compounds of the essential oil obtained from leaves of *E. camaldulensis* and *E. leucoxylon* (%)

N°	Compound	*E. cam. %*	*E. leuc. %*	KI
	Monoterpene hydrocarbons	23.38	25.73	
1	α-pinene	17.75	20.51	939
2	γ-terpinene	5.63	2.3	1075
	Oxygenated monoterpenes	27.93	31.23	
3	1,8-cineole	15.52	15.64	1084
4	Camphene	----	5.22	1206
5	Terpinene-4-ol	6.84	3.57	1217
	Sesquiterpene hydrocarbons	2.14	4.04	
6	Aromadendrene	2.14	4.01	1468
	Oxygenated sesquiterpenes	16.54	20.22	
7	Spathulenol	12.55	----	1618
8	Viridiflorol	3.28	4.9	1624
	Other compounds	11.33	10.73	
	Total	90.96	91.95	

Table 2. LC$_{50}$ values calculated for mortality within 24 h of exposure of *C. maculatus, B. rufimanus* and *B. lentis* adults to *E. camaldulensis* and *E. leucoxylon* essential oils

	E. camaldulensis	*E. leucoxylon*
C. maculatus	24.87 (11.80 - 34.85)	28.85
B. rufimanus	36.13 (28.06 - 43.03)	95.32 (76.55 - 145.08)
B. lentis	19.87 (8.90 - 27.38)	20.71 (9.10 - 28.82)

Fumigant toxicity

Results presented as percentage of mortality of adults after 24 h showed that essential *E. camaldulensis* and *E. leucoxylon* oils were toxic to *C. maculatus, B. rufimanus, B. lentis* adults (Fig. 1). These results corroborate the findings of Mahfuz and Khalequzzaman (2007) who reported the toxic effect of *Eucalyptus* essential oil on *C. maculatus.* Moreover*, E. camaldulensis* was more toxic than *E. leucoxylon* for all the concentrations and *B. lentis* was the most sensitive species. At the lowest concentration (26.31 µl/l air), adults of *B. lentis* all died after 24h of exposure for *E. camaldulensis* compared with respectively 66.66% and 80% mortality of *C. maculatus* and *B. rufimanus*.
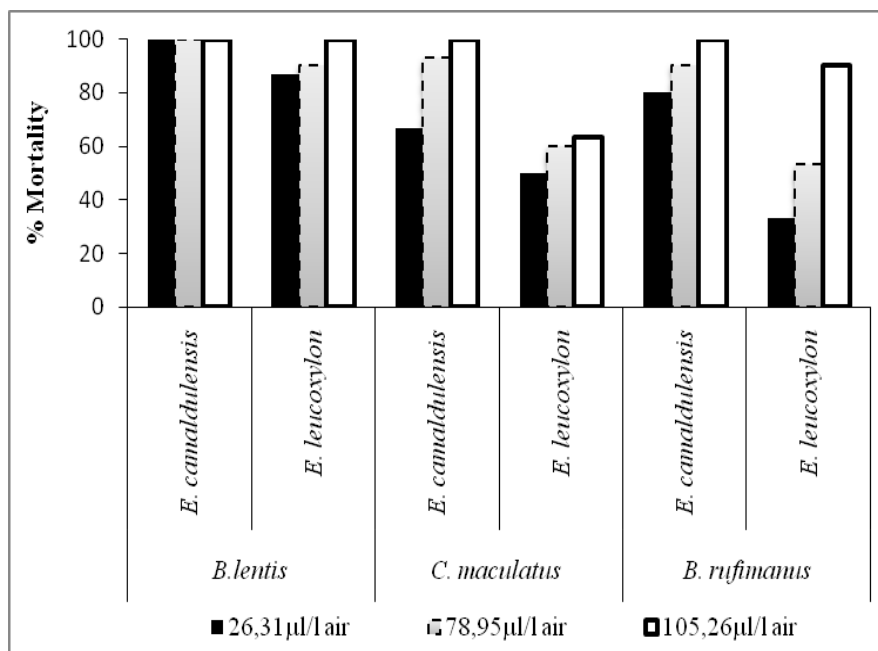
Fig. 1- Percentage of adult mortality after 24 hours exposure

For the concentration 78.95 µl/l air, *B. lentis* all died after 18h of exposure with *E. camaldulensis* and *E. leucoxylon* oils compared with 93.33% and 90% mortality of respectively *C. maculatus* and *B. rufimanus* after 24 h of exposure with *E. camaldulensis* (Fig. 1).

Probit analysis showed that *B. lentis* was more sensitive to *E. camaldulensis* essential oil than *C. maculatus* and *B. rufimanus*. The corresponding LC_{50} values were 19.87, 24.83 and 36.13 μl/l air, respectively (Table 2).

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IMPROVEMENT OF VACUUM EFFECT ON MORTALITY OF *SITOPHILUS GRANARIUS* (CURCULIONIDAE) USING OXYGEN CHEMICAL ABSORBER IN LABORATORY CONDITIONS

Zuzana Kucerova*, Karel Kyhos[1], Radek Aulicky, Vaclav Stejskal

Crop Research Institute, Drnovská 507, 161 06 Prague 6, Czech Republic
Food Research Institute Prague, Radiová 7, 10 231 Prague 10, Czech Republic[1]
*Corresponding author's e-mail: *zuzana.kucerova@vurv.cz*

ABSTRACT

The aim of this preliminary study was to find out if oxygen absorber can improve the efficacy of vacuum on mortality of stored product pests. The effect of vacuum (V) and vacuum in combination with the chemical absorber ATCO 400 (VA) on mortality of *S. granarius* adults was compared in dependence on temperature (15ºC, 25ºC), exposition time (1-7 days) and vacuum value (1.5 kPa). The airtight bags from aluminium and polyethylene films (size 10x25 cm) were used for laboratory experiments. Each bag contained a plastic 10 ml vial with 50 beetles (age 1-2 weeks) without grain substrate. The vials were closed with permeable caps. Seven replicates were used for each treatment. Bags were sealed using vacuum packing machine (KOMET Vacuboy). The results showed significant differences in efficacy of both V and V+A in dependence on temperature. Lethal time was 74 (LT_{50}) and 160 h (LT_{99}) of exposure in vacuum at 25ºC. Low temperature (15ºC) significantly prolonged the exposition time necessary for mortality of tested beetles in constant value of low pressure; LT_{50} and LT_{99} were 113 and 275 h, respectively. Suppression of exposition times was achieved with combination of vacuum and oxygen absorber (V+A); LT_{50} and LT_{99} were about 25h and 18h shorter at 25ºC and about 23h and 74h shorter at 15ºC in comparison with V treatment. Further research on comparison of V and VA effect is in progress including developmental stages in grain samples.

Key words: *Sitophilus granarius*, vacuum, oxygen absorber, MA storage, grain

INTRODUCTION

Relevant attention has been focused on bio-rational approach to managing stored-product insects in agricultural and food commodities to avoid use of chemical insecticides with their negative impact (Phillips and Throne, 2010). A non-chemical possibility is the utilisation of controlled and modified atmospheres (CA, MA) through the manipulation of physical environment (Longstaff, 1994; Stejskal and Adler, 1997; Navarro, 2006). One way is to suppress the oxygen level to a value that is lethal for insect pests. A low-oxygen control atmosphere can be achieved by applying low pressure (vacuum) to infested commodities (Phillips, 2006). Insect mortality depends on the value of vacuum, exposition time, temperature and also on pest species and their developmental stages. Generally, lethal environment for insect pests starts with O_2 content below 4.5 - 3% (Navarro, 1978; Phillips

and Throne 2010); the less amount of O_2 is present the better is the lethal efficiency to the most insect pests. On the other hand, some residual oxygen content still remains in the controlled commodities when using a vacuum packing machine.

The aim of the present preliminary study was to find out if the addition of an oxygen absorber can remove the residual oxygen content and improve the efficacy of vacuum on mortality of stored product pests for perspective use in CA, MA storage application technology by small organic farmers in the Czech Republic.

The effect of vacuum (V) and vacuum in combination with the chemical absorber ATCO 400 (V+A) on the mortality of *S. granarius* L. adults was compared in laboratory experiments in dependence on temperature, exposition time and vacuum value achieved by vacuum packing machine.

MATERIALS AND METHODS

Laboratory cultures of *Sitophilus granarius* L. (Curculionidae) were maintained in a rearing room at 25ºC and 75% relative humidity on grain cultivar Vánek (CZ).

The effect of the vacuum (V) and combination of vacuum and chemical absorber ATCO 400 (V+A) on beetle mortality was compared in relation to temperature (15 and 25ºC), and duration of exposure (24 to 168 hours) at a constant vacuum value (1.5 kPa). Airtight aluminium and polyethylene film bags (size 10x25 cm) were used for the laboratory experiments. Each bag contained 10-ml plastic vials with 50 beetles (age 1-2 weeks). The vials were closed with permeable caps. Seven replicates were used for each treatment, including the control samples. In V+A treatment one absorber ATCO 400 and 1 tablet of oxygen indicator were added in each bag just before sealing.

The bags were sealed using a vacuum packing machine (KOMET Vacuboy, KOMET Vakuumverpackungs-maschinen, Plochingen, Germany) and subsequently placed in incubators that were maintained at different temperatures for various exposure times. At the end of each exposure period, the vials were removed from the bags, supplied with a minimum amount of feeding substrate, placed in desiccators (75% r.h.) and returned to the incubators at the appropriate temperature. The mortality of the experimental and control beetles were checked on day 1, 3, 5 and 7 after the ending of exposure to the vacuum. The results were analysed by the logistic regression mortality model (χ^2 – test) for LT_{50} and LT_{99} using the statistical program XLSTAT (Addinsoft France, Paris, France). Statistical analyses were based on the mortality data obtained on day 7 after the ending of vacuum exposure. Non-parametric Nann-Whitney U test was used for statistical comparison of V and V+A treatments after the 1, 4 and 6 days of exposition and comparison of temperature influence (statistical program Statistica 10, StatSoft CR s.r.o, Czech Republic).

RESULTS AND DISCUSSION

VACUUM (V)

S. granarius adults´ mortality is shown in Figures 1A, B and regression models in Fig. 2A, B. Higher temperature significantly increased the mortality of the tested beetles (Z=2.81, p=0.005, 96 h exposition time; Z = 3.07, p = 0.002, 168 h exposition time) and simultaneously decreased the exposition time in vacuum. The lethal times were 74 (LT_{50}) and 160 h (LT_{99}) for V exposure at 25ºC, and 113 (LT_{50}) and 275 h (LT_{99}) at 15ºC (Table 1).
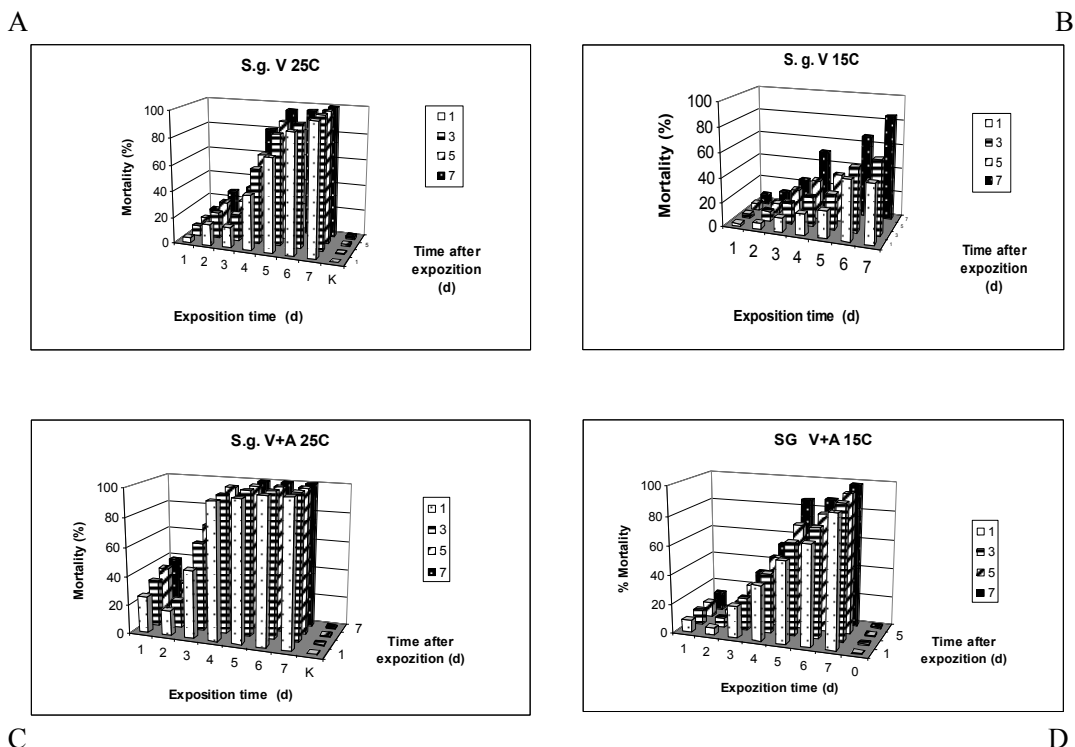
A

B

C

D

Fig. 1- Mortality comparison of *S. granarius* adults exposed to Vacuum (V) and Vacuum
with oxygen Absorber (V+A) in dependence on temperature.
(A - V 25ºC, B - V 15ºC, C - V+A 25ºC, D - V+A 15ºC).

VACUUM AND ABSORBER (V+A)

The absorber lowered the residual oxygen to < 0.1%, which was indicated by the pink colour
of the indicator tablets in all tested bags. *S. granarius* adult's mortality is shown in Figs.
1C, D and regression models in Fig. 2 C, D. Higher temperature also significantly increased
the mortality of the tested specimens (Z = 2.81, p=0.005, 96 h exposition time; Z = 3.17, p =
0.03, 168 h exposition time) and decreased the needed exposition time of modified
atmosphere. The lethal times were 48 (LT_{50}) and 142 h (LT_{99}) for V+A exposure at 25ºC and
90 (LT_{50}) and 200 h (LT_{99}) at 15ºC (Table 1).

Table 1. Regression model parameters´ of vacuum (V) and vacuum + absorber (V+A)
efficiency on mortality of *Sitophilus granarius*

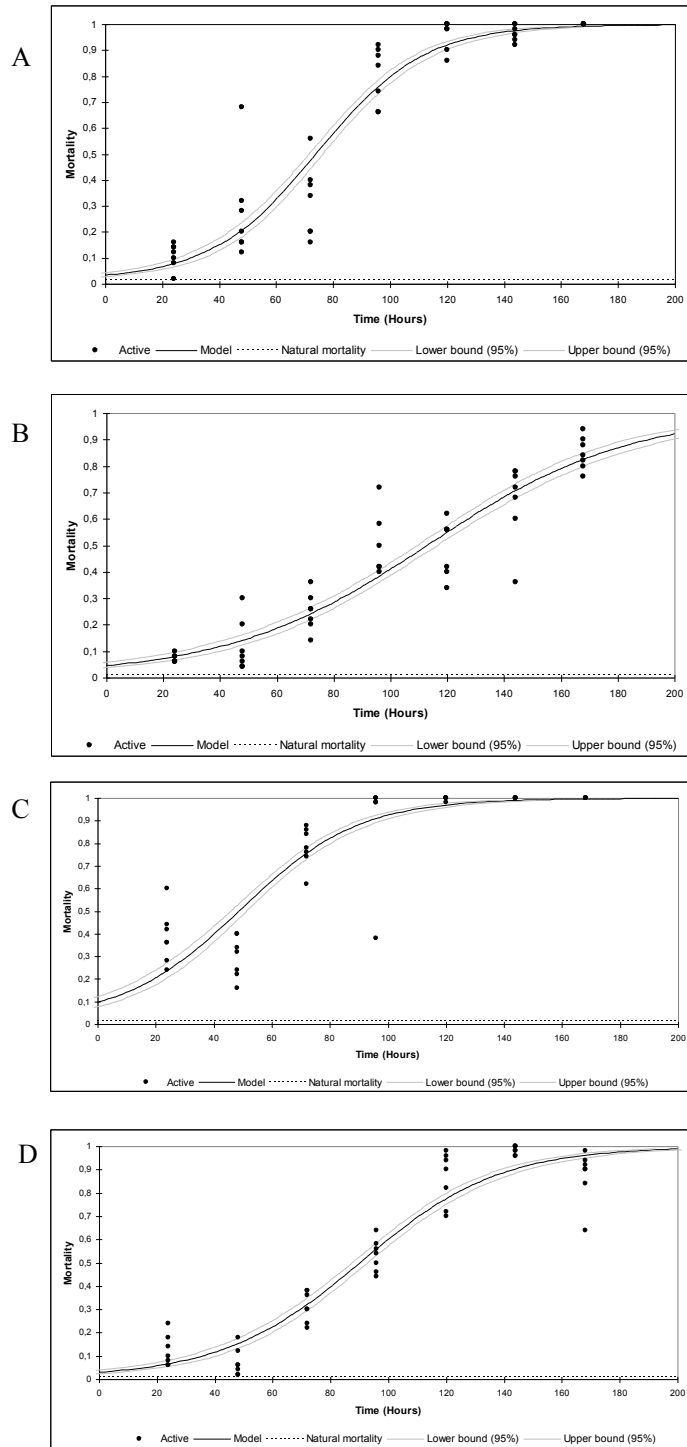|			Model parameters		Lethal time (h)		Model fitness		
		n	Intercept±SE	Slope±SE	LT50 (95% CL)	LT99 (95% CL)	$\chi2$	df	P
V	15°C	7	-3.22±0.14	0.03±0.01	112.67 (109.20-116.22)	274.68 (261.15-290.64)	768.47	1	<0.0001
V	25°C	7	-3.98±0.18	0.05±0.01	73.76 (71.36-76.10)	160.10 (153.63-167.63)	1494.30	1	<0.0001
V+A	15°C	7	-3.76±0.16	0.04±0.01	89.91 (87.24-92.54)	200.43 (192.40-209.70)	1278.53	1	<0.0001
V+A	25°C	7	-2.42±0.15	0.05±0.01	48.44 (45.62-51.09)	142.32 (135.06-150.94)	1049.46	1	<0.0001

Fig. 2- Regression model of *S. granarius* adult's mortality in vacuum (V) and vacuum with oxygen absorber (V+A)(A - V 25ºC, B - V 15ºC, C - V+A 25ºC, D - V+A 15º).

According regression model the addition of oxygen absorber to the vacuum (V+A) increased the insects mortality in both temperatures and shortened the treatment exposition time by 18 – 74 h. Statistically significant differences in the efficiency between both treatments (V+A and V) were found for the higher temperature (25ºC) in all tested exposition times (Z = -3.07, p = 0.002, 24 h; Z = -2.17, p = 0.03, 96 h; Z = -2.17, p = 0.03, 144 h exposition time). Statistically significant difference at low temperature (15ºC) was in 144 h exposition time only (Z = -3.07, p = 0.002, 144 h).

The fact, that both tested treatments of MA were more efficient at higher temperatures corresponded with the published results obtained for CA, MA and chemical fumigants (Phillips and Throne, 2010). Higher efficiency of treatments at higher temperatures is connected with more intensive respiration and metabolism of insect.

It is known that the active developmental stages of insects are generally more sensitive to hypoxia (adults, larvae) than inactive stages (eggs, pupae) (Hoback and Stanley, 2001). Particular developmental stages have different respiration rates at reduced oxygen levels (Emekci et al., 2002). The continuing research on comparison of the V and VA effect concerning various developmental stages of *S. granarius* in grain samples is therefore in progress.

ACKNOWLEDGMENTS

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FUMIGANT TOXICITY OF SOME VOLATILE BOTANICAL SUBSTANCES AGAINST THE WHEAT PEST *SITOPHILUS ORYZAE* AND TWO SEED-BORN FUNGI, *ASPERGILLUS WESTERDIJKIAE* AND *FUSARIUM GRAMINEARUM*

Guillaume Cardiet, Francis Fleurat-Lessard* and Christian Barreau

UR INRA 1264 Mycology and Food Safety (MycSA), INRA Bordeaux-Aquitaine Research Centre – BP No. 81 F-33883 Villenave d'Ornon, France
*Corresponding author's e-mail: *francis.fleurat-lessard@bordeaux.inra.fr*

ABSTRACT

Bioassays were performed to determine insecticidal and antifungal activity of volatile botanical substances, allylisothiocyanate (AITC) and ethyl formate (EtF), against the rice weevil, *Sitophilus oryzae*, and two mycotoxigenic fungi, *Aspergillus westerdijkiae* and *Fusarium graminearum*. Insect mortality rate was determined after 24 h exposure time in airtight enclosure. Antifungal activity was quantified either through fumigation toxicity assay (micro-atmosphere test) by measuring conidia germination inhibition and mycelium growth reduction rate after 72 h exposure time, or after incorporation of active compounds to fungi culture medium (agar diffusion plate method). A comparison with antifungal efficacy of clove oil, a less volatile botanical with remarkable antimicrobial properties, was also carried out with the latter method. Fungal growth reduction rate was determined according to dose mortality relationship model fit by a logistic regression. The lethal concentration of AITC vapour phase leading to 95% mortality of *S. oryzae* (LC_{95}) was 9.9 µL.L^{-1} compared with lower effect for EtF with LC_{95} determined at 52.1 µL.L^{-1}. For clove essential oil (EO), only the LC_{50} could be accurately determined after 24 h exposure time which one was observed at 311.5 µL.L^{-1} (*vs*. 6.3 µL.L^{-1} for AITC LC_{50}). AITC exhibited both antifungal and sporicide activity on the two fungal species. AITC LC_{99} for fungal growth total inhibition of *A. westerdijkiae* and *F. graminearum* was 14.4 and 7.8 µL per Petri dish culture, respectively, whereas it was 630 and 320 µL.L^{-1} of culture medium with clove EO. These results demonstrated that AITC may be an efficient fumigant helpful for sanitation of empty storage facilities or for preservation of grain stored in unsafe condition with a risk of fungal growth. However, these encouraging data must be consolidated by tests on grain and sorption studies.

Key words: Allylisothiocyanate, Clove oil, Ethyl formate, Fumigant activity.

INTRODUCTION

The search for new molecules with fumigant properties is essential in Europe in response to recent phasing out of active substances of common use in stored grain protection or stored-product warehouses disinfestation (Ciesla et al., 2008). During the last decade, several natural volatile substances were proposed as potential alternatives to these banned pesticides such as

methyle bromide, dichlorvos and malathion with more or less success. Allyl-isothiocyanate (AITC) is one of the predominant glucosinolates in cultivated *Brassicaceae* spp. (rape, radish, cabbage, mustard, celery, etc.). Insecticidal properties of AITC are well known for a long time but to date, this insecticidal activity is not practically exploited for stored grain protection according to toxicological properties and grain sorption behavior of vapor phase. Ethyl formate (EtF), a natural compound occurring in barley and beer with known fumigant activity, is registered in Australia for disinfestation of dried fruits and on grain, mixed with CO_2 (Ciesla et al., 2008; Rouzes et al., 2008). It was demonstrated that EtF is effective against stored grain pests (Muthu et al., 1984). Thus, ethyl formate (EtF) having appreciable vapor pressure at ambient temperature, the formulation of EtF in CO_2 as carrier gas was studied and developed as an alternative to phosphine treatments in grain farm storage facilities, especially where phosphine-resistant insect strains were identified (Haritos et al., 2003; 2006; Ryan and Bishop, 2003; Ren et al., 2005).

Beside this insecticidal activity, vapour phase of EtF and AITC was demonstrated with a promising antifungal activity against seed-borne fungi of *Aspergillus* or *Penicillium* genera (Mari et al., 2003) claimed to potentially limit fresh fruit decay rate after harvest (Wang et al., 2010). There are also numerous studies demonstrating antifungal properties of AITC for bio-fumigation of soil and suppression of several plant pathogenic fungi from black mustard (*Brassica nigra*) or wild radish (*Raphanus raphanistrum*) culture plough in (Mayton et al., 1996; Sarwar et al., 1998). The disinfection of storage structures and empty bins after cleaning is difficult to carry out with liquid antimicrobial compounds and fungitoxic activity of AITC or EtF in vapour phase may be useful when all parts of empty storage bins cannot be easily accessible for thorough cleaning before uploading a new harvest. For both stored grain disinfestation and empty bins antifungal treatment, the joint antifungal and insecticidal activity of these two volatile phytochemicals may be interesting for large grain bulks stored with poor control means with grain re-hydration risks and a lack of grain cooling equipment. These situations are relatively common with large grain bulks stored in 'flat' storage facilities, where *in situ* fumigation is the single disinfestation acceptable method.

The *in vitro* antifungal and insecticidal activities of AITC and EtF were investigated in order to determine the susceptibility of vapour phase exposure of the two kinds of target organisms: a stored grain primary pest, *Sitophilus oryza*e (L.) and two mycotoxigenic fungi: *Aspergillus westerdijkiae* Frisvad and Samson (= *A. ochraceus* NRRL 3174, Wilhelm strain) and *Fusarium graminearum* (Schwabe).

MATERIALS AND METHODS

Bioassay with grain insects
Tested compounds and application method
Mustard oil (AITC 95%) and pure EtF (Sigma, St. Quentin Fallavier, France) were tested for their fumigation toxicity in hermetic glassware of 237 mL capacity (Fig. 1). Insects (25 *S. oryzae* adults per replicate, 4 replicates per treatment) were placed on a filter paper at the bottom of the jar, the top hermetically sealed by a lid perforated by a 6 mm diameter hole in its centre, hermetically stopped with a rubber septum (Cardiet et al., 2011). The test substance was injected by a micro-syringe (Hamilton, Bonaduz, Switzerland) through the rubber septum into a cotton plug stick to the lid inside the vessel. The dose of compounds in the fumigation test was from 2 to 40 $\mu L.L^{-1}$. Water (40 $\mu L.L^{-1}$) was used for the untreated control.

Insecticidal activity determination
Mortality of insects after 24 h exposure time in airtight enclosure was assessed and related to the dose per volume ($\mu L.L^{-1}$). The lethal concentration 50% (LC_{50}) and 95% (LC_{95}) were calculated according to the model of correlation dose / mortality rate fit by a four-parameter logistic regression. The determination of lethal concentrations leading to 50% and 95% mortality rate and the standard deviation at this critical level were performed by XLstat data processing software (Addinsoft, France).

Antifungal screening bioassay

Tested compounds and application method on fungi culture
Mustard oil (95% AITC) and pure EtF were tested for conidia germination inhibition rate and fungal growth reduction rate for the two seed-contaminating and mycotoxigenic fungi: *F. graminearum* and *A. westerdijkiae*. A series treated with clove essential oil (EO) (Xeda International, Aubagne, France), a non-volatile botanical extract registered for use as a fungicide for sanitation of empty fresh fruit storage facilities (Bompeix et al., 2009), was added in the test as a reference compound. The fungi species were breed on two culture media: potato dextrose agar (PDA) and Czapek yeast extract agar (CYA) for *F graminearum* and *A. westerdijkiae*, respectively. The germination inhibition and growth reduction related to the dose of bioactive compounds (AITC, EtF and clove EO) was determined through micro-atmosphere diffusion bioassay in Petri dish and through agar diffusion bioassay (see Cardiet et al., 2011) enabling fungal growth inhibition zone measurement according to untreated condition. The fungal growth inhibition rate was assessed for the more active compound (AITC) in comparison to clove EO by agar diffusion test: a dose of tested substance was deposited on a 6 mm diameter 'cellulose test disk' and placed at the surface of PDA medium covered by a mixture of 1 mL spore suspension added to 4 ml PDA (1%) culture medium, *i.e.* 10^6 spore per Petri dish of 90 mm in diameter. Taking into account the high volatility of the tested compound (AITC), each series (dose) was enclosed in a hermetic glass jar (1.5 L capacity) during the incubation period of 72 h before control of fungal growth inhibition rate.

The germicide activity of AITC and clove EO on the spores of the two fungal species was assessed from fungi culture medium in Petri dish (90 mm in diameter), inoculated by a spore suspension and incubated during 16 h allowing spore germination in normal conditions. Then, the Petri dishes were returned upside down and the dose of tested substances deposited on a cellulose disk (6-mm in diameter) placed inside the dish lid (Cardiet et al., 2011). The dose range in the test for conidia germination inhibition was from 0.04 to 40 µL for mustard oil (AITC) and 31 µL to 1 mL for clove EO. All treatments were done in four Petri dishes (replicates) per dose, each series enclosed in a hermetic glass jar to avoid vapour transfer between series during incubation period at 25°C.

Quantification of fungal spore germination and growth inhibition rate
The effects of the active substances were checked up to 72 h after the assay begun. Fungal spore germination or total inhibition was visually measured, allowing the assessment of critical inhibitory concentrations (IC_{50} and IC_{99}) of AITC and EtF per Petri dish.

RESULTS

Insecticidal activity

The three compounds differed greatly in their ability to kill *S. oryzae* adults when used as a fumigant in a hermetic chamber and after 24 h exposure time. The regression equation of

mortality rate with the dose of tested compounds allowed to deduce the CL_{50} and CL_{95} (Table 1). There was observed that AITC had the most potent fumigant effect after 24 h exposure compared to EtF and that the fumigant effect on *S. oryzae* of clove EO was very poor after 24 h. Thus, CL_{50} after 24 h exposure was observed at 6.3, 36.2 and 311.5 µL.L^{-1} for AITC, EtF and clove EO, respectively. The CL_{95} was in the same order: 9.9 and 52.1 µL.L^{-1} for AITC and EtF respectively. CL_{95} for clove EO used as a fumigant could not be reached at the maximum dosage of 1 mL.L^{-1}. The confidence interval at 95% either at CL_{50} or at CL_{95} was low and the determination coefficient was very highly significant, especially for the logistic regression model with four parameters (Table 1). The logistic curve fit cannot be obtained with clove EO according to its very low vapor pressure compared to the two other volatile compounds (Figs. 1-3).
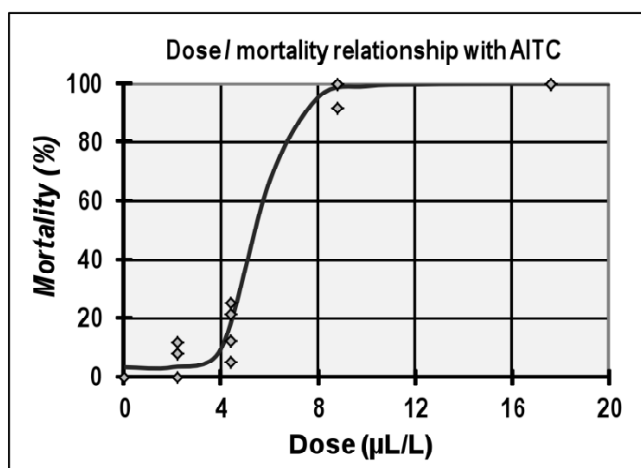
Fig. 1- Logistic regression of mortality rate of *S. oryzae* adults exposed during 24 h to different doses of AITC vapour in a hermetic enclosure (4 replicates of 25 insects per series).
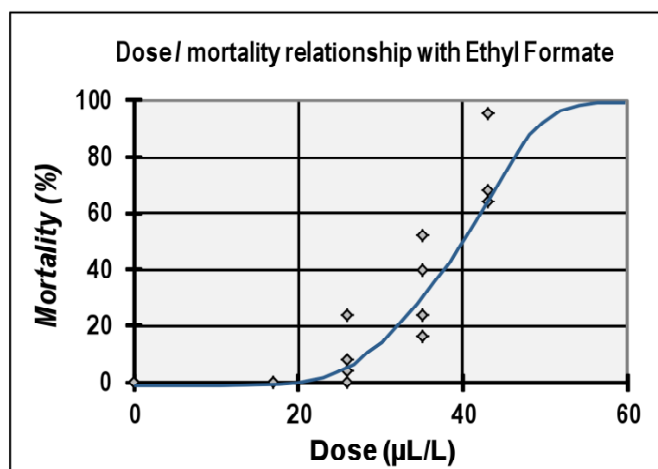

Fig. 2- Logistic regression of mortality rate of *S. oryzae* adults exposed during 24 h to different doses of ethyl formate vapour in a hermetic enclosure (4 x 25 insects per series).
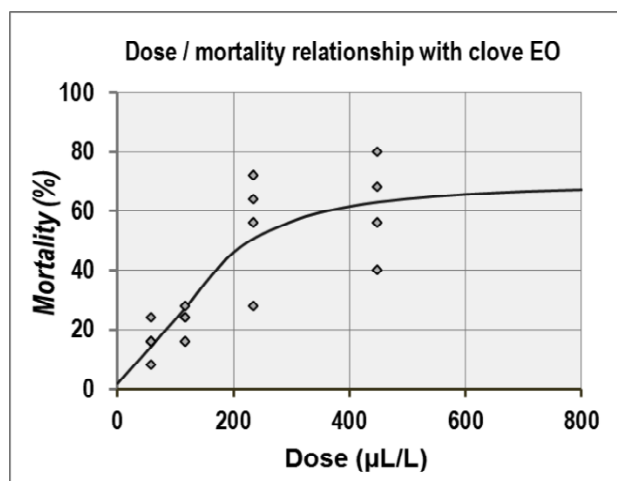
Fig. 3- Logistic regression of mortality rate of *S. oryzae* adults exposed during 24 h to different doses of clove oil vapour in a hermetic enclosure (4 x 25 insects per series).

Table 1. Logistic model of regression of *S. oryzae* mortality rate with the dose of AITC, EtF and clove EO in vapour phase and critical dose / mortality rate ratios determination

Substances	Logistic or polynomial regression model	R^2	LC_{50}	CI 50% lower limit	CI 50% upper limit	LC_{95}	CI 95% lower limit	CI 95% upper limit
AITC	$y = 100.086 + (3.461 - 100.086) / (1 + (x / 5.548)^{8.179})$	0.989	6.3	5.9	6.8	9.9	9.2	10.9
Ethyl formate	$y = 0.0011\ x^2 - 0.0385\ x + 0.3392$	0.902	38.2	36.9	39.8	52.1	49.2	56.3
Clove EO	$y = 69.434 + (1.866 - 69.434) / (1 + (x / 145.422)^{1.988})$	0.809	311.5	269.3	367.3	IND	IND	IND
IND = indeterminable								

Antifungal activity

Fungal growth and in vitro development inhibition

From the results of micro-atmosphere test method, it was shown that the seed-borne fungal species *A. westerdijkiae* was more tolerant to AITC in vapour phase than the hydrophilic plant pathogen *F. graminearum*. Thus, from the probit conversion of growth rate inhibition measurements (*vs.* untreated control), IC_{50} was determined by statistical analysis at 7.1 and 3.1 µL for *A. westerdijkiae* and *F. graminearum*, respectively; whereas CI_{99} (assimilated to minimum inhibition concentration, MIC) was determined at 14.4 and 7.8 µL per culture plate (Table 2 and Fig. 4).
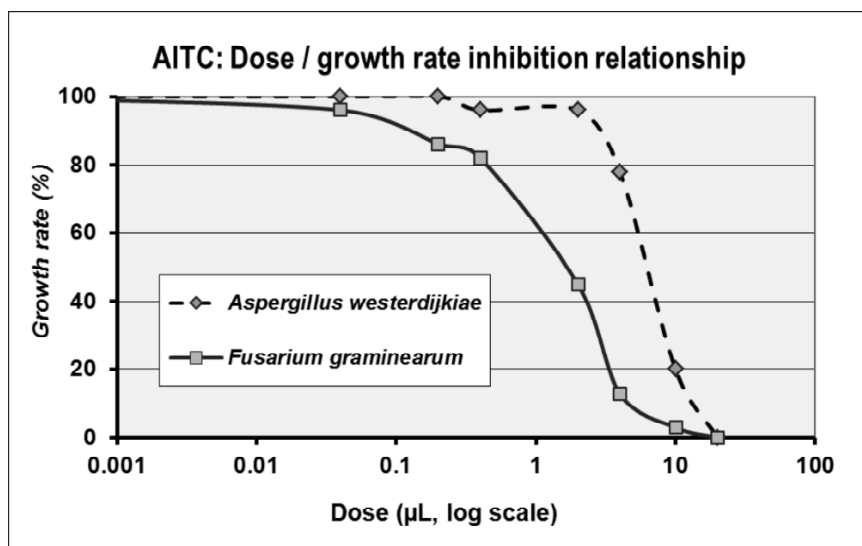
Fig. 4- Logistic regression of mycelial growth of the mycotoxigenic fungi *A. westerdijkiae* and *F. graminearum* exposed to vapour of AITC in Petri dish cultures (4 replicates per series)

Table 2. Regression of mycelial growth of *A. westerdijkiae* and *F. graminearum* grown on agar medium with AITC dose exposure as a fumigant: determination of CL_{50} and CL_{99} and their confidence interval ($P \leq 0.05$)

Fungus	CL_{50} (µL per dish)	Lower limit	Upper limit	CL_{99} (µL per dish)	Lower limit	Upper limit
Aspergillus westerdijkiae	7.07	6.5	7.74	14.4	12.9	16.2
Fusarium graminearum	3.05	2.22	3.81	7.83	6.5	8.84

The sum of the doses used in each treatment (4 fungi culture plates in a glass vessel of 1.5 L) allowed the calculation of the effective dose in vapour phase inducing complete inhibition of fungal growth of the two fungi. Thus MIC was assessed at 60 µL for 1.5 L container, *i.e.* 40 µL.L^{-1}. The treatment with clove oil led to antifungal efficacy at much higher dose than AITC according to the very low vapour pressure developed by clove essential oil. Thus, IC_{50} with clove EO treatment was observed at 218 and 133 µL.L^{-1} of agar culture substrate (Table 3 and Fig. 5).
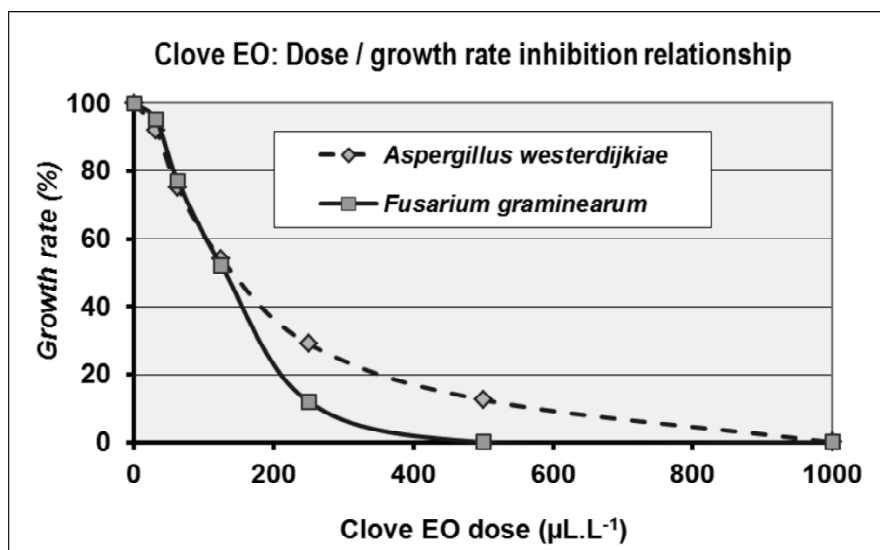
Fig. 5- Logistic regression of mycelial growth of the mycotoxigenic fungi *A. westerdijkiae* and *F. graminearum* exposed to clove oil dilution in Petri dish cultures (4 replicates per series).

Table 3. Regression of mycelium growth diameter of *A. westerdijkiae* and *F. graminearum* grown on agar medium with clove oil dose incorporated in *in vitro* culture medium ($\mu L.L^{-1}$): determination of CL_{50} and CL_{99} and their confidence interval ($P \leq 0.05$)

Fungus	CL_{50} (μL)	Lower limit	Upper limit	CL_{99} (μL)	Lower limit	Upper limit
Aspergillus westerdijkiae	218	199	265	630	557	739
Fusarium graminearum	133	125	169	320	303	387

Fungal spore germination inhibition

The complete inhibition of spore germination of the two fungi species was observed through micro-atmosphere diffusion test at 1 μL of AITC per culture plate, *i.e.* about 3 $\mu L.L^{-1}$ of air in the fungal spores exposure chamber (Fig. 6).
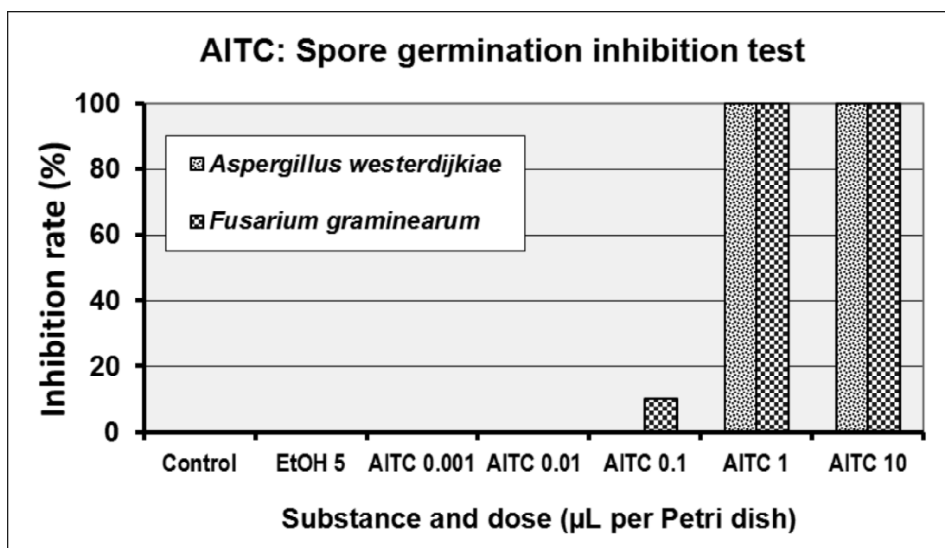
Fig. 6- Regression of spore germination rate of spores of *A. westerdijkiae* and *F. graminearum* on culture medium in Petri dish exposed to progressive dose of AITC in vapour phase (micro-atmosphere diffusion test) – 4 replicates per series.

As a reference with clove EO incorporation to fungal culture medium, only 50% inhibition of fungal spore germination was observed at a concentration of 15 $\mu L.L^{-1}$ agar culture substrate (Fig. 7).
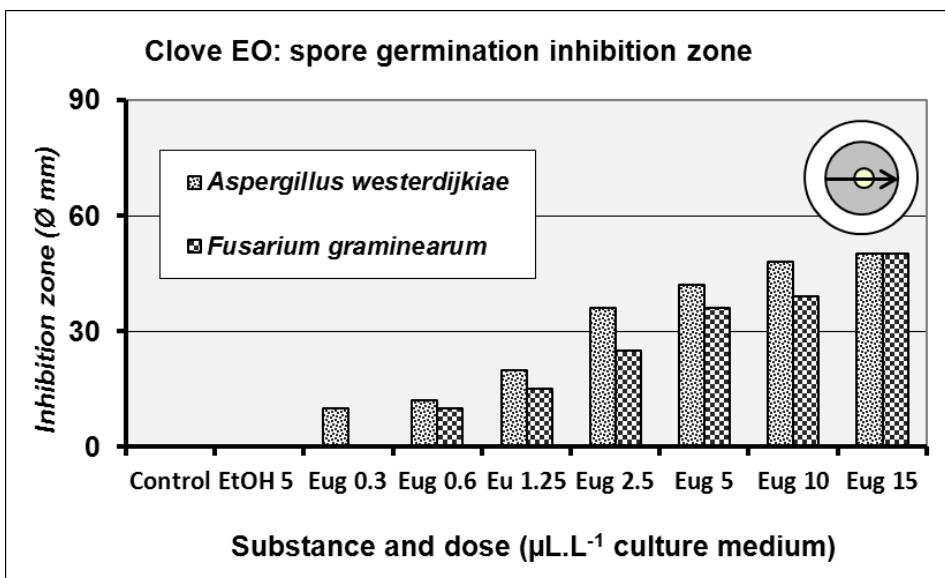

Fig. 7- Regression rate of germination rate of spores of *A. westerdijkiae* and *F. graminearum* on culture medium in Petri dish spiked with increasing doses of clove oil (agar diffusion bioassay) – 4 replicates per series.

DISCUSSION

The mustard oil major compound, allylisothiocyanate (AITC) exhibited remarkable activity both against grain insect pest *S. oryzae* (adult stage) and against spore germination and mycelial growth of the two mycotoxigenic fungi *A. westerdijkiae* and *F. graminearum*. At a concentration in vapour phase of 40 μL.L^{-1}, the germination of fungal spore of the two fungi is completely inhibited and adults of the rice weevil are killed after 24 h exposure at less than 10 μL.L^{-1}.

The insecticidal activity of AITC is five times above the one of ethyl formate (LC$_{95}$ of EtF observed at 52 μL.L^{-1} to be compared to 10 μL.L^{-1} for AITC). The sporicide activity of AITC against the seed-borne and pathogenic fungi tested in the present study is remarkable at a dose as low as 40 μL.L^{-1} air volume. These antifungal properties should be developed for the fumigation of empty storage facilities or grain bins with a good airtightness in situations where the access to thorough cleaning and insecticide treatment before loading with grain is complicated or impossible in some locations (*e.g.* inside perforated aeration duct). Generally, these "uneasy cleanable" points are accumulating broken grain, dust and impurities and are often humid and favourable to fungal development and secondary insect pests population multiplication. The use of AITC for the sanitation of empty grain bin or sealable grain storage facilities when access inside is difficult will be profitable for efficient limitation of the risks of infestation or contamination of the new sound harvest coming into these storage facilities. For grain disinfestation purposes, the use of AITC as a new fumigant is more problematic. The combination of AITC and EtF was tentatively proposed in Australia and in France some years ago (Ciesla et al., 2008), but the barrier of registration was not overcome today. New advances on the joint antifungal and insecticidal activity of AITC will require more studies with infested grain and mouldy grain aiming at a confirmation of the present promising results.

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FUMIGANT TOXICITY OF TWO MEDICINAL PLANT ESSENTIAL OILS ON *TRIBOLIUM CASTANEUM* (HERBST.)

Akrami, H.[1*], Moharramipour, S.[2]

[1]Department of Entomology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[2]Department of Entomology, Faculty of Agriculture, Tarbiat Modares University
*Corresponding author's e-mail: *hd.akrami@yahoo.com*

ABSTRACT

In recent years, interests in researches of medicinal plant essential oils have been growing. In this study, the fumigant activity of essential oils from *Thymus kotschyanus* Boiss and Hohen. and *Mentha longifolia* L. were tested on *Tribolium castaneum* (Herbst.) The flowering dried parts of the plants were subjected to hydrodistillation using a Clevenger-type apparatus for 3 h. Experiment was carried out at 26±1 ◦C and 65±5 r.h.% in dark condition on 1-7-day-old adult insects. The mortality of insects was tested in different concentrations. The mortality was increased with concentration from 384 - 769 µL/L air for *T. kotschyanus* and 0.2-1 µL/L air for *M. longifolia*. Data analysis demonstrated that lethal concentration to kill 50% of the population (LC_{50}) of *T. kotschyanus* and *M. longifolia* were 573.94 and 1.46 µL/L air respectively and lethal time to kill 50% of the population (LT_{50}) of *T. kotschyanus* at 615-1076 µL/L air were estimated for 7-4 h. In case of *M. longifolia*, LT_{50} value at 18-46 µL/L air were estimated from 0.73 to 2 h. The present study showed that essential oil of *M. longifolia* was more toxic than *T. kotschyanus*. Also these medicinal plants can use as a safe insecticidal to control of stored product pests.

Key words: *Thymus kotschyanus*, *Mentha longifolia*, *Tribolium castaneum*, essential oil, fumigant toxicity

INTRODUCTION

Insects cause a significant nutritional and economical problem to products in most countries. The red flour beetle *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) is a serious and cosmopolitan pest (Via, 1999; Weston and Rattlingourd 2000). Many synthetic chemicals are being used to control of this insect can adversely affect non-target animals (Jamber et al., 1995; Jovanovic et al., 2007; Kamali Hatil Hashim, 2009). Resistance to synthetic insecticides, economic costs to conform with pesticide laws, consumer expectations and preferences, are important considerations for the management of *T. castaneum* and other stored-product pests. The imminent loss of the fumigant methyl bromide through compliance with the Montreal Protocol (Anonymous, 2004) will certainly further affect management programs for *T. castaneum*, accelerating the demand for new control strategies (Zettler et al., 1973; Phillips et al., 2000; Lee et al. 2001). Therefore, studies on insecticides that are safe and ecologically acceptable seem to be necessary.

In recent decades, global research has focused on plant secondary metabolites, especially essential oils, in protection of stored agricultural products. Essential oils and their major components have attracted research attention as potential alternatives to classical fumigants (Ogendoet al., 2010). More than 100,000 secondary metabolites from about 200,000 plant species have been identified, which include alkaloids, terpenoids, flavonoids and others which can have insecticidal properties (Vendramim and Castiglioni, 2000; Potenza et al., 2004). *Thymus kotschyanus* Boiss. and Hohen. (Labiatae) and *Mentha longifolia* L. (Lamiaceae) are medicinal plants that grow throughout the Iran. In the present research, toxicity of essential oils from these medicinal plants has been studied against *T. castaneum*.

MATERIALS AND METHODS

Plant materials

Aerial parts of *T. kotschyanus* and *M. longifolia* were collected at full flowering stage from Tehran, Iran in June 2007. The collected plants were dried naturally on laboratory benches at room temperature 23-27°C for a week until crisp dry. The dried parts of the plant were separately hydrodistilled in a Clevenger-type apparatus for 4 h. The resulting oil was dried over anhydrous sodium sulfate. The oil was kept at 4°C in the sealed brown vials until required. Oil yield for *T. kotschyanus* ($1.66 \pm 0.13\%$ w/w) and for *M. longifolia* ($2.08 \pm 0.17\%$ w/w) was calculated on a dry weight basis.

Insect rearing

Tribolium castaneum was used in this study and was reared on wheat flour mixed with yeast (10:1, w/w). The insect was obtained from laboratory cultures maintained in the dark in incubators set at 26 ± 1°C and $65 \pm 5\%$ r.h. All experiments were carried out under the same environmental conditions as the cultures.

Fumigant toxicity

The method used to determine the fumigant activity of extracted compounds was based as Negahban et al. (2006). Ten adult insects (1-7 days old) were put into glass bottle. Filter papers (2.0 cm diameter) were impregnated with an appropriate concentration of the oil with dichloromethane as a solvent. After evaporating the solvent for 2 min, each filter paper was placed on the underside of the screw cap of a glass vial. The cap was then screwed on tightly. Control insects kept in the same conditions without any essential oil. Each concentration and control was replicated five times. After 24 h from commencement of exposure to the essential oil, the insects were transferred to another glass bottle without any essential oil and the number of dead and live insects in each bottle was counted. When no leg or antennal movements were observed, insects were considered dead. Probit analysis (Finney, 1971) was used to estimate LC_{50} and LC_{90} values, SAS program (SAS Institute, 1997).

Another experiment was designed to determine the median effective time causing mortality of 50% of the test insects (LT_{50} values) at 615.4, 769.2, 923.1 and 1076.9 µL/L air of *T. kotschyanus*, and at 18.5, 27.7, 36.9 and 46.2 µL/L air of *M. longifolia* essential oil. The mortality was assessed by direct observation of the insects over the exposure time. Time-mortality data for each experiment was analyzed by the method of Finney (1971) with time as the explanatory variable to estimated the number of hours required for 50% mortality. Estimates were compared to see if there was overlap of the 95% fiducial limits.

RESULTS

The results showed that *T. kotschyanus* and *M. longifolia* essential oils were toxic to *T. castaneum* adults. LC_{50} values for the essential oils were 574 and 0.56 µL/L air for *T. kotschyanus* and *M. longifolia*, respectively. Also the probit analysis showed that the LC_{90} values of *T. kotschyanus* and *M. longifolia* oils were 908 and 1.46 µL/L air, respectively (Table 1). Therefore the *M. longifolia* oil was clearly more toxic to *T. castaneum* than that of *T. kotschyanus*.

Table 1. Fumigant toxicity of *Thymus kotschyanus* and *Mentha longifolia* essential oils on adults of *Tribolium castaneum*

Essential oil	χ^2 (df)	P-Value	Intercept ± SE	Slope ± SE	LC_{50} (µL/L air)[1]	LC_{90} (µL/L air)[1]
T. kotschyanus	3.486(4)	0.480	-17.74±2.27	6.43±0.82	573.94 (542.65 -607.69)	908.15 (816.98 -1075)
M. longifolia	0.78(3)	0.854	0.78±0.13	3.06±0.40	0.56 (0.49-0.63)	1.46 (1.16-2.10)

1) 95% lower and upper fiducial limits are shown in parenthesis

Median effective time to cause mortality of 50% of the test insects again showed that *T. castaneum* was more sensitive to *M. longifolia* than *T. kotschyanus*. LT_{50} and LT_{90} values for the highest dose of *T. kotschyanus* (1076.9 µL/L air) were 4.14 and 13.92 h, and for the lowest dose (615.38 µL/L air) were 7.56 and 17.62 h respectively. Also, LT_{50} and LT_{90} values for the highest dose of *M. longifolia* (46.2 µL/L air) were 0.73 and 2.97 h, and for the lowest dose (18.5 µL/L air) were 1.95 and 4.68 h respectively (Table 2).

DISCUSSION

In this initial exploratory work, the essential oil of *T. kotschyanus* and *M. longifolia* demonstrated fumigant toxicity against the red flour beetle *T. castaneum* within 24 h. The insecticidal activity depends on oil concentration. Other researches have shown that essential oils and their constituents may potentially be used as alternative compounds to synthetic fumigants in present usage (Shaaya et al., 1997; Regnault- Roger et al., 1993; Shakarami et al., 2005). It has been shown that *Artemisia tridentata* and *Artemisia vulgaris* essential oils have significant fumigant activity against adults, larvae and eggs of *T. castaneum* (Dunkel, and Sears, 1998; Wang, et al., 2006). The essential oil of *Artemisia annua* showed fumigant activity against *T. castaneum* (Tripathi, et al., 2000). The present results indicate that the essential oil of *M. longifolia* may be more effective than the other essential oils tested.

Rise of problems relating to the use of modem synthetic chemical insecticides, such as persistence of residues, environmental concerns and insect populations becoming resistant to conventional chemicals have generated attention towards naturally occurring products. The outcome of the results presented in this study indicate that *M. longifolia* oil or its major constituents could provide an effective fumigant and be considered for integration with other pest management procedures. More studies are needed to evaluate the fumigant activity of these essential oils and their constituents to other insects. Also, the mechanism of action of essential oils in target pests could be an attractive area of research.

Table 2. Regression analysis and $LT_{50/90}$ values of *T. kotschyanus* and *Mentha longifolia* essential oils on *Tribolium castaneum*

Essential oil	Conc. (µL/L air)	χ^2 (df)	P-Value	Intercept ± SE	Slope ± SE	LT_{50} (h)[1]	LT_{90} (h)[1]
T. kotschyanus	615.38	2.50(4)	0.648	-9.26±1.25	3.49±0.47	7.56 (6.80-8.37)	17.62 (14.52-24.17)
	769.23	0.72(4)	0.948	-8.05±1.11	3.10±0.43	6.64 (5.92 -7.47)	17.22 (13.66-21.35)
	923.08	1.76(4)	0.781	-7.32±0.98	2.92±0.39	5.35 (4.71-6.04)	14.70 (11.74-18.13)
	1076.9	2.84(4)	0.585	-5.83±0.86	2.43±0.35	4.14 (3.47-4.78)	13.92 (10.76-15.42)
M. longifolia	18.5	0.74(4)	0.947	-6.99±0.94	3.38±0.45	1.95 (1.75-2.17)	4.68 (3.87-6.36)
	27.7	1.00(4)	0.910	-4.90±0.67	2.60±0.35	1.28 (1.11-1.47)	3.99 (3.11-6.00)
	36.9	3.10(4)	0.541	-4.16±0.57	2.38±0.32	0.93 (0.79-1.08)	3.23 (2.46-5.02)
	46.2	6.19(4)	0.186	-3.45±0.46	2.10±0.28	0.73 (0.62-0.87)	2.97 (2.12-5.12)

1) 95% lower and upper fiducial limits are shown in parenthesis

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FUMIGANT TOXICITY OF *CARUM COPTICUM* ESSENTIAL OIL AGAINST *SITOPHILUS GRANARIUS*

Masumeh Ziaee, Saeid Moharramipour*

Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
*Corresponding author's e-mail: *moharami@modares.ac.ir*

ABSTRACT

Laboratory bioassays were conducted to assess fumigant toxicity of *Carum copticum* (L.) essential oil against *Sitophilus granarius* (L.). Essential oil was extracted from *C. copticum* seeds by hydrodistillation using a modified Clevenger type apparatus. The concentrations of 6, 9, 12, 15 and 18 µL L^{-1} air were applied for the bioassays. Adults were exposed to the concentrations impregnated to the filter papers, placed into the under surface of the screw lids of glass vials. The vials were kept in incubator set up at 27±1°C and 55±5% r.h. in continuous darkness. The mortality was counted after 3, 6, 10, 24, and 48 h of exposure. The mode of action of essential oil was characterized by insect's knockdown, hyperactivity, convulsion, paralysis; and ultimately insect death. Mortality increased with increasing concentration levels and time exposing to the oil. However the insect mortality did not exceed 89% even after 48 h exposure to the highest concentration (18 µL L^{-1} air). The present study suggests that *C. copticum* essential oil may have the potential to apply as grain protectants against stored-products insect pests. However, further investigations are necessary to confirm these findings.

Key words: Essential oil, fumigant toxicity, *Sitophilus granarius*, *Carum copticum*, stored-products protection

INTRODUCTION

The granary weevil, *Sitophilus granarius* (L.), is a serious insect pest of various food grains under storage. This species has global distribution; usually found in grain storage facilities and damage harvested grains that are being stored. The larvae feed inside the grain until pupation, after which they bore a hole out of the grain and emerge (Rees, 1996). With regards to the importance of stored products, protection of them from insect-pests infestations should be considered. Natural pesticides based on plant-essential oils may be one of the most promising alternatives to synthetic insecticides and fumigants; because they are of natural origin and they don't have hazards to human and environment (Isman, 2000).

Carum copticum (L.) (Apiaceae) is a medicinal plant grown in Iran, Pakistan and Egypt. The plant bears white flowers and small brownish fruits. It is used as household medicine, and the oil is used as pharmaceutical applications (Sahaf et al., 2007). The insecticidal efficacy of *C. copticum* essential oil has been investigated against *Callosobruchus maculatus* (F.), *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst) and *Plodia interpunctella* (Hubner) (Sahaf and Moharramipour, 2008; Sahaf et al., 2007; Shojaaddini et al., 2008). However, fumigant toxicity of *C. copticum* oil has not been assessed against *S. granarius*.

The aim of the present study was to investigate fumigant toxicity of *C. copticum* essential oil against adults of *S. granarius*.

MATERIALS AND METHODS

Rearing of tested insects
Sitophilus granarius was obtained from the cultures maintained in the Entomology Laboratory-Tarbiat Modares University for at least 3 years with no history of exposure to insecticides. Adults were reared on wheat (variety Pishtaz Madary, m.c. ≈ 12%) at 27±1°C and 65±5% r.h. in continuous darkness.

Collection and preparation of essential oil
Carum copticum seeds were purchased from a research farm in Ferdowsi University-Mashhad-Iran. Seeds were packed in bags and kept in the refrigerator at 4°C. Oil extraction was performed using a Clevenger type apparatus. About 40 g of seeds were ground and put into the Round-bottom flask over water at a temperature around 100°C. As the water was heated the steam passed through the plant material, vaporizing the volatile compounds. Volatile oil assembled in the reservoir was collected after the 4 h distillation process. Subsequently, anhydrous sodium sulphate was applied to remove water. Essential oil was stored in self standing screw cap microtubes covered with foil at 4°C until beginning of the experiment.

Bioassay test for fumigant toxicity
Fumigant toxicity of essential oil was assessed against adults of *S. granarius*. Glass vials with the volume of 280 ml were used for the experiments and 25 adults were introduced into each vial. Filter papers (Whatman No. 1) were placed into the under surface of the screw lids of glass vials and impregnated with different concentrations of essential oil. The concentrations of 6, 9, 12, 15 and 18 μL L^{-1} air were applied for the experiment. Each concentration and control was replicated four times. All vials were placed in incubators set at 27±1°C and 55±5% r.h. in continuous darkness. The adult mortality was counted after 3, 6, 10, 24, and 48 h of exposure.

Statistical analysis
There were no mortality in control groups; so, there was no need to correct the mortality data. Mortality percentages were transformed to square root of arcsine to normalize the data. The data were subjected to one-way analysis of variances to determine significant differences between exposure time and concentration levels (SPSS, 2007). Data was subjected to Probit analysis (Finney, 1971) to estimate Lethal Concentration$_{50}$ (LC$_{50}$) and Lethal Time$_{50}$ (LT$_{50}$)values and their 95% confidence limits using SAS 6.12 software (SAS Institute, 1997).

RESULTS

Percentage mortalities of *S. granarius* adults exposed to different concentrations of *C. copticum* essential oil are presented in Fig.1.

No mortality was observed 3 h after exposing to different concentrations of the oil. However, the mortality increased with increasing the exposure time. Based on LC$_{50}$ values, 46.37 and 10.85 μL L^{-1} air of *C. copticum* essential oil was required to obtain 50% mortality of *S. granarius* adults after 24 and 48 h of exposure, respectively (Table 1).
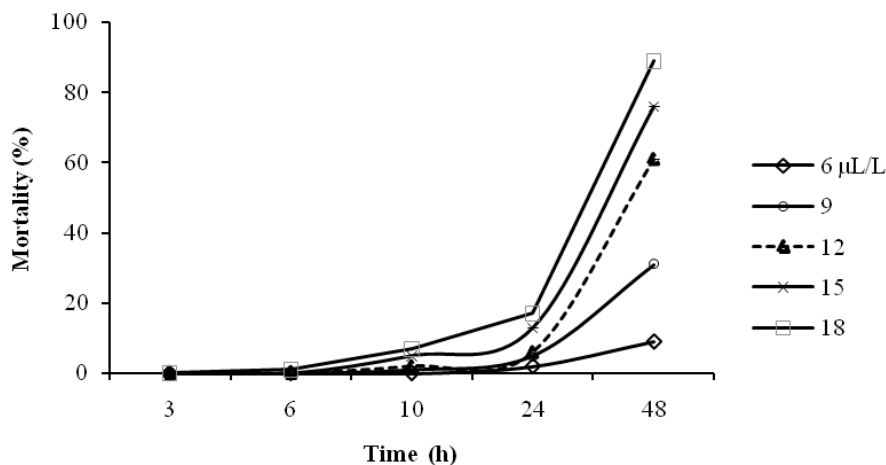
Fig. 1- Percentage mortality (%) ± SE of *Sitophilus granarius* adults exposed to different concentrations of *Carum copticum* essential oil for 3, 6, 10, 24, and 48 h.

Table 1. Lethal Concentration$_{50}$ (LC$_{50}$) values for *Carum copticum* essential oil against *Sitophilus granarius* adults resulting from 24 and 48 h laboratory fumigation

Time (h)	LC$_{50}$ (µL/L air)	95% Cl (µL L^{-1}air)		Slope	d.f.	χ^2	*P* value
		Lower	Upper				
24	46.37	30.06	156.9	2.37	3	0.790	0.852
48	10.85	10.26	11.45	5.38	3	0.899	0.586

CI: Confidence intervals

LT$_{50}$ estimates indicated no significant differences (no overlap in 95% confidence limits) between concentrations of 15 and 18 µL L^{-1} air (Table 2).

Table 2. Lethal time$_{50}$ (LT$_{50}$) values of *Sitophilus granarius* adults exposed to 15 and 18 (µL L^{-1}) concentration of *Carum copticum* essential oil

Concentration (µL L^{-1})	LT$_{50}$ (h)	95% Cl (h)		Slope	d.f.	χ^2	*P* value
		Lower	Upper				
15	35.74	21.84	111.6	3.96	3	18.6	0.01
18	30.01	14.60	144.6	3.99	3	28.3	0.01

CI: Confidence intervals

DISCUSSION

Observation of *S. granarius* exposed to *C. copticum* essential oil indicated their knockdown, hyperactivity, convulsion, paralysis; and finally insect death. Isman (2006) stated that rapid effect of essential oils is because of their neurotoxic mode of action.

According to the results obtained from present study insects mortality increased with increasing concentration levels and exposure time. This is in agreement with Sahaf and Moharramipour (2008). However, in most cases the efficacy of essential oil decreased over time, which can be attributed to their high volatility and low stability (Mikhaiel, 2011; Ogendo et al., 2008; Rozman et al., 2007; Sahaf et al., 2007). In our study, if the experiment was continued up to 48 h, may be adults mortality declined due to the reduction of essential oil efficacy.

Insect's susceptibility to the same essential oil differs from species to species. According to the Sahaf and Moharramipour (2008), LC_{50} values of *C. copticum* essential oil against adults of *C. maculatus* after 24 h exposure time was 0.90 µL L^{-1} air. In the other study Sahaf et al., (2007) stated that 0.91 µL L^{-1} air of *C. copticum* oil was required to obtain 50% mortality of *S. oryzae*, and 33.14 µL L^{-1} air in the case of *T. castaneum*. Shojaaddini et al., (2008) also estimated 257.83 and 91.36 µL L^{-1} air of *C. copticum* oil to control 50% of *P. interpunctella* adults and larvae, respectively. Based on LC_{50} values, *S. granarius* seems to be more tolerant to *C. copticum* oil than other Coleopteran species.

Most of the plants and herbs are locally available and can be applied for pest control programs in small scales. However, there is need to conduct further studies to assess their insecticidal efficacy against stored-product insect pests.

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FUMIGANT PROPERTIES OF NANO-ENCAPSULATED ESSENTIAL OIL FROM *ARTEMISIA SIEBERI* ON *TRIBOLIUM CASTANEUM*

Maryam Negahban[1], Saeid Moharramipour[1*], Mojgan Zandi [2], Seyed Ali Hashemi[3]

[1]Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
[2]Department of Biomaterials, Iran Polymer Institute, Tehran, Iran
[3]Department of Dug Delivery, Paints and Coatings, Iran Polymer Institute, Tehran, Iran
*Corresponding author's e-mai*l: moharami@modares.ac.ir*

ABSTRACT

Various policies and initiatives exist to reduce the effects of chemical pesticides in the environment. Recent research has focused on insecticidal properties of plant essential oils in biological control of insects. Controlled release by nano-encapsulated formulations allows the quantities of the oils to be used more effectively over a given time interval, enhances the suitability to mode of application and minimizes the environmental damage. In this research, the essential oil of *Artemisia sieberi* Besser was encapsulated by in-situ polymerization of oil/water emulsion in nano-scale. Then fumigant toxicity and persistence of produced nano-encapsuled essential oil (NEO) were examined against *Tribolium castaneum* (Herbst) and compared with pure essential oil (PEO) (not encapsulated). The fumigant toxicity of nano-capsules (LC_{50}= 11.24 ppm) was significantly higher than that of PEO (LC_{50}= 15.68 ppm). At sub-lethal doses after 7 days exposure, allowed the essential oil to be entrapped without any changes in their composition and control release. Also, the half-life time of the NEO (LT_{50}= 28.73 days) was significantly much longer than that of the PEO (LT_{50}= 4.27 days). Overall, it seems that the findings of present study could be promising to make practical use of plant essential oils. As the new technology in NEO allows the control release of active ingredients, it could be overcome the restrictions of plant essential oils usage in storage and farms.

Key words: Nano-capsule, plant essential oil, *Artemisia sieberi, Tribolium castaneum*, fumigant toxicity, stored product insects, formulation

INTRODUCTION

Several studies have focused on the potential use of botanicals applications in biological control of different insect pests since some are selective, biodegrade to nontoxic products, and have few effects on non-target organisms and the environment (Singh and Upadhyay, 1993; Isman, 2000; Kim et al., 2010). In the past few years, several studies have focused on the potential use of essential oil applications in biological control of different insect pests. The essential oils may be more rapidly degraded in the environment than synthetic compounds, and some have increased specificity that favors beneficial insects. Their action against stored-

product insects has been extensively studied (Negahban et al., 2007a; Sahaf and Moharramipour, 2008).

In spite of the fact that essential oils have most promising properties, problems related to their volatility, poor water solubility, and potential for oxidation have to be resolved before used as alternative pest control means (Moretti et al., 2002). In this endeavor the use of encapsulated formulations seem to be useful tools to answer those problems (Clancy et al., 1992; Passino et al., 2004).The propagation of poly urea-formaldehyde (PUF) nano-encapsulation techniques would supply insecticide formulations of essential oils without widespread destruction effects on their major compounds. Urea formaldehyde is used in agriculture as a controlled release source of nitrogen fertilizer. Urea formaldehyde's rate of decomposition into CO_2 and NH_3 is determined by the action of microbes found naturally in most soils (Martin and Trenkel, 1997). Studies relating to the activity of PUF nano-capsules of *Artemisia sieberi* as an insecticide have not been carried out. The purpose of this study was to test the fumigant toxicity and persistence of produced nano-encapsulated essential oil (NEO) against *T. castaneum* (the most economically deleterious pest of stored grain throughout the world) and compared with pure essential oil (PEO).

MATERIALS AND METHODS

Plant materials and preparation of essential oil formulation
Aerial parts of *A. sieberi* were collected at full-flowering stage in October, 2011 from Qom province in Iran. The plant material was dried naturally on laboratory benches at room temperature (23–24 ºC) for 5 days until crisp. The dried material was stored at 24 ºC until needed and then hydrodistilled to extract its essential oil. Essential oil was extracted from the plant samples using a Clevenger-type apparatus where the plant material was subjected to hydrodistillation. Conditions of extraction were: 50 g of air-dried sample; 1:10 plant material/water volume ratio, 4 h distillation. Anhydrous sodium sulphate was used to remove water after extraction. Oil yield (2.86%w/w) was calculated on a dry weight basis. Extracted essential oil was stored in a refrigerator at 4 ºC.

The nano-encapsulation process was carried out by polymerization technology. Essential oil was used as a core material, and Urea (U) and formaldehyde (F) as shell materials. Sulphuric acid solution (10 % w/w) was prepared in our laboratory to control the pH of emulsion and tween 80 (Polysorbate 80) was used as emulsifier (Merck, Germany). The obtained suspension of nano-capsules was cooled down to ambient temperature, rinsed with deionized water, filtered and finally dehydrated by freeze-drying using a LIO-5P apparatus (CinquePascal, Trezzano SN, Milan, Italy). Scanning and transmission electron microscopy were used to observe surface morphology of the nano-capsules.

Test insects
T. castaneum was reared on wheat flour mixed with brewer yeast (10:1, w/w). Adult insects, 1 to 3 days old, were used for toxicity tests. The cultures were maintained in dark in a growth chamber set at 27±1°C and 65±5% r.h. All experiments were carried out under the same environmental conditions.

Bioassay
Fumigant toxicity bioassay was deliberated (Negahban et al., 2006) to assess 50% lethal doses of its essential oil content. At first, concentration ranges of fumigant toxicity of nano-encapsulated oil (NEO) and pure essential oil (PEO) were determined by using a preliminary

experiment and logarithmic distance. Then, 20 adults (1 to 3 days old) were put into 280 mL glass bottles with screw lids, the experimental apparatus was designed in order to obtain *T. castaneum* kept 10 cm away from the oil formulation. A series of concentrations from 3 to 25 ppm of the NEO and PEO was tested on *T. castaneum* adults. Control insects were kept under the same conditions without any oil. Each dose was replicated five times. The number of dead and live insects in each bottle was counted 7 days after initial exposure to the essential oil. Probit analysis (Finney, 1971) was used to estimate LC_{50} values.

The mortality half-life of the NEO and PEO, as opposed to chemical half-life, involves determining the mortality caused by a pesticide over time (Stark and Wennergren, 1995; Negahban et al., 2007b). Determination of chemical half-life involves quantization of actual residue levels over time. Our approach involved exposing 20 adults (1 to 3 days old) to glass vial (27 mL) treated with the essential oil at 30 ppm. Thereafter, new adults were introduced in vials every 2 days. The following time steps were used in this study: 3, 5, 7, 9 and 30 days (5 replicates per time step). For each time step (2 days), the adults were removed after 24 h and the mortality was recorded after 48 h later. The mortality half-life data for each experiment were analyzed by the method of Finney (1971), indicating the loss of essential oil activity over time.

RESULTS

As shown in Fig. 1, nano-capsules appeared to be made up of single spherical units of about 80 nm diameter. The external surface of each unit was almost regular and smooth, showing that the Poly (urea-formaldehyde) forms a continuous film surrounding the essential oil droplets.
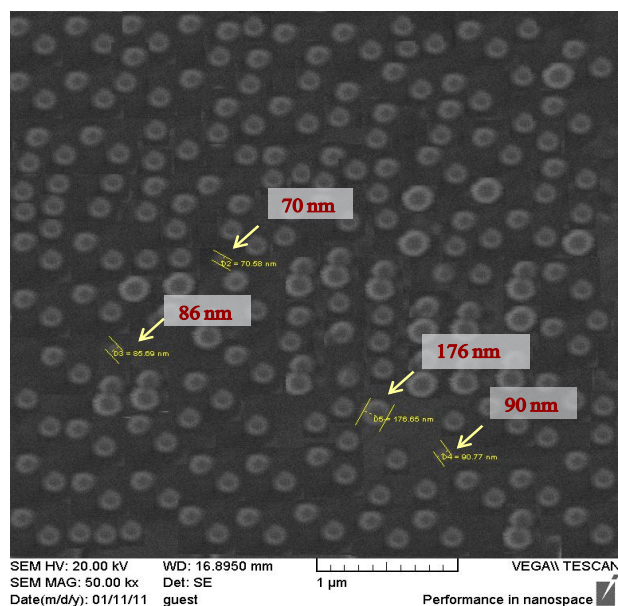

Fig. 1- The scanning electron micrograph shows the external surface of each unit of nano-capsule.

On the basis of the LC$_{50}$s, NEO shows higher mortality than PEO as a case in point LC$_{50}$ values were 11.24 ppm for NEO and 15.68 ppm for PEO for adult insect after 7 days exposure time (Table 1).

Table 1. Fumigant toxicity of nano-encapsuled essential oil (NEO) and pure essential oil (PEO) against *Tribolium castaneum* adults after 7 days exposure at 27°C and 65% r.h.

Type	N	LC$_{50}$ (ppm) (95% fiducial limits)	Slope ± SE	df	P-value	Chi square (χ^2)
NEO	600	11.24 (10.92 - 11.58)	1.73 ± 1.11	4	0.86	1.15
PEO	600	15.68 (15.26 - 16.13)	7. 3 ± 0.78	4	0.96	0.67

The estimate of LT$_{50}$s for *T. castaneum* showed that the half-life time of the NEO was significantly longer than that of PEO (Table 2).

Table 2. LT$_{50}$ values expressing persistence of nano-encapsuled essential oil (NEO) and pure essential oil (PEO) on *Tribolium castaneum* adults exposed to 30 ppm of each essential oil at 27°C and 65% r.h.

Type	n	LT$_{50}$ (h)[1] (95% fiducial limits)	Slope ± SE	df	P-value	Chi square (χ^2)
NEO	700	28.73 (28.40 - 29.95)	1.61 ± 0.39	5	0.88	1.15
PEO	700	4.27 (4.47 - 6.42)	-7. 21 ± 1.04	5	0.99	0.67

[1] LT$_{50}$ values indicate half-life time of the essential oil

DISCUSSION

Essential oil contains compounds that show ovicidal, repellent, antifeedant and toxic effects in insects (Arabi et al., 2008). The toxicity may act by fumigant action (Negahban et al., 2007a). Focus on the insecticidal toxicity of essential oils of plants and their constituents have sharpened since the 1980s specifically on essential oils (Rajendran and Sriranjini, 2008). There are many reviews dealing with the use of plant products in general, against insect pests (Isman, 2006; Rajendran and Sriranjini, 2008). Therefore, it was considered appropriate to look into the status of research on plant essential oils and their constituents as insecticides. The present study examines the work conducted and addresses the prospects and problems of the use of plant products as fumigants within these parameters. The nano-capsule of *Artemisia* oil was shown here to possess fumigant toxicity, as well as its longer persistence compared to *Artemisia* oil before formulation. In consistence with studies of Moretti et al. (2002) and Passino et al. (2004), our findings showed higher mortality rates in nano-capsule than in pure essential oil due to controlled-release formulations allowing smaller quantities of essential oil to be used more effectively over a given time interval.

The reasons for nano-encapsulating the essential oil have been to improve its stability to reduce side effects or to reduce dosing frequency and total dosing amount, to obtain better

toxicity activity, and for long-lasting release (Huang et al., 2006). Generally, the modifications of nano-capsules prepared by Poly (urea-formaldehyde) (PUF) are required in order to improve their insecticidal toxicity stability, strength or sustained release.

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POSSIBILITY OF USING SOME MONOTERPENOID COMPOUNDS AS A FUMIGANT FOR CONTROLLING STORED-WHEAT INSECTS

Özgür Sağlam[1*], Nihal Özder[1]

[1]Namık Kemal University, Faculty of Agriculture, Department of Plant Protection Tekirdağ-Turkey
*Corresponding author's e-mail: *osaglam14@hotmail.com*

ABSTRACT

This study was carried out to determine fumigant toxicity of monoterpenoid components; α-pinene, *p*-cymene, eugenol, cuminaldehyde, linalyl acetate, linalool, α-terpinene, gamma terpinene, limonene, β-pinene, allyl isothiocyanate and diallyl disulphide against all life stages of *Tribolium confusum* du Val. and *Ephestia kuehniella* Zell. at 25±1 °C and % 65±5 r.h. In preliminary biological tests all life stages were exposed to 100 µl/l concentration of monoterpenoid compounds for 24 h. Preliminary bioassay tests indicated only allyl isothiocyanate and diallyl disulphide had high fumigant toxicity to all lifestages of *T. confusum*. Whereas, fumigant toxicity to *E. kuehniella* varied with tested monoterpenoid components. Cuminaldehyde, allyl isothiocyanate and diallyl disulphide were highly toxic to *E. kuehniella* eggs while only allyl isothiocyanate had high fumigant toxicity to *E. kuehniella* larvae. For *E. kuehniella* pupae, only allyl isothiocyanate and diallyl disulphide had high fumigant toxicity while all tested monoterpenoid compounds except linalyl acetate and eugenol were highly toxic to *E. kuehniella* adults. The toxicities of the most effective compounds (cuminaldehyde, allyl isothiocyanate and diallyl disulphide) to different life stages of both species in absent and present of commodity were varied. Cuminaldehyde was highly toxic to only egg stage in absent of the commodity, however it had a low toxicity to the eggs placed at bottom position in present of commodity. Diallyl disulphide was shown high toxicity to all life stages of *T. confusum* and *E. kuehniella* (except of larva stage) in absent of the commodity, but it had a very low toxicity in present of the commodity. In conclusion, these results indicated that allyl isothiocyanate would be a potential compound in controlling stored-product insects.

Key words: Monoterpenoids compounds, *Tribolium confusum* duVal.*, Ephestia kuehniella* Zell. fumigant toxicity

INTRODUCTION

Insect control in stored products at present relies upon the use of gaseous fumigants and residual insecticides, both of which pose serious hazards to mammals and the environment (Shaaya et al., 1997; Ren et al., 2008). Fumigation is still one of the most effective methods for prevention of storage losses. Aluminum phosphide is the major compound for disinfestation of stored grains. But unlike residual pesticides where new compounds have become available and continue to do so, the development of new fumigants have not been forthcoming (Bell, 2000; Zettler and Arthur, 2000). Recent studies of attention among

alternative fumigants are the biofumigants, which reflects the growing attention received by bio- pesticides or biorational pesticides (Isman, 2006; Rajendran and Sriranjini, 2008).

Monoterpenoid compounds had potent fumigant activities against the adults of various stored-product insects (Regnault-Roger and Hamraoui, 1995; Erler, 2005; Huang et al., 2000); however, only limited information is available on the efficacy of monoterpenoids against the immature stages and had been limited information in absent or present of commodity. The present study was carried out to determine the fumigant toxicity of twelve monoterpenoids with different chemical groups; α-pinene, p-cymene, eugenol, cuminaldehyde, linalyl acetate, linalool, α-terpinene, γ-terpinene, limonene, β-pinene, allyl isothiocyanate and diallyl disulphide in absent and present of commodity against all life stages (adult, egg, larva and pupa) of confused flour beetle, *Tribolium confusum* Jacquelin Du Val. and Mediterranean flour moth, *Ephestia kuehniella* Zell. and to discuss the possible use of these monoterpenoids as bio-fumigants against stored-grain insects.

MATERIALS AND METODS
Test insects
Bioassays were carried out on all stages (egg, larva, pupa and adult) of *T. confusum* and *E. kuehniella*. *Tribolium confusum* were obtained from the cultures reared on wheat flour mixed with dry brewer's yeast (17:1, wt:wt) using standard culture techniques (Donahaye, 1990) at 1 l glass jars. *Ephestia kuehniella* were collected from cultures reared on a diet of a 1:1:1 mixture of wheat flour: bran: craked maize at 14 x 20 cm plastic box. Insect rearing and all experimental procedures were carried out in room set at 65± 5 % r.h. and 25± 1 °C, under continuous darkness. The experiments of *T. confusum* were conducted with eggs (24-48 h old), old larvae (30-35 d old), pupae (1-2 d old) and adults (6-7 d old) while for *E. kuehniella;* eggs (24-48 h old), old larvae (30-35 d old), pupa (1-3 d old) and adult (1-2 d old). Eggs were separated from the flour by using a sieve with 70 mesh and old larvae were separated from the other life stages using a sieve with 1 mm openings while pupae and adults were separated by using a sieve with 25 mesh. *Ephestia kuehniella* eggs were obtained from empty oviposition jars containing 40 to 50 adults. Larvae and pupae of *E. kuehniella* were collected by fine pens and adults were collected by vacuum machine.

Tested monoterpenoid compounds
Twelve monoterpenoid compounds; α-pinene (Aldrich, 147524, 98 %), *p*-cymene (Sigma-Aldrich, C121452, 99 %), eugenol (Fluka, 46129, *Ph eur*), cuminaldehyde (Fluka, 28210, 85 %), linalyl acetate (Fluka, 45980, 95 %), linalool (Fluka, 62140, 95 %), α-terpinene (Aldrich, 223182, 85 %), γ-terpinene (Fluka, 86478, 97 %), limonene (Sigma-Aldrich, 183164, 97 %), β-pinene (Aldrich, 112089, 99 %), allyl isothiocyanate (Merck, 800260, 95 %) and diallyl disulphide (Sigma-Aldrich, 317691, 80 %) were tested against all life stages of *T. confusum* and *E. kuehniella*. After purchase, the monoterpenoid compounds were collected in sealed glass containers with frangible septum which allows easy penetration by a syringe needle to permit withdrawal of the compounds and refrigerated in the dark at 4°C until use.

Bioassay and experimental procedures
The single-dose test in absent and present of commodity was carried out to determine the effective concentrations of each compound against the all life stages of *T. confusum* and *E. kuehniella*. Each stage of *T. confusum* and *E. kuehniella* were exposed to a constant concentration of 100 µl l^{-1} air of each compounds for 24 h. For single-dose tests in present of commodity, each stage of *T. confusum* was exposed to a concentration of 100 µl l^{-1} air of

monoterponoid compounds while egg and pupa stage of *E. kuehniella* were exposed to concentration of 100 µl l^{-1} and its larva and adult were exposed to concentration of 80 µl l^{-1} and 55 µl l^{-1} of tested monoterponoids respectively. The biological tests of highly toxic compounds were conducted for different life stages of both species placed into two different positions (top and bottom) in present of 2 kg wheat (*Triticum aestivum* L var Flamura 85). Tested stages of *T. confusum* and *E. kuehniella* were collected from the cultures and placed in the glass vials covered with a fine mesh to allow penetration of any volatiles emanating from the monoterpenoid compounds. Fifty eggs and twenty five larvae, pupae and adults were used in each replicate. Single-dose tests in absent and present of commodity were carried out in 3-l and 5-l glass jars respectively. Both closed with metal screw-on lids, which served to as fumigation chambers and were kept at 25±1 °C and 65±5 % r.h. An aqueous saturated magnesium nitrite solution (Mg (NO$_3$)$_2$) was placed in small glass petri dishes of 7 cm diameter to provide 65±5 % r.h. in the glass jar (Greenspan, 1977). Monoterpenoid compounds were applied on filter paper (2 x 5 cm) attached to the under-side of the lids of fumigation chambers by using 50 µl micropipette. Eggs, larvae, pupae and adults of each species kept in the glass vials were transferred separately into fumigation chambers. The fumigation chambers were closed with screw-on lids. Each treatment and control was replicated three times. For the control treatment, all life stages of each insect species were exposed to atmospheric conditions and were kept at 25±1 °C and 65±5 % r.h.

Data processing and analysis

After each treatment, larvae, pupae, and adults (except *E. kuehniella* adults) were transferred to 50-ml jars containing food medium and were held at 25±1 °C and 65±5 % r.h. until examined for mortality. The eggs in their Perspex slides were held under the same conditions until the oviposition sites were examined for egg hatch. Mortality counts for adults were made 4-5 d after exposure; for larvae they were based on those insects that when failed to pupate 9 d after exposure; pupal mortality was based on those pupae that failed to produce adults 9 d after exposure, and egg hatch was counted 7 d after treatment. Mortality data obtained from preliminary bioassay tests were normalized using arcsine transformation and then were analyzed using two-way analysis of variance (ANOVA). The means were separated by using the Duncan's test at the 5 % level (SPSS, 2009).

<div align="center">RESULTS AND DISCUSSION</div>

In present study, the single-dose tests in absent and present of commodity indicated that tested monoterpenoids with different chemical groups (concentration of 100 µl l^{-1}) had significantly different fumigant activity on all life stages of *T. confusum* and *E. kuehnilla* (*P*<0.05). Allyl isothiocyanate and diallyl disulphide indicated a strong fumigant activity on all life stages of *T. confusum*, whereas other tested monoterpenoids (expect cuminaldehyde for only egg stage) had very low fumigant toxicity (Table 1).

Compared with the investigation of Lee et al. (2003) our results indicated a similar low toxicity of limonene and linalool against red flour beetle, *Tribolium castaneum* (Herbst) adults. The high fumigant activity of allyl isothiocyanate to adults of *T. confusum* were similar to other studies with the maize weevil *Sitophilus zeamais* (Motsch.), the lesser grain borer, the book louse *Liposcelis entomophila* (Enderline) , the confused flour beetle and one other study with red flour beetle (Worfel et al., 1997; Demirel et al., 2009; Wu et al., 2009). In this study, cuminaldehyde had a high fumigant activity on *T. confusum* eggs. Isikber et al. (2009) reported that cumin essential oil of which cuminaldehyde is major monoterpenoid

compound had high fumigant toxicity to *T. confusum* eggs, which it is similar to our results. The results obtained from single-dose tests in absent of commodity for *E. kuehniella* indicated that cuminaldehyde, allyl isothiocyanate and diallyl disulphide were highly toxic to *E. kuehniella* eggs while only allyl isothiocyanate had high fumigant toxicity to *E. kuehniella* larvae. On the other hand all tested monoterpenoid compounds except linalyl acetate and eugenol were highly toxic to *E. kuehniella* adults (Table 2).

Table 1. Percent mortality of *Tribolium confusum* eggs, larvae, pupae and adults exposed to $100\,\mu l\ l^{-1}$ concentration of tested monoterpenoid compounds for 24 h in absent of commodity

Monoterpenoid compounds	Means of Mortality (%) ± S.E.				F and P value
	Egg	Larva	Pupa	Adult	
α-pinene	17.3±4.6 BCDa*	5.4±1.3 CDE b	21.7±3.89 BC a	1.3±1.33 B b	$F_{3,8}$=12.65 P< 0.002
p-cymene	12±1.15 CD a	6.8±1.33CDE bc	9.5±1.5DEF ab	4±0 Bc	$F_{3,8}$=9.90 P< 0.005
Cuminaldehyde	100±0 A a	4.2±2.4 E b	6.7±2.63EF b	1.3±1.33B b	$F_{3,8}$=137.94 P< 0.0001
Linalool	27.3±1.76 B a	5.7±1.32 CDE b	4.1±2.31F b	0±0 B c	$F_{3,8}$=24.93 P< 0.0001
β-pinene	14.7±2.85 CD a	5.3±2.66 DE bc	9.8±1.1DEF a	1.3±1.33 B b	$F_{3,8}$=5.21 P< 0.027
Eugenol	16.7±3.52 BC a	18.7±3.52 C a	24±2.3B a	0±0B b	$F_{3,8}$=17.64 P< 0.001
α-terpine	15.3±3.71CD a	14.1±1.63CD a	17.3±5.8 BCDa	5.3±3.52B a	$F_{3,8}$=2.32 P=0.152
Linaly acetate	16±4.16 CD ab	5.5±1.41CDE bc	21.8±3.89 BC a	2.7±2.66 B c	$F_{3,8}$=7.56 P< 0.01
D. disulphide	100±0 A a	92 ±4 B b	100±0 A a	100±0 A a	$F_{3,8}$=4.00 P= 0.05
A. isothiocyanate	100 ± 0 A a	100 ± 0 A a	100 ± 0 A a	100 ± 0 A a	---**
γ- terpinen	8.7±1.33 DE bc	17.3±4.8 C a	12±2.3CDE a	2.7±2.66B b	$F_{3,8}$=4.96 P< 0.031
Limonene	9.3±3.52 DE a	17.3±4.8 CD a	9.3±1.33DEF a	8±4 B a	$F_{3,8}$=1.02 P=0.433
Control	2.7±0.66 E a	1.4±1.38 E a	0±0 G a	2.7±1.33 B a	$F_{3,8}$=2.17 P= 0.16
F and P value	$F_{12,26}$=133.03 P< 0.0001	$F_{12,26}$=51.87 P< 0.0001	$F_{12,26}$=129.31 P< 0.0001	$F_{12,26}$=68.51 P< 0.0001	

*Two-way ANOVA was applied to the data. Means within a row with the same lower-case letter and a column with the same upper-case letter do not differ significantly (Duncan test at 5% level).

Single-dose test in present of commodity ($100\ \mu l\ l^{-1}$) showed that cuminaldehyde had a low toxicity to the eggs of *T.confusum* and *E. kuehniella*, placed at bottom position of 2 kg of wheat, since it could have weak penetration into the commodity. Diallyl disulphide had a low toxicity to adults, pupae and adults of both tested species placed at bottom position in present of 2 kf of wheat (Fig. 1).

Table 2. Percent mortality of *Ephestia kuehniella* eggs, larvae, pupae and adults exposed to $100\,\mu l\,l^{-1}$ concentration of tested monoterpenoid compounds for 24 h

Monoterpenoid compounds	Means of Mortality (%) ± S.E.				F and P value
	Egg	Larva	Pupa	Adult	
α-pinene	2±1.15 CD c[*]	4±0 BC bc	9.4±3.5 CD b	100±0 A a	$F_{3,8}$=259.5 P < 0.0001
p- cymene	3.3 ±1.33 CD b	6.7 ±2.66 BC b	8.2 ±2. 26 CD b	100 ± 0 A a	$F_{3,8}$=223.38 P < 0.0001
Cuminaldehyde	100±0 A a	10.7±1.33 BC b	13.9±3.46 CD b	100±0 A a	$F_{3,8}$=635.98 P < 0.0001
Linalool	14.7±3.52 B b	14.7±5.81 BC b	13.3±5.85 CD b	100±0 A a	$F_{3,8}$= 74.18 P < 0.0001
β-pinene	6.7 ± 1.33 BC c	16 ±2.30 BC b	17.4 ±3.34 C b	100 ± 0 A a	$F_{3,8}$=397.31 P < 0.0001
Eugenol	4±2.3 CD a	2.9±2.89 BC a	6.8±2.83 CD a	2.7±1.33 C a	$F_{3,8}$= 0.43 P = 0.73
α-terpine	2.7±0.66 CD c	7.7 ±2.33 BC b	7.7 ±2.05 CD b	100 ± 0 A a	$F_{3,8}$=451.27 P < 0.0001
Linaly acetate	2.7±1.33 CD b	6.7±1.33 BC ab	13.1±5.81 CD a	13.3±3.5 B a	$F_{3,8}$= 5.14 P = 0.02
D.disulphide	100±0 A a	22.7±1.33 B b	90.7±6 B a	100±0 A a	$F_{3,8}$= 26.90 P < 0.0001
A. isothiocyanate	100 ± 0 A a	100 ±0 A a	100 ± 0 A a	100 ± 0 A a	---[**]
γ- terpinen	6±2 C a	12±0 BC b	18±2.66 C b	100±0 A a	$F_{3,8}$=346.92 P < 0.0001
Limonene	4±1.15 C c	10.7±2.66 BC b	9.3±2.66 CD bc	100±0 A a	$F_{3,8}$=322.18 P < 0.0001
Control	1 ±0.68 D a	4 ± 2.30 C a	2.7 ± 1.33 D a	0 ± 0 D a	$F_{3,8}$= 1.63 P = 0.25
F and P value	$F_{12,26}$=187.126 P< 0.0001	$F_{12,26}$=29.27 P< 0.0001	$F_{12,26}$=44.85 P< 0.0001	$F_{12,26}$=697.79 P< 0.0001	

*Two-way ANOVA was applied to the data. Means within a row with the same lower-case letter and a column with the same upper-case letter do not differ significantly (Duncan test at 5% level).

Study determined that the toxicity of diallyl disulphide in descending order according to life stages in commodity was egg > larva > pupa > adult for *T. confusum*. Allyl isothiocyanate had high fumigant toxicity to all life stge of tested species placed in each position of 2 kg od wheat. All tested monoterpenoid compounds except 2 of them (allyl isothiocyanate and dially disulphide) showed low toxicity. Similar results were obtained for *E. kuehniella* eggs and pupae at concentration of 100 μl l^{1}. Larvae and adults of *E.kuehniella* were tested at concentration of 80 μl l^{-1} and 55 μl l^{-1}. Allyl isothiocyanate for *E. kuehniella* larvae and 10 monoterpenoid compounds (α-pinene, *p*-cymene, cuminaldehyde, linalool, α-terpinene, gamma terpinene, limonene, β-pinene, allyl isothiocyanate and diallyl disulphide) for *E. kuehniella* adults showed high fumigant toxicity. Cuminaldehyde against to *E. kuehniella* egg indicated same result at single-dose tests in absent and present of commodity. It was

determined that the toxicity of diallyl disulphide in descending order according to life stages in present of commodity adult > egg > pupa for *E. kuehniella* respectively (Fig. 2).
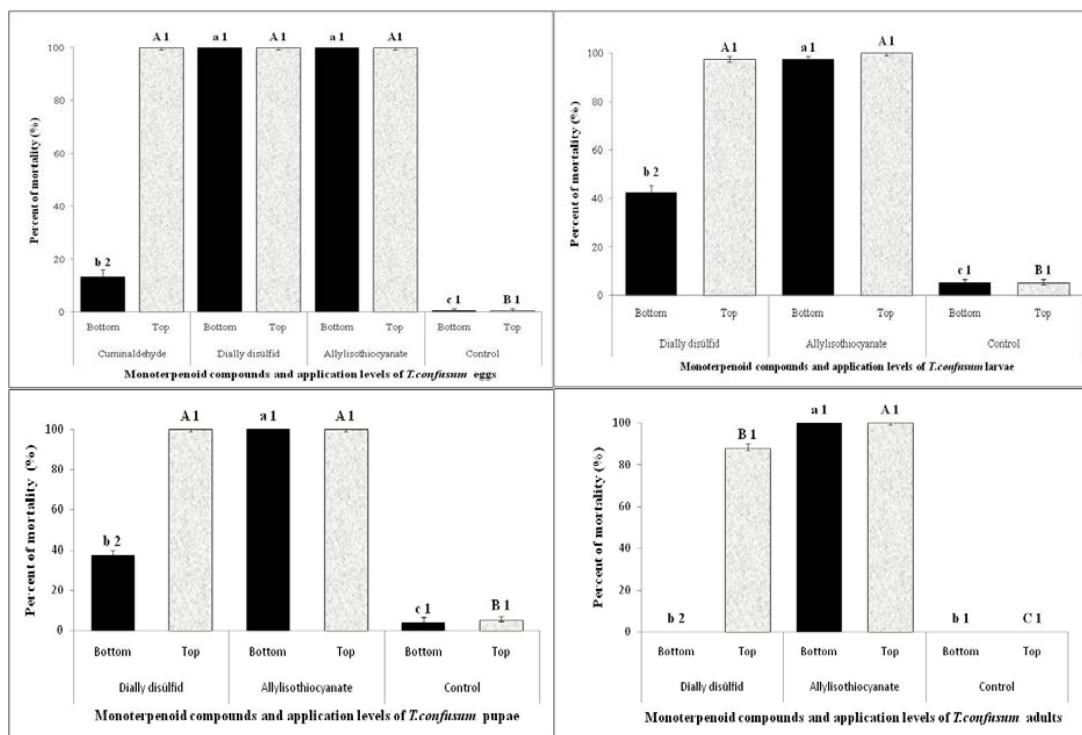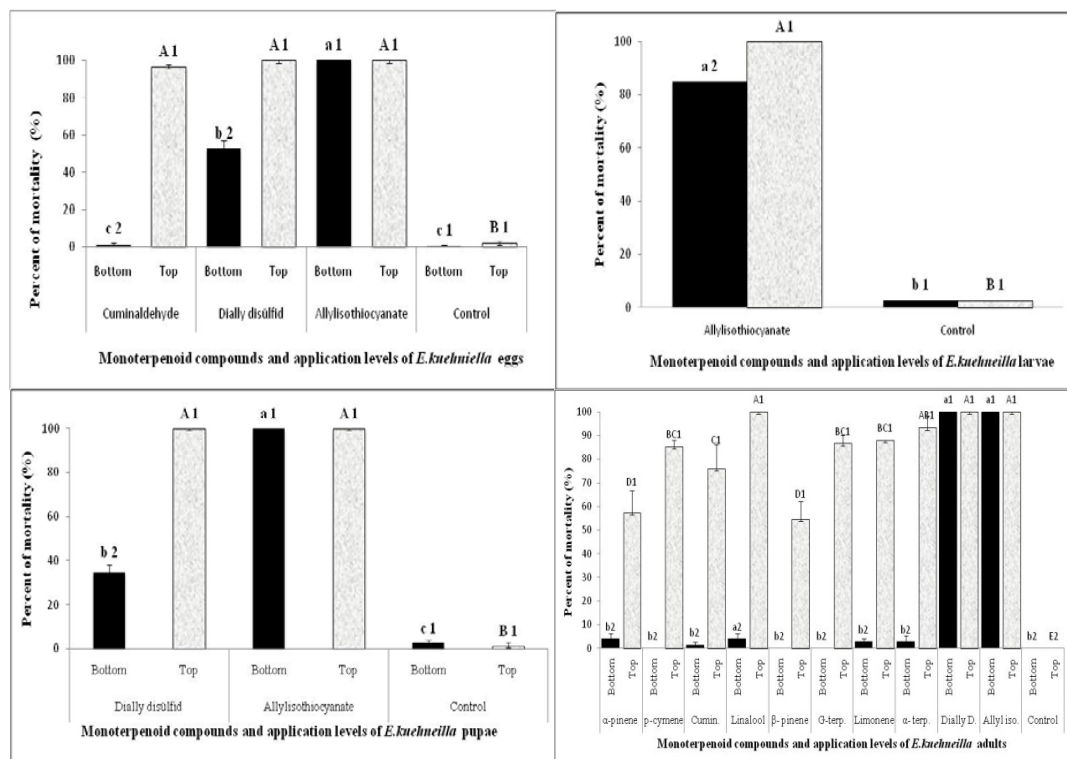

Fig. 1- Percent mortality of *Tribolium confusum* eggs, larvae, pupae and adults placed at different positions (bottom and top) in present of 2 kg of wheat, which were exposed to 100 µl l^{-1} concentration of tested monoterpenoid compounds. (Two-way ANOVA was applied to the data. Means within a row with lower-case letter, a column with upper-case letter and each numbers indicated that differences of percentage mortality. Same characters do not differ significantly (Duncan test at 5% level).

Fig. 2- Percent mortality of *Ephestia kuehniella* eggs, larvae, pupae and adults placed at different positions (bottom and top) in present of 2 kg of wheat, which were exposed to 100, 80, 100 and 55 µl l^{-1} concentration of tested monoterpenoid compounds respectively. (Two-way ANOVA was applied to the data. Means within a row with lower-case letter, a column with upper-case letter and each numbers indicated that differences of percent of mortality. Same characters do not differ significantly (Duncan test at 5% level).

Diallyl disulphide had a very low toxicity to larvae, pupa and eggs of *E. kuehniella* in present of commodity indicated poor penetration through the commodity. Allyl isothiocyanate was more toxic than diallyl disulphide to both *T. confusum* and *E. kuehniella* egg, larva, pupa and adult in present and absent of commodity.

In conclusion, it was determined that allyl isothiocyanate would be a potential compound in controlling stored-product insects, since it had high toxicity to all biological stages of both species in both absent and present of commodity.

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SESSION 2

Quality preservation, safety and protection of the environment

Chairpersons:
Uygun Aksoy, Turkey
Hagit Navarro, Israel
William Anthony Jonfia-Essien, Ghana

AN INSIDE LOOK AT THE SILO-BAG SYSTEM

Ricardo Bartosik*

National Institute of Agricultural Technologies (INTA), Ruta 226 km 73,5 (7620), Balcarce, Buenos Aires Province, Argentina
*Corresponding author's e-mail: *rbartosik@balcarce.inta.gov.ar*

ABSTRACT

The silo-bags are a hermetic type of storage widely adopted. This paper summarizes the results of the effect of silo-bag storage on the commercial quality of corn, soybean, wheat, sunflower, malting barley, canola and beans. The effect of the modified atmosphere on insect population and storage fungi, and recommendations for proper storage conditions in the silo-bags are also presented.

Overall, when dry grain is stored in silo-bag, the CO_2 ranges from 3 to 10% and the O_2 from 18 to 10%. The degree of modification of the interstitial atmosphere increases with the grain m.c. and temperature having typical CO_2 concentration of 15-25% and O_2 of 2-5% for wet grain.

There are few reports of insect presence in silo-bags. Analysis of data indicates that unfavorable environmental conditions negatively affect insect development. Thus, storage in silo-bags under the analyzed climate conditions help to maintain grain without notable insect populations.

When grain is stored in silo-bags at m.c. that would allow for mold development, the mold activity is lower compared with that of normal atmosphere storage conditions. Additionally, grain temperature inside the silo-bag is mainly affected by the ambient temperature. Silo-bags have a high heat exchange rate with the air and soil (double surface/volume ratio than regular bins), so no heat damage is observed, even when wet grain is stored in temperate weathers.

The overall results indicate that dry grain (equilibrium relative humidity below 67%) can be stored in silo-bag for more than six months without losing quality (measured as percentage of mold damaged grain, test weight, germination, fat acidity, and nutritional and organoleptic parameters, among others). When grain m.c. increases, commercial quality could be maintained for up to six months in winter time, to less than three months in summer time. In all cases, maintaining the airtightness of the bag is a key factor for successful storage. A monitoring system for silo-bags based on measuring CO_2 concentration was also developed.

Key words: hermetic storage, modified atmosphere, storage, quality, cereal, oilseeds

INTRODUCTION

The silo-bags are a hermetic type of storage made with a plastic bag, with the shape of a tube, of 60 m long and 2.74 m diameter. The plastic cover is made of three layers (white outside and black inside) with 235 μm of thickness.

Each bag can hold approximately 200 tonnes of grain and with the available handling equipment is very easy to fill. The new generation of high capacity combines found in the

silo-bag system is the ideal partner, since the loading capacity of the bagging machine is basically limited to the transportation capacity between the combine and the place where the bag is filled. Several companies also developed machineries to unload the plastic bag transferring the grain directly from the silo-bag to the truck or wagon with a high capacity (more than 180 tonnes/h).

Argentina is the country in which the silo-bag was developed for storing dry grains. Since mid 1990's when it was introduced, the silo-bag system gained rapid adoption in the agricultural and industrial sector. Each year, more than 40% of the total production of the country is stored in the silo-bags (more than 40 million tonnes in year 2011).

Due to the successful experience in Argentina, the silo-bag system is now being adopted in more than 40 countries worldwide, from countries with tropical weather (i.e. Sudan) to countries with cold weather (i.e., Russia).

There was an important development regarding to the bagging (loading) and unloading equipment. The operating capacity of the loading and unloading equipment is higher than 180 tonnes/h. Fig. 1 shows a picture of a typical loading and unloading equipment.

Fig. 1- Images of loading (left) and unloading (right) machines.

A LOOK INSIDE OF THE SILO-BAG

Environment and Relationships

Fig. 2 shows a diagram of the main factors affecting the ecosystem of the silo-bag and the relationship among them. Based on this model, the respiration of grains, fungi, insects and other microorganisms present in the grain ecosystem consume the O_2 and generate CO_2, heat and water. The respiration process also consumes the grain energy sources (starch, oil or protein), which could be quantified as dry matter loss (DML).

The respiration rate is affected by grain type and condition, m.c., temperature, storage time, and O_2 and CO_2 concentrations. These last two factors make a difference between the respiration rate of grains in regular storage structures and hermetic structures.

The temperature of the grain depends on the initial grain temperature (this effect is less important as the storage period increases), the effect of the sun radiation, the heat release from the respiration process, and the transfer of heat with the air and soil. The grain m.c. depends on the initial grain m.c., the entrance of moisture from the outside (through openings after a rain event into broken or poorly sealed silo-bags), and the moisture released from the respiration process. Additionally, due to the day and night temperature differential, some moisture condensation can occur in the top grain layers resulting in a localized spot of wetter grain.

For any particular time, the CO_2 and O_2 concentration in the silo-bag depends on the balance between respiration (consumption of O_2 and generation of CO_2), the entrance of external O_2 to the system, and the loss of CO_2 to the ambient air. The movement of gases in and out of the silo-bags depends on the gas partial pressure differential and the permeability of the system (through openings in the plastic cover, or through the natural permeability of the plastic material to the gases).
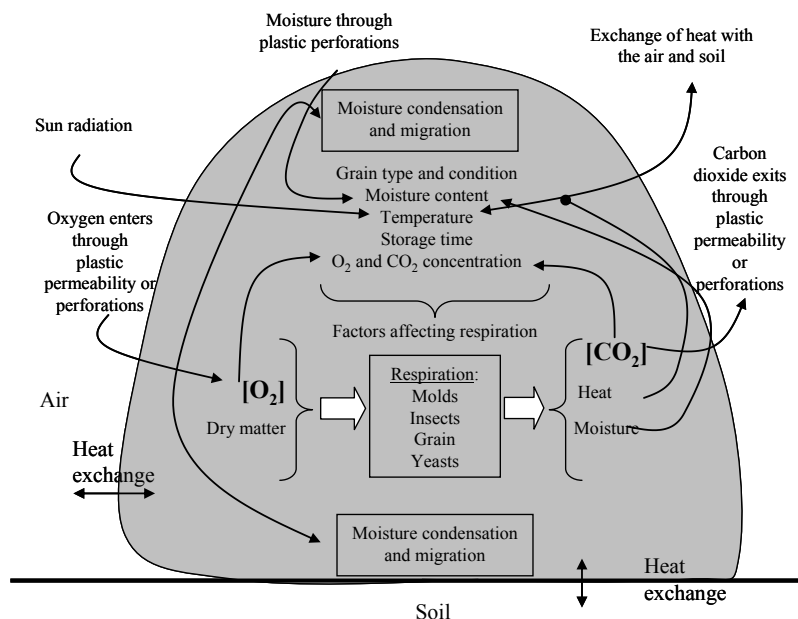
Moisture through plastic perforations

Exchange of heat with the air and soil

Sun radiation

Moisture condensation and migration

Grain type and condition
Moisture content
Temperature
Storage time
O_2 and CO_2 concentration

Carbon dioxide exits through plastic permeability or perforations

Oxygen enters through plastic permeability or perforations

Factors affecting respiration

[O₂]

[CO₂]

Air

Dry matter

Respiration:
Molds
Insects
Grain
Yeasts

Heat

Moisture

Heat exchange

Moisture condensation and migration

Heat exchange

Soil

Fig. 2- Section diagram of the silo-bag showing the main factors affecting the grain ecosystem, the relationship among them and with the external environment.

EFFECT OF AMBIENT TEMPERATURE

Bartosik et al. (2008a) indicated that the grain temperature at the surface showed the distinctive pattern of the ambient air temperature, reaching its maximum at noon and minimum during the early morning (Fig. 3). The daily temperature oscillation decreased with the grain depth, being not noticeable after 0.7 m depth. It was also demonstrated that the average grain temperature in the silo-bags followed the pattern of the average ambient temperature through the season.

In a field experiment, silo-bags with wheat were set up during the summer time with grain temperatures close to 40°C. The silo-bag was able to dissipate the heat in the grain to the ambient air and the soil in a couple of months, reducing the grain temperature to less than 17°C by early May (Fig. 3). This could be explained with the relation volume/surface, which is substantially lower for silo-bags (0.7 for a 200 tonnes silo-bag) than for a regular bin of similar storage capacity (1.27 for a 7 m diameter and 9 m height bin of 200 tonnes capacity). On the other hand, soybean and corn, harvested during the fall and winter, were able to maintain the temperature below 17°C until early November. Similar results were reported by Barreto et al. (2012) simulating the effect of ambient conditions on wheat silo-bags temperature in different regions of Argentina.
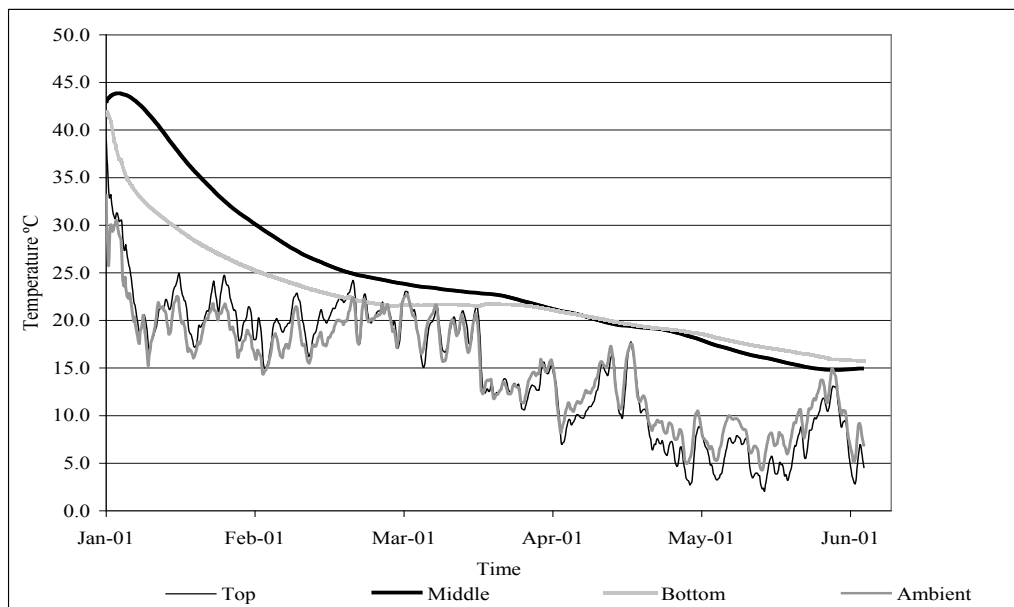
Fig. 3- Temperature pattern at different grain depths (top, middle and bottom) during storage of wheat in a silo-bag from January to June. Source: Bartosik et al. (2008).

Effect of Grain Moisture Content

Since the silo-bag is made of a hermetic plastic cover, no moisture variation should be expected during storage, unless rainwater enters to the bag through openings. Gaston et al. (2009) mentioned that the temperature differential between the top layer and the rest of the bag caused migration of moisture from the core of the grain mass to the top layer, and, to a lesser extent, to the bottom layer. Moisture migration can lead to m.c. rise in some grain layer, increasing the risk of grain spoilage (and grain quality deterioration) in localized areas of the silo-bag. Up to the present, it is not clear the magnitude of the moisture stratification process during storage in the silo-bag. Gaston et al. (2009) considered that grain m.c., grain temperature, grain temperature fluctuation magnitude and storage time affect the magnitude of m.c. stratification.

Darby and Caddick (2007) reported moisture stratification during storage of dry barley (≤ 11% m.c.) under Australian conditions in non-punctured silo-bags. This stratification increased m.c. in the peripheral layer up to 13% over winter, but remained dry over summer with temperatures above 30°C, indicating that the grain could be stored in perfect condition for up to 6 months. On the other hand, Ochandio et al. (2009) did not find m.c. stratification in 12% m.c. barley silo-bags, even after 1 year of storage.

Respiration of Biological Components

Grain, insects, fungi and other microorganisms respire, consuming grain constituents and O_2 from the environment, and releasing to the interstitial environment CO_2, water and heat.

Grain type, m.c., temperature, storage time and O_2 and CO_2 concentrations affect the respiration rate. Most of the factors influencing respiration in silo-bags could be modeled. However, there are no correlations available for predicting respiration rate of grains stored under hermetic conditions (oxygen depleting environments). In order to further improve the

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modeling of modified and controlled atmospheres it is necessary to generate suitable correlation for predicting respiration in O_2 restricted environments.

Permeability

The transfer of gases between the inside and outside of the silo-bag depends on the gas partial pressure differential and the effective permeability of the silo-bag to gases (permeability of the plastic layer film and perforations). While the permeability of the plastic cover could be measured or estimated based on the characteristics of the plastic material (most of the silo-bags are made of similar materials and have similar thickness), the permeability due to perforations is more difficult to estimate since the size, shape, location and number of perforations differ substantially among different silo-bags.

Plastic Cover

The permeability of the silo-bag plastic cover depends on the thickness and material composition, both set by the manufacturing process. The silo-bag is made of a three layer plastic of 230 to 250 µm thickness, black inside and white outside. The plastic layers are a mixture of high density (HDPE) and low density polyethylene (LDPE). The plastic layer has a differential permeance to O_2 and CO_2. For a silo-bag with an average thickness of 240 µm, Abalone et al. (2011) estimated that the permeance to O_2 was 4.06×10^{-4} $m^3 d^{-1} m^{-2} atm^{-1}$ and to CO_2 was of 1.34×10^{-3} $m^3 d^{-1} m^{-2}$ atm^{-1}.

Perforations

Perforations in the plastic cover increase the exchange rate of gases between the inside and the outside. Simulations were performed by Abalone et al. (2011) to explore the effect of structural damage of the silo-bag. It was shown that even a small perforation can significantly change the evolution of gas composition, from 1 percentage point for one perforation of 1 mm diameter per linear meter of a silo-bag, to more than 5 percentage points for one perforation of 10 mm diameter.

The effect of number of perforations on gaseous composition was also investigated. Wheat at 13% m.c. and 25°C stored in a completely airtight silo-bag reached a CO_2 concentration of 6.5% and a O_2 concentration of 12%. One perforation of 3 mm diameter per meter of silo-bag reduces the CO_2 concentration to 4.5% and increases the O_2 concentration to 15%, while 5 perforations per meter resulted in a decrease in the CO_2 concentration to 1.5% and an increase in the O_2 concentration to 19.5% (Abalone et al., 2011).

Oxygen and Carbon Dioxide Concentration

The CO_2 and O_2 concentration in any given time is the result between the respiration rate (depletion of O_2 and generation of CO_2) and the gas exchange rate with the outside (entrance of O_2 and exit of CO_2). Gas concentration data were measured over time for different grains and storage conditions (m.c.) (Fig. 4). Typically, for dry grains, the O_2 concentration equilibrates between 10 and 18%, while the CO_2 concentration equilibrates between 3 and 10%. For wet grains (equilibrium relative humidity higher than 67%) the O_2 concentration drops to 2 to 5%, while the CO_2 rises to 15 to 25%. In some cases, with exceptionally wet grain, the CO_2 concentration can reach values as high as 70% (O_2 close to 0%).

Silo-bags would act as a typical modified atmosphere system when the grain is wet enough to hold biological activity that would consume the O_2 at a higher rate than O_2 is entering to the bag from the outside through the plastic cover. Under this situation the O_2 concentration will drop below the limit at which aerobic respiration starts to be limited. This

observation is in agreement with Darby and Caddick (2007) in their comprehensive report made about silo-bags in Australia.
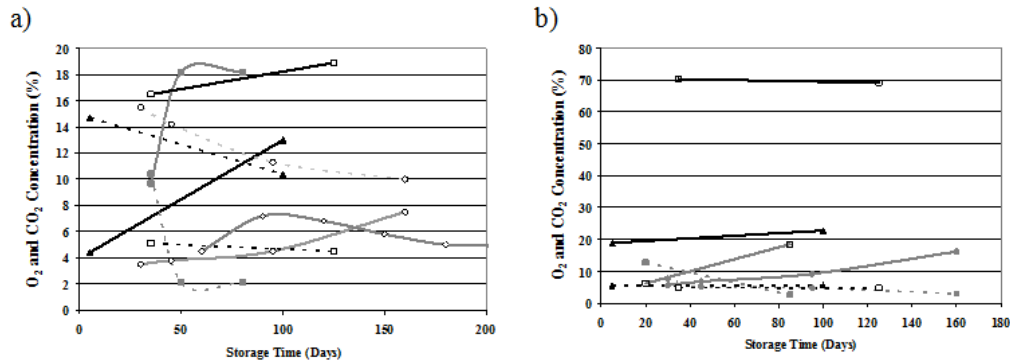

Fig. 4- O_2 and CO_2 concentration during storage of dry (a) and wet (b) grains in silo-bags. Adapted from Bartosik et al. (2008). Legends: solid line, CO_2; dashed line, O_2; ▲, wheat; ■, corn; ○, soybean; □, sunflower; ◊, barley.

The temperature also has a positive effect on the biological activity, but the interaction with m.c. shows that the effect of temperature is higher in wet grain storage than in dry grain storage (Fig. 5). This would imply that dry grain would not hold significantly different biological activity in winter or summer, but storing wet grain could be substantially more challenging (affected by biological activity) in summer than in winter time.
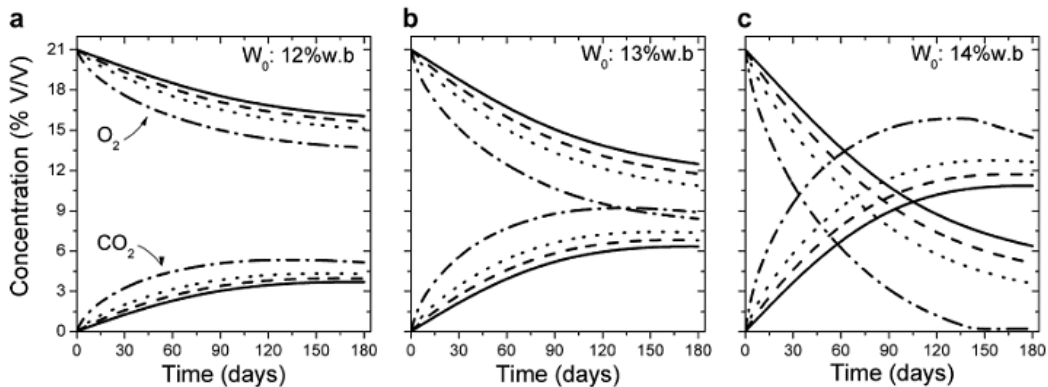
Fig. 5- Predicted evolution of O_2 and CO_2 concentrations during storage from summer (January 1) to winter (July 30) for different initial storage temperatures of grains. Initial grain moisture content: a) 12% w.b; b) 13% w.b; c) 14%w.b. Initial grain temperature:— , 20°C; - - , 25°C; …, 30°C; –.– , 40°C. Source: Abalone et al. (2011) (with permission).

Effect on Quality
Wheat
The storage of dry wheat (12.5% m.c.) during 6 months in a silo-bag resulted with no substantial reduction in the test weight, neither affecting the baking quality parameters (loaf

volume, gluten %, w, etc). When 16.4% m.c. wheat was bagged in January the average grain temperature was of 42°C. The combination of high m.c. and high temperature resulted in a substantial decrease on most of the quality parameters evaluated. Test weight decreased from 78.7 to 77.3 kg/hl, although this decrease did not change the commercial grade of the wheat. Additionally, all the baking quality parameters were negatively affected, making this wet wheat not suitable for flour milling purposes.

Corn

The grain bagged at 14.8% m.c. resulted with a slightly higher test weight after 150 days of storage, while the percentage of damaged kernels increased by 1.3 percentage points (the initial percentage of damaged kernel was greater than 3%). The wet corn samples (19.5% m.c.) resulted with a reduction in the test weight of 2 kg/hl, and a substantial increase of the damaged corn faction of 4.4 percentage points.

Soybean

The soybean bagged at 12.5% m.c. did not substantially modify the test weight and oil percentage of the samples after 150 days. On the other hand, the oil acidity index and the germination were, slightly affected. The wet soybean samples (15.6% m.c.) did not result in effect on the test weight and the percentage of oil, but resulted with an increase in the oil acidity index from 1.7% to 2.3%.

Barley

Malting barley stored dry (below 12% m.c.) for a storage period from 6 to 12 months did not have negative effect on the germination (always remained above 98%). In one study including 56 silo-bags, only 2 resulted with germination test values of 94%, and one with values of 86%. The protein content typically did not change during storage, being the highest change observed of 1 percentage point after 6 months of storage (Ochandio et al., 2009; Cardoso et al., 2010; Massigoge et al., 2011).

Sunflower

When sunflower was bagged at 8.4% m.c. no reduction in oil composition was observed, while the oil acidity index slightly increased from 0.9 to 1.4%. This increase in the oil acidity index did not affect the commercialization standard grade of sunflower, since the oil acidity index limit for the argentine standard is 1.5% until August 31, and 2% thereafter. Thus, storage of dry sunflower (below 11% m.c.) is a safe practice, since the industrial quality parameters were not affected after 150 storage days. Storing of wet sunflower (16.4%) resulted in a reduction of oil composition of 1.3 percentage points (from 47.0 to 45.7%) after 150 storage days, and a more substantial increase in the oil acidity index (0.9 to 3.9%).

Canola

The r.h. in the interstitial air of canola remained below 50% along the entire storage period (canola m.c. of 6%). The m.c., foreign matters and fat values remained unchanged throughout the storage period. The fat acidity increased during storage in 0.7 % points, reaching a final value of 1.4%, but did not represent a commercial quality loss (Ochandio et al., 2010).

Seeds

When seeds are stored with low m.c. (equilibrium r.h. below 67%), no substantial reduction in the germination was observed for wheat (Bartosik et al., 2008a) and barley (Ochandio et al.,

2009; Cardoso et al., 2010; Massigoge et al., 2010). In the case of soybean it was observed that when the initial germination values were low, there was a substantial decrease of this parameter during storage, even for m.c. as low as 12.5% (Bartosik et al., 2008a). Additional data showed that when the initial germination value was high (i.e., above 95%), the soybean seed viability did not change during storage when the m.c. was below 12.5%. However, when the seed was stored at a m.c. higher than 12.5%, the number of samples in which a reduction in the germination was observed increased.

Molds and Mycotoxins

In grain ecosystems, the most important abiotic conditions that influence mold growth and mycotoxins production are a_w, temperature, and gas composition. Fungal species involved in the deterioration of stored grain are obligate aerobes, but they can grow under conditions of reduced levels of oxygen, and some species can tolerate high levels of CO_2. Additionally, modified atmospheres also had been reported to have control effect on mycotoxin production at both, high CO_2 concentration and low O_2 concentration (Chulze, 2010).

Pacin et al. (2009) reported fumonisin in corn silo-bags. The contamination levels recorded at the closing of the silo suggest that contamination with molds and fumonisins are more dependent on the grain conditions at the moment of entrance to the silo bags than on the duration of storage.

Castellari et al. (2010) indentified two potential producers of aflatoxins (*A. flavus* and *A. parasiticus*) and a potential producer of fumonisins (*F. verticillioides*) in corn silo-bags with m.c. from 14 to more than 20%, although toxins levels were not tested.

Most of the mold species typically present in grains cannot develop in environments with r.h. below 67-65%, which corresponds with an equilibrium m.c. of 14% in wheat and corn, 12.5% in soybean and 8-9% in sunflower. Under this storage condition in the silo-bag the mold activity is basically stopped, and hence the mycotoxin production.

When storing grain at a m.c. that would support mold growth (equilibrium r.h. higher than 67%), the mold activity and the mycotoxin production would be affected by the atmosphere composition. If the grain is wet, thus the microbial activity would deplete the oxygen rather quickly (few hours), preventing mold damage and mycotoxin production. However, if the grain is slightly wet, the modification of the interstitial atmosphere would be rather slow, and many days (and may be months) would be required to reach the level of mold suppression. Under this condition mycotoxin production could be possible. Additionally, if the grain is wet (high biological activity) but the silo-bag has a low airtight level (i.e., bad sealing of the closing end, perforations, etc), oxygen will enter from the outside allowing mold development and mycotoxin production. The relationship among grain m.c., the effect on biological activity, the resulting CO_2 and O_2 concentration and how this affect the mycotoxin production is yet not fully understood for typical silo-bag storage conditions and more research is needed.

Insects in the Silo-bag

There are relatively few reports of insect infestation of grain stored in silo-bag. Massigoge et al. (2010) reported that insects were observed in 10 barley silo-bags out of 56 monitored. The wheat milling industry, which uses silo-bag for storing dry wheat, indicates that the presence of insects is more frequent during summer time and in silo-bags filled with grain that has been previously stored in regular bins (not coming from the field).

Conditions that Affect Insect Development in Silo-bags

The insect development in silo-bags is limited because: 1) most of the silo-bags are filled with grain coming directly from the field. The presence of stored grain insects in the field is rather scarce, depending on the ambient condition of the harvest time (temperature, r.h.), proximity to storage structures, etc, but most of the time the grain comes form the field free of insects. Additionally, during the harvest operation the grain passes through the combine, then to a truck or wagon and then to the bagging machine, reducing the risk of infestation when compared to grain stored at the elevators. 2) The plastic bag itself comes free of insects, in comparison with regular bins which could be infested prior to the harvest. 3) Once the grain is stored in the silo-bag, the plastic cover acts as a physical barrier, preventing the entrance of insects. 4) The temperature of the grain inside of the silo-bag follows the average ambient temperature throughout the year. Thus, in temperate and cold climates, during the fall and winter the grain temperature will drop below the range of insect activity (15-17°C), reducing substantially their development. 5) When grain is stored with m.c. above the mold activity limit, the O_2 concentration can drop below the 2% and the CO_2 concentration can rise above 20%, creating a lethal environment for insects.

Based on these considerations, the most critical situation that would favor insect development (and damage) in the silo-bags is when the bag is filled with previously infested grain, the grain is stored over summer time (grain temperature between 25 and 30°C), and the grain is too dry to create a lethal atmosphere for insects.

Phosphine Fumigation

Phosphine fumigation in silo-bags has been successfully implemented when insect control is required. Cardoso et al. (2009) showed that applying aluminum phosphine pellets each 5 m along the silo-bag with a dose of 1 g of PH_3 (3 g of aluminum phosphide) per tonne was sufficient to hold 200 ppm during 5 days in the almost entire grain mass. The critical point was the closing end, where a re-application after 3-4 days was recommended. In a similar study using a phosphine dose of 1.5 g m^{-3} in wheat, Ridley et al. (2011) found that complete control of all life stages of *R. dominica* was achieved at all locations in the fumigated silo bags.

Monitoring Grain Quality (CO_2 Monitoring)

The respiration of the biotic components of the grain mass (fungi, insects, and grain) increases CO_2 and reduces O_2 concentrations. Thus, the degree of modification of the gas composition in the interstitial air could be related to the biological activity inside the silo-bag, and can be used as a monitoring tool to detect early spoilage problems (Bartosik et al., 2008b). INTA developed the CO_2 monitoring technology with a private company (Silcheck, Lincoln, Argentina). Trained personnel with a portable CO_2 meter measures interstitial atmosphere CO_2 composition every 6 m along the bag, perforating the plastic cover with a needle (this operation takes less than 10 min for the entire bag). The information is uploaded to a server where the data are automatically analyzed and processed, a storage risk index is elaborated for each environment of the silo-bag, and the storage condition of the silo-bag can be monitored through internet. In case of detecting unsafe storage conditions, an automatic report is sent to the owner of the silo-bag through e-mail, fax or by cell phone SMS.
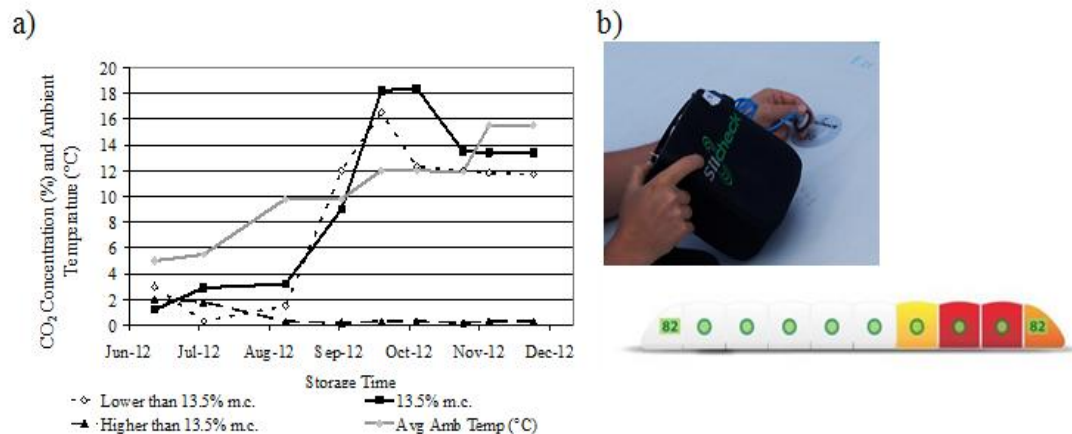
Fig. 6- a) CO_2 concentration during storage of one silo-bag without storage problems (-▲-) and two silo-bags with spoiled grain (−■− and -◊-) with soybean at m.c. around 13.5% (Source: Bartosik et al., 2008b). b) CO_2 meter and internet report with visual information showing in a color scale the storage risk index.

Recommendation for Successful Storage with Silo-bags

The overall results indicate that dry grain (equilibrium r.h. below 67%) can be stored in silo-bag for more than six months without losing quality (measured as percentage of mold damaged grain, test weight, germination, fat acidity, and nutritional and organoleptic parameters, among others). When grain m.c. increases, commercial quality could be maintained from three to six months in winter time, and from one to three months in summer time.

Silo-bags storing dry grain will not create a lethal environment for insects. However, low temperatures during winter in temperate climates will affect insect development. Storing grain at m.c. that can hold mold activity would create a lethal environment for insects, but the storage time will be limited due to effects on grain quality. Phosphine fumigation in silo-bags is a simple and effective insect control methodology.

Prior to set up the silo-bag, the site selection is a key factor. The piece of land should be high and with a slight slope to avoid rain water accumulation that, potentially, could enter into the silo-bag through perforations. A smoothing and leveling operation of the ground should be done. The soil should not have materials that could damage the bottom of the silo-bag during the filling operation, such as stones, residues of the crop, etc. Additionally, sites that are close to trees should be avoided to place silo-bags, since falling branches can damage the bag.

Maintaining a high airtightness level is a key factor for successful storage. Good care should be taken to maintain the plastic cover integrity during the bag filling operation and during storage. It is also critical to make a proper sealing of the closing end. Thermo sealing seems to be the most appropriated technique for ensuring a high airtightness level.

Place the silo-bags in pairs, leaving one open road for the unloading operation before the next pair of silo-bags. With this configuration, any silo-bag could be unloaded at any time (i.e., because a spoilage problem was detected), without having to unnecessary unload an extra bag.

Set up a fence around the silo-bags to keep out the animals, either wild or domestics (i.e., dogs and cats). The fence could be permanent, or made with electrified wires, such as those used for cattle. The wires should be placed at different heights, according to the typical animals of the location.

Some animals, such as birds and rodents, cannot be controlled by a fence. Thus, a rodent monitoring and control program must be implemented. Keeping clean and mowing or spraying herbicide in the silo-bag area will also help to prevent animal activity around the silo-bags.

The silo-bags should be periodically inspected. Any perforation should be properly sealed immediately. Avoid probing the silo-bag, since the patches often get detached. It is convenient to collect grain samples for quality control during the bag filling operation. Monitoring of the grain storage condition should be done by measuring CO_2 concentration, since it does not affect the physical integrity of the bag.

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RECENT DEVELOPMENTS IN THE STORAGE OF DRY COCOA BEANS IN GHANA

William Anthony Jonfia-Essien

Quality Control Company Limited (COCOBOD), Ghana.
*Corresponding author's e-mail: *wajonfiaessien@gmail.com*

ABSTRACT

In the past, methyl bromide was used in the fumigation of stored cocoa beans in Ghana but following the signing of the Montreal Protocol by 183 countries including Ghana, methyl bromide was phased out. Being an international treaty the Protocol was aimed at reducing or eliminating the use of chemicals that contribute to the depletion of the atmosphere's ozone layer and mitigate some of the harmful effects that could also have some negative effects for producers and consumers. As an alternative, phosphine fumigant which is more user and environmental friendly was adopted for the fumigation of dry cocoa beans in Ghana. However, much effort is being put in to minimise the use of agro-chemicals in Ghana. Hence, hermetic control has been adopted alongside phosphine fumigation in storage as a national policy as part of integrated pest management (IPM) with great success, resulting in a 50% reduction of agro-chemical application. There is a reduction in the frequency of fogging and spraying. Organic cocoa beans from Ghana are now fully treated using the principles of hermetic storage and as a result, the quality of the cocoa beans is maintained with 100% insect mortality.

Key words: cocoa beans, storage, phosphine, hermetic, fumigation, fogging, spraying, IPM, insect mortality

INTRODUCTION

In Ghana dry cocoa beans are first packed in jute bags and stacked into warehouses, depots and sheds. Dry cocoa beans are usually stored in warehouses at three Take -Over Centres namely; Tema port, Takoradi port and Kaase Inland Port in Kumasi. Storage of dry cocoa beans in Warehouses mainly at the ports is designed for longer storage of cocoa compared to that of depots and sheds being practiced up-country.

Cocoa, like other tropical crops, is often ravaged by insects, diseases and other pests that must be controlled effectively as well as safely. Their control involves intensive use of agro-chemicals which is now facing some international challenges in terms of rigid international standards on Maximum Residue Levels (MRLs).

Insect infestations in dry cocoa beans can be controlled effectively by the application of Integrated Pest Management (IPM), but insect pest populations could be reduced drastically through efficient cultural practices in combination with the IPM. The quality of cocoa beans can be sustained by ensuring general good sanitation, maintaining low Moisture Content (MC) to as low as 6.0 – 7.5% to curtail metabolic activity of any organism present. The recent approach in controlling insect infestation in dry cocoa in Ghana is the application of modified

atmospheres (MAs) in combination with other IPM strategies. By this approach, oxygen availability is reduced while carbon dioxide concentration increases. This approach was not used in the storage of cocoa beans but it was initiated when methyl bromide, an important fumigant was withdrawn under the international Montreal Protocol agreement due to its ozone depletion property and also when there were sporadic reports that the phosphine fumigant alternative did not offer 100% mortality of insect pests. Treatments involving MAs have been investigated and accepted as viable alternative treatments (Villers *et al.* 2001; Navarro *et al.,* 1996; Navarro *et al.,* 1994; 1989; Calderon and Navarro 1980; Navarro and Calderon, 1980) for further development in the immediate future including hermetic treatment.

Hermetic storage consists of a sealed storage system containing a modified atmosphere. This means that, as a result of respiration effects, there is generally depletion of oxygen (O_2) and production of high carbon dioxide (CO_2) atmosphere. Pioneering modern hermetic storage, Calderon and Navarro (1980), Navarro and Calderon (1980), Navarro *et al.* (1989; 1994) used safe, pesticide-free hermetic material made of flexible Polyvinyl Chloride (PVC) plastic liner (also known as GrainPro Cocoon^{TM}) for the storage of many commodities and seeds, particularly in hot and humid climates. PVC plastic liner is suitable for maintaining a constant moisture environment.

Ghana has successfully adopted the hermetic control alongside phosphine fumigation of dry cocoa beans in storage. Hermetic storage of cocoa beans in Ghana has led to 50% reduction in the use of agro-chemicals. Ghana has also maintained its premium grade cocoa with 100% insect mortality with hermetic storage facility (Jonfia-Essien *et al.* 2008a). Quality parameters of dry cocoa beans as well as mortality level of stored insect pests of cocoa beans under hermetic storage were closely monitored alongside those under the phosphine fumigation by the Research Department, Quality Control Company Limited (COCOBOD) (QCC).

MATERIALS AND METHODS

PRE-STORAGE QUALITY DETERMINATION

When cocoa beans graded, sealed and certified by QCC arrived from depots up-country, they were laid and samples of the cocoa beans were drawn from each bag for re-examination (i.e. 100% sampling). The samples were thoroughly bulked, quartered and the process repeated until final sample was obtained for thorough physical quality analysis. Quality parameters determined were moisture content, bean count, bean size uniformity and grade (i.e. mould, slate and all other defects including flat beans, germinated beans, as well as insect infested beans). Five bags of cocoa beans were randomly selected and sieved for insect infestation analysis at the Entomology laboratory before treatment.

Moisture content of cocoa beans was determined using AquaBoy moisture meter while mould, slate, purple and insect infestation were determined through cut test. Bean size uniformity was determined by counting number of whole cocoa beans in a weighed 100g beans. The unusual cocoa beans were counted and expressed as a percentage of total number of beans from the 100g weighed beans. A tolerance level of 10% was used as an index of bean size uniformity for any particular category of cocoa beans. Only cocoa beans that passed the quality parameters were accepted into stack and stored under either gas proof sheet with full dose of phosphine gas treatment or hermetic condition with half dose of phosphine gas at the take over centres (Port) warehouse.

PRE-SHIPMENT QUALITY DETERMINATION

Physical quality parameters of cocoa beans stored under both hermetic and gas proof sheet condition were determined in the same manner as that of pre-storage above.

Five bags each of cocoa beans were randomly selected from fumigated stacks (a bag each from the four sides and one from the top) under both the hermetic and gas proof sheet storage. With the aid of a sieving box selected bags of cocoa beans were sieved. Insect infestation analysis was performed at the Entomology laboratory and both live and dead forms of insects were identified.

RESULTS AND DISCUSSION

Physical assessment conducted on cocoa beans arriving from up-country to the Take Over Centre (TOC) during the period under review showed that the cocoa beans were of main crop size with all the MC within the threshold level of 7.5%. The average tolerance level (ATL) for all the cocoa beans was far below the 10% threshold level.

The importance of the ATL is to ensure good segregation of cocoa beans into various categories of sizes and the consignment is fairly uniform. High MC beyond 8% promote the growth of moulds but excessively low MC especially below 5.5% makes the cocoa beans too brittle and turn to break up during roasting for product processing and this account for the setting of national standard of MC between 6.0% and 7.5%.

Insect infestation of cocoa beans begins from the drying mat and it is essential to know the type of insects in the cocoa beans even before accepting the consignment into storage. Analyses of sieving samples revealed that cocoa beans received at all the TOCs (Tema, Takoradi and Kaase inland ports were found to be infested with *Cryptolestes ferrugineus, Ephestia cautella, Tribolium castaneum, Lasioderma serricorne, Araecerus fasciculatus, Rhyzopertha dominica and Carpophilus dimidiatus*. However the percentage infestation differed from the TOCs (Fig. 1).

With the exception of 2010/2011 crop year, Kaase inland port remained the TOC with the least insect infestation and Tema port being the TOC with the highest infestation level. This is an indication that distance of up-country depots to the TOC plays a pivotal role in infestation level of cocoa beans. The covering of cocoa beans in trucks during evacuation from the up-country depots to the TOC which is very critical to the sustainability of the cocoa beans also provides conducive environment for the multiplication of insect pests in the cocoa beans. It is therefore important to check the infestation level of cocoa beans and deal with it before accepting the consignment into storage.

Cocoa beans from different districts and up-country depots were brought together to construct a stack. However, physical assessment conducted on cocoa beans prior to shipment showed that the Bean Count (BC), ATL and the MC of the cocoa beans were still within the threshold levels. These physical parameters of the cocoa beans were not affected by the type of storage, whether the storage was under gas proof sheet of hermetic cocoon^{TM}. It can therefore be concluded that a thoroughly dry cocoa beans with MC between the 6.0 % and 7% will aid the sustainability of the quality of cocoa beans if good storage management practices are put in place.

Another factor that is critical to the sustainability of the quality of cocoa beans in storage is insect infestation. It was evidenced from the study that 100% mortality was recorded in most of the treatment of cocoa beans under gas proof sheet. However, a few percentages of the cocoa beans under the gas proof were found to be infested with insect pests, both adults and larvae (Fig. 2).
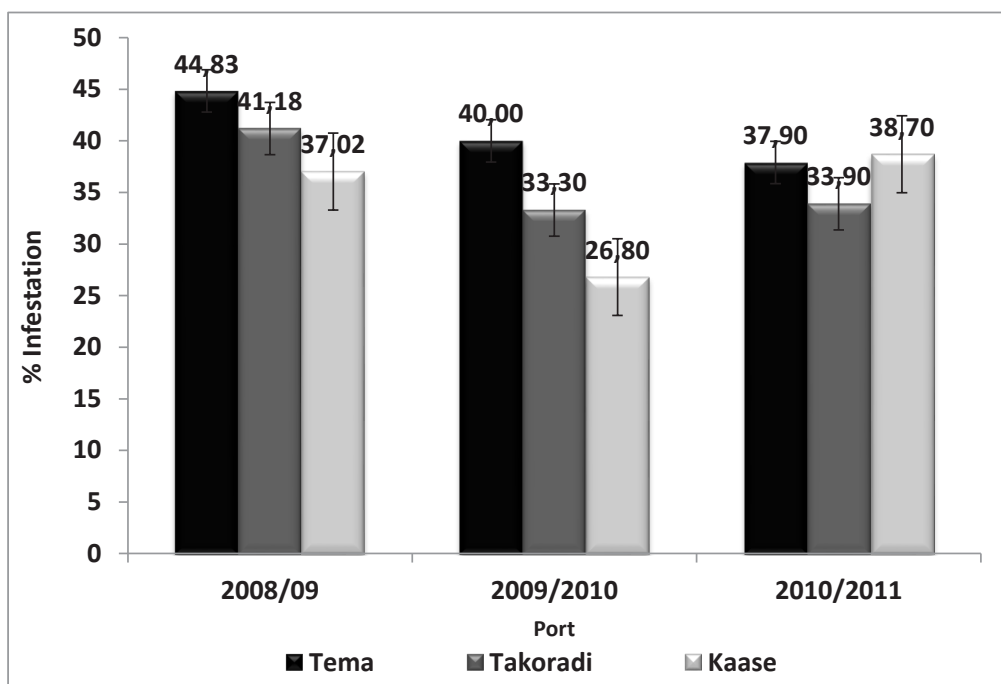
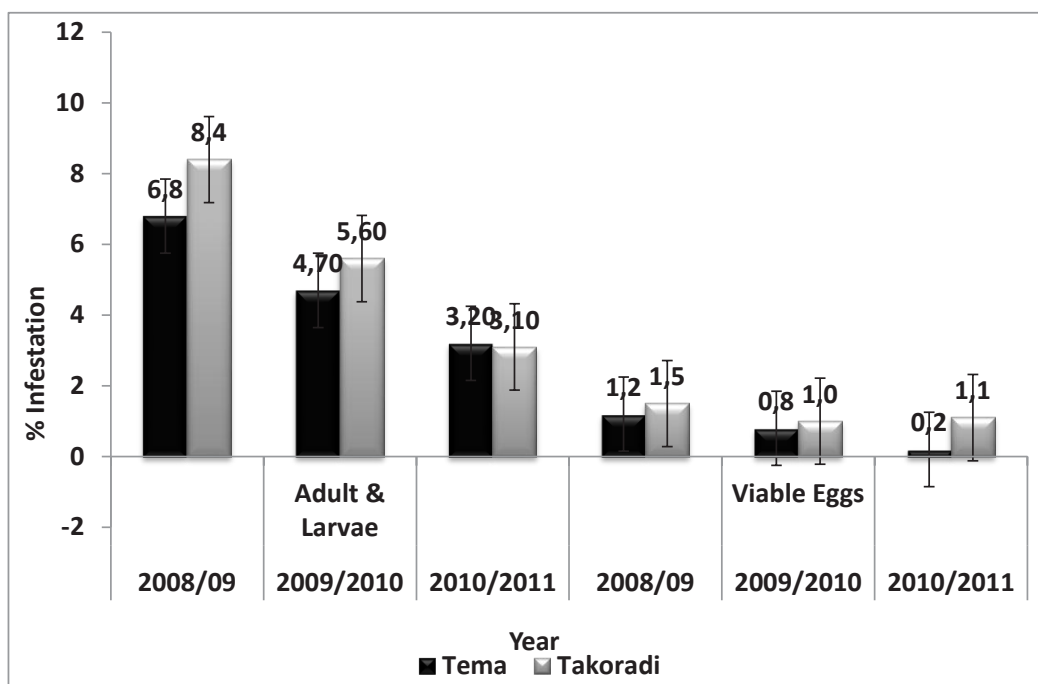Fig. 1- Percentage of arrival cocoa beans infested with insect pests

Fig. 2- Percentage of shipment cocoa beans with live insect pests after fumigation under gas proof sheet

Predominant among them were *Cryptolestes ferrugineus, Tribolium castaneum, Ephestia cautella,* and *Araecerus ferrugineus.* Takoradi port recorded the highest incidence of insect pest infestation contrary to the population of insects in the cocoa beans at the time of arrival. In very exceptional cases, isolated incidence of viable eggs was observed in post culture sieving analysis. Often than not the post culture sieving insect pests were identified to be *Cryptolestes ferrugineus* and *Ephestia cautella*.

Treatment under the hermetic Cocoon[TM] was a great success. The 100% mortality of insects in both pre and post culture sieving analysis confirms the findings on hermetic storage of cocoa beans by Jonfia-Essien *et al.* (2008a) and in consequence there have been a 50% reduction in the use of agro-chemicals on stored cocoa beans in Ghana. No viable egg was observed in post culture sieving analysis throughout the period under review. These findings informed a policy shift by Ghana Cocoa Board to store and treat cocoa beans using the hermetic Cocoon[TM]. At the moment the hermetic Cocoon[TM] treatment is running concurrently with the treatment of cocoa beans under gas proof sheet. However the hermetic Cocoon[TM] were installed only in two TOCs (Tema and Takoradi ports). In total, almost eight hundred and seventy three tons of organic cocoa beans (872.94 tons) treated under pure hermetic cocoon[TM] has been exported between 2008/09 and 2010/11 crop years. The success of hermetic cocoon[TM] contributed the decision by a buyer that every organic cocoa beans shipped to them should be treated under hermetic cocoon[TM].

The quality of cocoa beans prior to storage under gas proof sheet was maintained after storage, though there was a decline in some of the quality parameters especially the total mould and total slate (Table 1). Notwithstanding, the grade of the cocoa beans remained the same

Table 1. Quality of cocoa beans before and after treatment under gas proof sheet storage

Port	Crop year	Gas proof treated cocoa beans									
		Arrival analysis					Shipment analysis				
		TM	TS	AOD	Purity	Grade	TM	TS	AOD	Purity	Grade
Tema	1ST	1.0	2.0	1.0	96.0	I	1.0	2.7	1.3	95	I
	2ND	0.0	1.7	2.7	95.7	I	1.0	2.0	2.0	95	I
T'di	1ST	0.0	2	2.3	95.7	I	1.0	2.0	1.0	96	I
	2ND	0.0	1.3	2.7	96.0	I	1.0	2.3	1.7	95	I

MC- Moisture content   TM- Total mould   TS- Total slate   AOD- All other defects

On the contrary, there was no decline in any of the quality parameters in the cocoa beans stored under the hermetic cocoon[TM] and the quality was sustained (Table 2). The grade also remained the same.

Table 2. Quality of cocoa beans before and after treatment under hermetic cocoon[TM] storage.

Port	Crop year	Hermetic cocoon[TM] treated cocoa beans									
		Arrival analysis					Shipment analysis				
		TM	TS	AOD	Purity	Grade	TM	TS	AOD	Purity	Grade
Tema	1ST	0.0	2.7	2.3	95	I	0.0	2.7	2.3	95	I
	2ND	0.0	2.7	2.3	95	I	0.0	2.0	2.0	96	I
T'di	1ST	0.0	2.0	2.0	96	I	0.0	2.3	1.7	96	I
	2ND	0.3	2.7	2.0	95	I	0.3	2.0	1.7	96	I

MC- Moisture content   TM- Total mould   TS- Total slate   AOD- All other defects

The observed differences between the use of the gas proof sheet and the hermetic cocoon[TM] in the treatment of cocoa beans could be attributed to type of material used in the manufacturing of the sheet and the cocoon[TM]. The gas proof sheet allows the exchange of gas / air between the inner and outer environment whereas the hermetic cocoon[TM] does not allow for exchange of gas /air. This explains why carbon dioxide concentration in the hermetic cocoon[TM] increased while the oxygen concentration was being depleted.

CONCLUSIONS

As a leading producer of quality cocoa beans, implementing pragmatic measures such as IPM including hermetic control and good storage management to sustain the quality has been the hallmark of success in the storage of cocoa beans in Ghana. The 50% reduction in the use of agro-chemical will contribute to effective management of pesticide residues and therefore the effort is worth maintaining and possibly must be stepped up.

ACKNOWLEDGMENTS

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EMERGING INTERNATIONAL STANDARDS IN COCOA TRADE: RECENT TREATISE ON MRL

William Anthony Jonfia-Essien*

Quality Control Company Limited (COCOBOD), Ghana.
*Corresponding author's e-mail: *wajonfiaessien@gmail.com*

ABSTRACT

Contracts for the sale of cocoa beans basically require that the beans should be well fermented, dry, free from smoky and other abnormal odours. The cocoa beans should also be reasonably free from flat beans, germinated beans, fragments and pieces of shell. It should be reasonably free from insect, rodent and any other type of infestation. The cocoa beans should be reasonably uniform in size and virtually free from foreign matter and adulteration. Additionally the contracts require that good fermented beans should not be more than 4% visibly mouldy or 6% insect damaged and not have more than 8% slaty beans. This however, constitutes the basic requirement for trading on the international market. In recent time, the use of agro-chemicals to curb the population of insect pests and diseases has ushered in a new requirement. The Japanese published a "Positive List System for Agro-chemical residues" on cocoa which requires that cocoa beans with pesticide residue above prescribed limit (MRL) would not be accepted for processing. This was followed by EU with the introduction of pesticide residue legislation (EC No. 396/2005). To enforce and promote compliance to these requirements or emerging international standards, cocoa beans are routinely monitored and screened for pesticides residue in Ghana before export. Not with standing, the cocoa sector faces a lot of challenges and require arduous task to confront them. Some of these challenges emanate from methodology differentials and equipment use. Hence, this paper is an attempt to alleviate the bottomless in MRL analysis of cocoa beans.

Key words: cocoa beans, Agro-chemical, pesticides residue, MRL, international standards

INTRODUCTION

Cocoa, like other tropical crops, is often ravaged by insects, diseases and other pests that must be controlled effectively as well as safely. Pesticides can provide useful control solutions, but must be approved for use on the basis of Good Agricultural Practices (GAP).

In recent times, the extent of the use of pesticides and their mode of application including their abuse, especially in agriculture, has been of much concern to environmental scientists. Alongside their uses are also the residual effect of these pesticides and particularly their replicating effect on human health.

When a pesticide product is applied on the field, the chemical is gradually lost as a result of breakdown, leaching and evaporation and the residue is the amount that remains after application (Cox, 1995). Some pesticides have long residual activity and therefore persist in the environment whereas others have short residual activity and therefore disappear from the

environment or produce low residue concentration. It is therefore not surprising to find or detect residues of pesticides in the environment. Pesticide residues on crops are monitored with reference to Maximum Residue Level (MRL) which are based on analysis of quantity of a given active ingredient remaining on food product samples (Bateman, 2008) and it is the maximum concentration of pesticide residue likely to occur in or on food and feedstuff after the use of pesticide according to GAP and will not cause any health effect or hazard (Cabtas and Martin, 1992). In International circles food crops with pesticide residue level above the stipulated MRL are likely to be rejected.

Pesticide residues determination in food crop allows us to know the quality of the food in terms of pesticide contamination. Chromatographic techniques such as Gas Chromatography (GC), High Performance Liquid Chromatography (HPLC) and Thin Layer Chromatography (TLC) have been recommended for the determination of pesticides residues. Spectrophotometry could also be used for many pesticides, and colorimetric kits are available for cholinesterase inhibiting insecticides and fungicides (Afful, 2002; Lowor, 1999). Nowadays, techniques such as Gas Chromatography-Mass Spectrometry (GC-MS), Liquid Chromatography-Mass Spectrometry (LC-MS) is becoming popular and fast gaining grounds for pesticide residues analysis (Balinova and Balinova, 2006). Gas Chromatography has traditionally, however, been used widely for analysis of pesticide residues in plants tissues, soils and water samples (Yeboah *et al.*, 2003; Roseboom and Herbold, 1980).

Pesticide residue monitoring programmes are the only tool to control the quantity of pesticides on food and to enforce tolerances. In view of increasing consumer awareness of food safety issues, traceability is becoming an important agenda for the global cocoa market. Markets now require MRLs of pesticides as an additional standard in cocoa beans.
The European Parliament amended Regulation (EC) No 396/2005 and replaced it with (EC) No 149/2008 of 29 January 2008 which set maximum levels on the amount of pesticides permitted on imported foods including cocoa beans. Consequently, all cocoa beans imported into the EU from September 2008 must conform to the new Regulation. Also, the Food Quality Protection Act (FQPA) in the U.S.A was passed and was signed into law on August 3, 1996 (WWW – 1), empowering the Environmental Protection Agency (EPA) with an enormous challenge of implementing the Nation's pesticide and food safety laws. The FQPA amended the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the Federal Food Drug, and Cosmetic Act (FFDCA) by fundamentally changing the way EPA regulates pesticides (WWW – 2) and the amount of pesticide residues permitted on food for consumption.

In Japan, the Ministry of Health, Labour and Welfare (MHLW) established a new legislation that came into effect from May 2006, setting new MRLs for food products. Codex, European Union, Japan, Canada, New Zealand, South Africa Malaysia, Hong Kong, Korea, India, Israel, Russian Federation and Singapore set default pesticides MRLs for cocoa beans to be 0.01mg/kg if no MRL exist (Azhar & Rahmat, 2011).

MATERIALS AND METHODS

Problems however arise from the fact that cocoa importing countries use different methodologies to establish MRLs and different analytical methods to determine pesticide residues in cocoa beans. Different MRL levels in different countries and different measurement technologies constitute complications for international trade in cocoa (ICCO, 2011). These issues call for harmonization in legislation and its implementation. Examples of some of the different methodologies applied in pesticide residues analysis are the Classical

Multi Residue Method (Luke *et al*., 1999; Anastassiades *et al.,* 2003), the Japanese MHLW Method and the QuEChERS Method (Anastassiades *et al.,* 2003).

The Japanese MHLW method is adapted by the Ghana Standards Authority and Ghana Cocoa Board for the analysis of organochlorines and organophosphates for the cocoa beans from Ghana.

Currently, Ghana is complying with the Japanese legislation – which is more stringent than the others, since it requires the use of whole cocoa beans (beans with both nib and shell) in analysis, which is more likely to result in residue violations, whereas in the EU and USA, samples of cocoa beans are first de-shelled before residue analysis takes place. A comparison study of the two methods carried out by Azhar and Rahmat (2011) in Malaysia revealed that the QuEChERS method is more effective, less time and chemical consumption, simple, safe and more environmental friendly than the Japanese MHLW method.

Since 2008, the Ghana Cocoa Board has been determining the MRLs of the cocoa beans shipped to the international (Japanese) markets**.** The Japanese pesticides of interest were: Chlorpyrifos, Fenvalerate, Pirimiphos-Methyl, Endosulfan, Promecarb and Fenitrothion. These pesticides according to the new EU legislation on pesticide residues in foodstuffs of vegetable and animal origin imported into the European Union are not to be used on cocoa. Imidacloprid and Thiametoxam active ingredient in insecticides were recently added to the Japanese list for MRL analysis.

PESTICIDE RESIDUE ANALYSIS OF COCOA BEANS FROM GHANA

The annual production of cocoa beans in Ghana between 2008 and 2010 was around 750,000 metric tons. About 40% of the cocoa beans produced were analysed for pesticide residue and only those whose residue were within the Japanese MRL were shipped. All cocoa beans are re-analysed for pesticide residue on arrival at Japan. Any pesticide residue that is above the MRL is recorded as violation. This study examined the violations in the MRL of pesticide residues in cocoa beans.

RESULTS AND DISCUSSION

The MRLs of Chlorpyrifos, Fenvalerate, Pirimiphos-Methyl, Endosulfan, Promecarb, Fenitrothion, Imidacloprid and Thiametoxam in cocoa beans set by the Ministry of Health, Labour and Welfare (MHLW) in Japan are shown on Fig. 1- . The Japanese MRLs are very stringent and any pesticide residue in cocoa beans within the MRL certifies also the European and America market.

In total, one hundred and thirteen (113) violations of MRL were recorded in Japan between 2008 and 2010 (Figure 2). High violations were recorded in 2009 with Endosulfan leading followed by Pirimiphos-Methyl and then Fenvalerate. The least violations were recorded in 2008 with Chlorpyrifos leading followed by Pirimiphos-Methyl and then Endosulfan. In 2010, Fenvalerate recorded the highest violation followed by Imidacloprid and then permethrin.

Mean concentration of Endosulfan residue in cocoa beans was far higher than the MRL in 2008. May recorded the highest followed by April and December (Figure 3). Profenofos and Fenvalerate recorded the lowest concentration in December. In all violations occurred in seven out of the twelve months of shipment of cocoa beans.
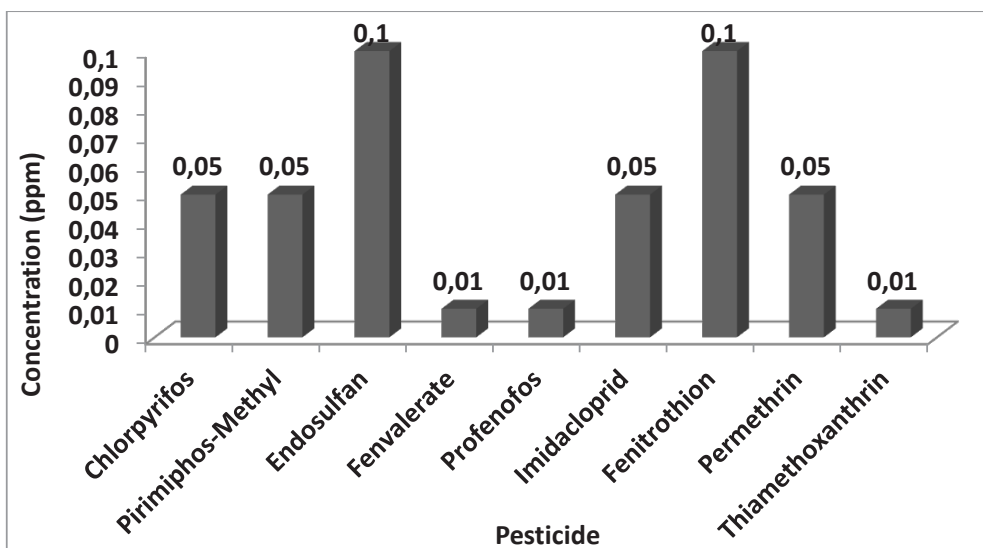
Fig. 1- MRL set by the Ministry of Health, Labour and Welfare of Japan.
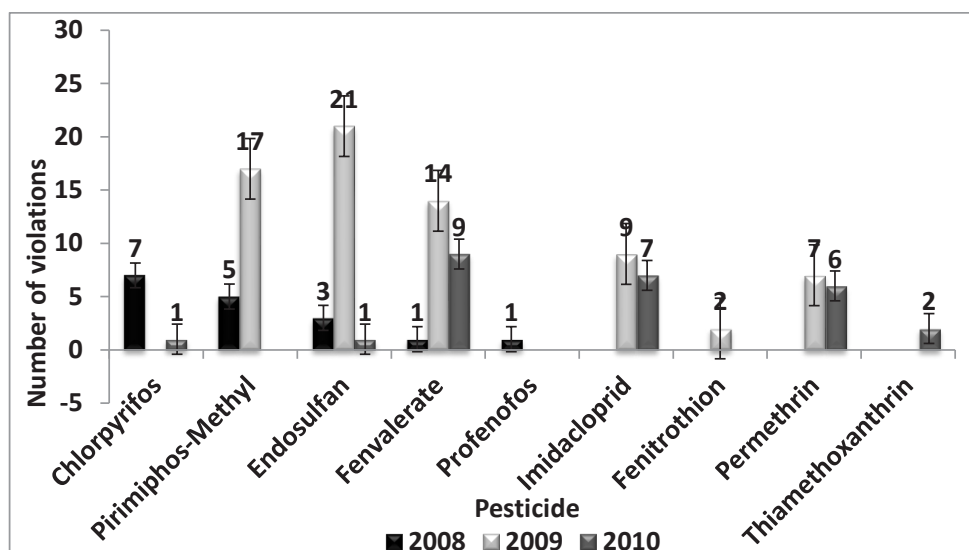

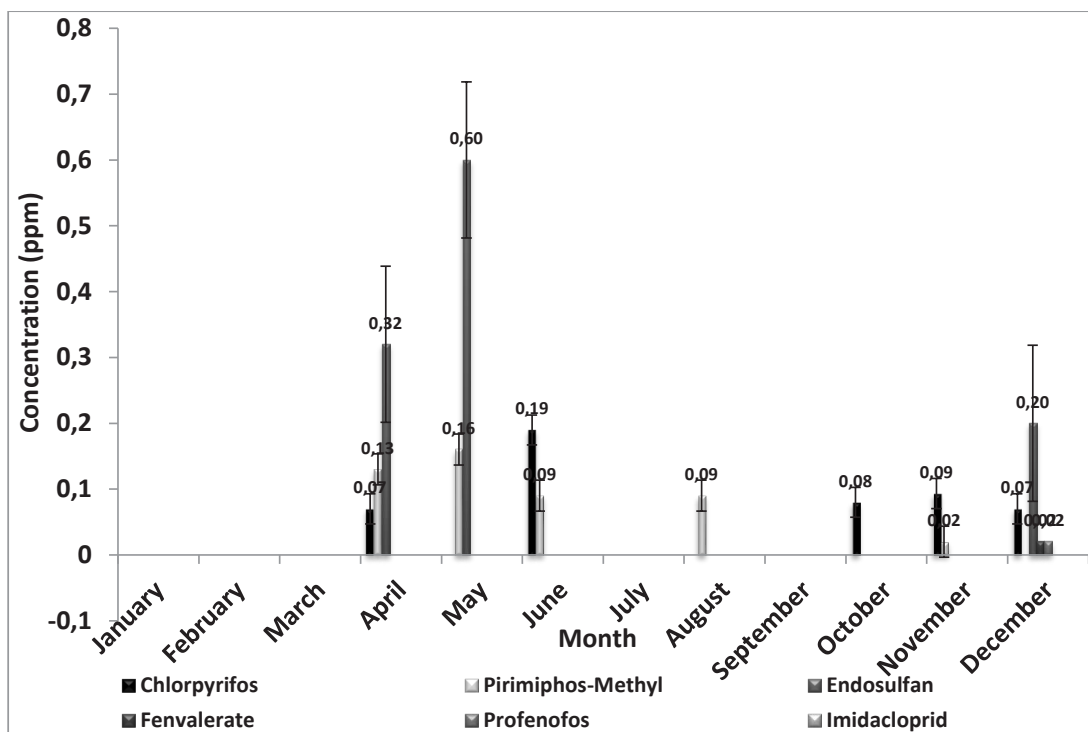Fig. 2- Number of MRL violations of pesticide residues in cocoa beans shipped to Japan between 2008 and 2010.

Fig. 3- Mean concentration of pesticide residue in cocoa beans shipped to Japan in 2008 in violation of MRL.

The trend was different in 2009 and the occurrence of violations was higher in this year. With the exception April, all the other eleven months in 2009 recorded violations (Fig. 4).
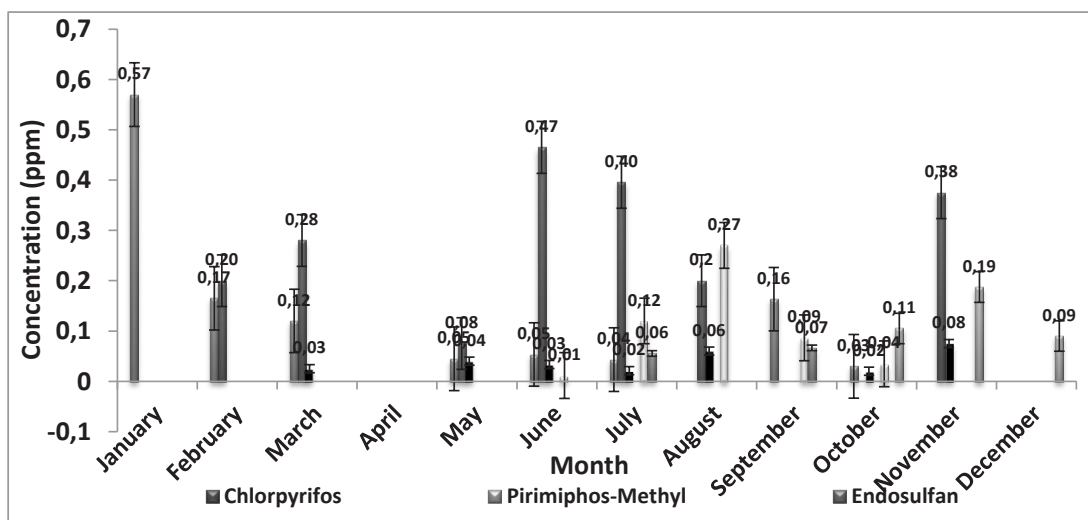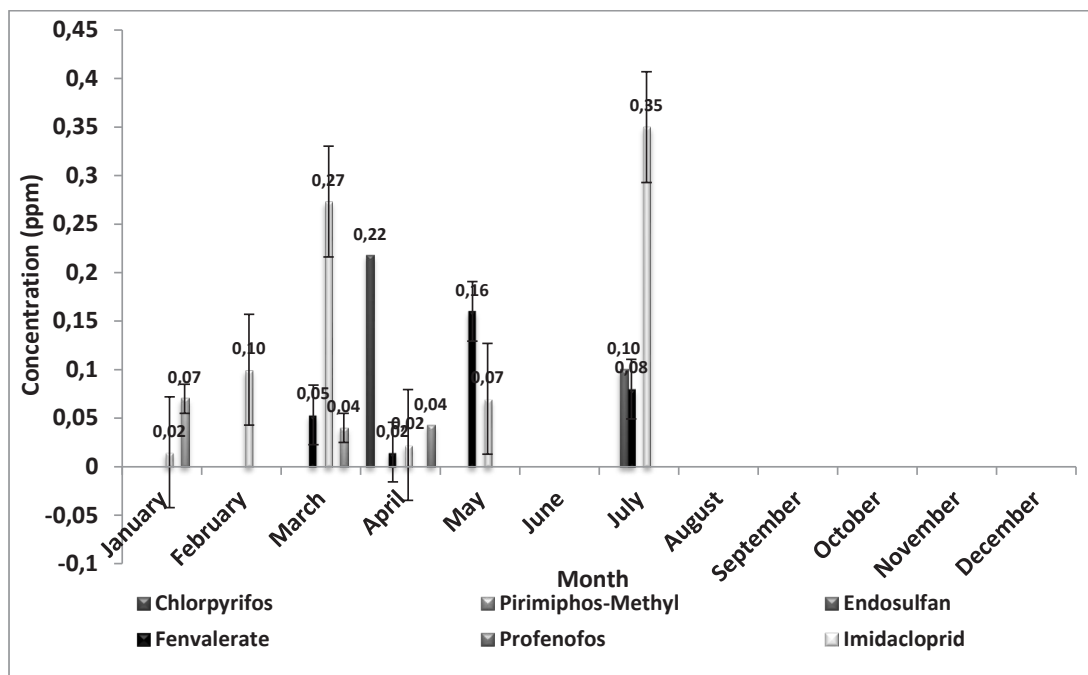

Fig. 4- Mean concentration of pesticide residue in cocoa beans shipped to Japan in 2009 in violation of MRL.

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Pirimiphos-Methyl and Endosulfan had the highest frequency in violation of MRL but Pirimiphos-Methyl had the highest concentration of residues in the cocoa beans though Endosulfan generally had high concentration of residues. In 2010, violation occurred only in half of the year, thus from January to May and in July but no violation in MRL occurred in the cocoa beans at all in June and from August to December (Fig. 5).

Fig. 5- Mean concentration of pesticide residue in cocoa beans shipped to Japan in 2010 in violation of MRL.

Imidacloprid recorded the highest concentration and high frequency with residue above the MRL in all the cocoa beans shipped every month. The frequency of residue above the MRL in cocoa beans was followed by Fenvalerate.

It was observed that the violation in MRL of Fenvalerate occurred frequently in cocoa beans obtained from Enchi, Dadieso, Wassa Akropong and Ashanti Bekwai Sefwi Wiawso Districts whereas that of Imidacloprid occurred frequently in cocoa beans obtained from Wassa Akropong, Sefwi Wiawso and Takoradi Districts. There was no trend in violations in MRL of other pesticides.

One major pitfall in the MRL was the frequency of violations in cocoa beans analyzed outside Ghana whilst only cocoa beans with low pesticide residue below the MRL were shipped. During the study, it was observed that the Japanese MHLW method adapted by the Ghana Standards Authority and Ghana Cocoa Board for the pesticide residue analysis was not exactly the same method that was used. The method used had gone through slight modification. The Japanese has kept on up-grading their methodology and also their instrumentation in terms of pesticide residue analysis. Currently GC-MS and HPLC recommended by the Japanese for the pesticide residue analyses are being used in Ghana. However Japan is not using GC-MS and HPLC for confirmatory pesticide residue analysis.

They are using GC-MS/MS and LC-MS/MS which have relatively lower detection limit for the confirmatory pesticide residue analysis performed in Japan. This implied that pesticide residues that were not detectable with the GC-MS and HPLC in Ghana were detected by the GC-MS/MS and LC-MS/MS in Japan. Thus rendering the MRL of pesticide residues in cocoa beans, which is an emerging international standard endless. This therefore explains why violations in the MRL of pesticide residues in cocoa beans occur frequently.

The rising cost of both instrumentation and chemicals is also a major factor to be looked at critically if MRL of pesticide residues in cocoa beans should be considered as international standard. All the various regulations notably, EU Council Directive 91/414/EEC, EU Regulation (EC) NO 396/2005, EU Commission Regulation (EC) No 149/2008, USA Food Quality Protection Act (FQPA), or H.R.1627, USA Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) and the USA Federal Food Drug, and Cosmetic Act (FFDCA) and the Japanese MHLW has an inherent mandatory force for pesticide residue to be conducted without recourse to cost implication.

CONCLUSIONS

To alleviate the bottomless in MRL of pesticide residues in cocoa beans with all the pitfalls, all methodologies must be harmonized. The methodologies developed in Japan, EU and USA must be harmonized into one standardized method. Such a method should be used for all confirmatory pesticide residue analysis on cocoa beans.

ACKNOWLEDGMENTS

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QUALITY PRESERVATION OF SOYBEANS STORED AT LOW-OXYGEN CONDITIONS

Yanyu Li [*], Yang Cao, Fuping Xu, Huiyi Zhao

Academy of State Administration of Grain, №11 Baiwanzhuang Street, Beijing, 100037, P.R. China, Tel: 86-10-68623707. Fax: 86-10-58523599
[*]Corresponding author's e-mail: *lyy@chinagrain.org*

ABSTRACT

A pilot-scale experiment for the evaluation of the effect of different low oxygen concentrations (2%, 4%, 6%, 8% and 21%) on the quality of stored soybean was carried out. The various oxygen concentrations were tested at temperatures of 20°C and 30°C. Monthly samples were taken for testing the quality parameters of soybeans stored at low oxygen conditions. The test results showed that: the low oxygen concentration in the system could be controlled within ±0.3% accuracy. The brightness of soybean powder did not change at different temperatures and oxygen concentrations; lightness remained between 67 and 69, hue -1.4 and -1.8, and chroma 15 and 17. The acid value of soybeans increased as the oxygen concentration increased and/or the storage time prolonged. Peroxide value of soybean increased with increase in oxygen concentration. The activity of the urea enzyme decreased; with temperature, oxygen concentration and storage time. The total protein, moisture and oil content remained constant, 38.57%, 8.50% and 20.87%, respectively. The results indicated that at 30°C, low oxygen could partly delay the change in soybean quality and at 20°C, the oxygen concentration should be sufficiently low to keep the soybean quality.

Key words: Argentina soybean, quality, storage, low oxygen

INTRODUCTION

Soybean is an important oil crop with a high nutritional value. To meet the increasing demand, soybean production has been increasing year by year in China. The different storage methods and storage time have obvious effect on the quality of soybean (Cao and Cui, 2005; Liu and Su, 2003; Lu, 1999). Because soybean is rich in protein (35%-40%) and fat (17%-22%), the storage stability is poor due to moisture absorption, mold development, increase in free fatty acids, and germination loss during storage. Compared with other crops, soybean storage needs more attention to prevent spontaneous heating for quality preservation (Cao and Cui, 2005). To meet the demand for high quality of food and without the use of chemicals, the storage technology of control atmosphere (CA) has reached the historic moment. CA storage technology is a green grain storage technology, which is an economic and effective method developed in P.R. China and abroad for commercial applications. There are several methods for generating modified atmospheres (MA), among them are; by the biological consumption of oxygen, oxygen absorption, catalytic converters that results in the alteration of the ratio of oxygen, nitrogen or carbon dioxide concentration in the grain bulk. Such MA leads to the

control of pests and inhibits mould activity for retaining food quality during storage (Li, 2006). The objective of this investigation was to determine the quality parameters of soybeans stored under nitrogen atmosphere.

MATERIALS AND METHODS

Materials
Soybean imported from Argentina.

Reagents
Hydrogen gas, purity ≥99.995%; Helium gas, purity ≥99.995%; Aspartic acid, purity ≥99%, Ethyl ether, ethanol, acetic acid, methanol, petroleum ether, chloroform, strong sulfuric acid, strong hydrochloric acid, potassium iodate, potassium iodide, potassium hydroxide, sodium hydroxide, sodium thiosulfate, potassium dihydrogen phosphate, dipotassium hydrogen phosphate, sodium chloride, sodium molybdate, sulfuric acid hydrazine, phenolphthalein, anthrone, perchloric acid, trichloride ferric. All above were analytical reagents, obtained from Beijing chemical works. Deionized water was chemical pure.

Equipments and apparatus
FOSS 2055 crude fat radiomete, FOSS 2300 automatic protein radiometer (Denmark FOSS TECATOR company); PL403-IC electronic balance (accuracy 0.001 g), ML204/02 electronic balance (accuracy 0.0001 g)(METTLER TOLEDO (Shanghai) instrument Co., LTD.); THZ-D desktop constant temperature oscillator (HUAMEI biochemical instrument factory, Taicang); RJ-TDX-50A centrifuge (RUN JIANG company, Jiangsu); LAMBDA35 ultraviolet-visible spectrophotometer (PerkinElmer instrument Co., LTD., USA); CR-400 brightness instrument (KONICA MINOLTA Co., Japan).

Experimental Methods
The oil content, crude protein, water-soluble protein, fatty acid value, peroxide value and moisture content were determined according to GB/T14488.1-2008, GB/T5511-2008, NY-T1205-2006, GB5510-85, GB/T 18868-2002 and GB/T 5497-1985, respectively. The color was analyzed by color meter.

Low oxygen atmosphere storage of soybeans
The volume of the gas tank was 200 L, equipped with the sampling port, air inlet and air outlet, and sensor connecting port for detecting oxygen concentration (a monitoring system with recorder), and temperature-humidity(Safestore[@] made in USA). The sensor could record the change of the oxygen, temperature and humidity in the system at real-time. Tank gas tightness was tested by half-life pressure decay time, in which pressure from 300 Pa to 150 Pa was longer than 5 min. .

Soybeans were stored at two temperatures, 20°C and 30°C, in climate controlled chambers for testing the bean qualities under MA.

The moisture content of the stored soybeans was 8.5% wet basis. The corresponding equilibrium relative humidity of the beans was 40% (20°C) and 50% (30°C). To ensure prevent moisture loss during storage, the relative humidity of the filled nitrogen was also kept in the range of the corresponding equilibrium relative humidity.

A machine produced nitrogen based on membrane-separation system (MSS) with maximum flow of nitrogen (N_2) 35 m^3/h and maximum N_2 concentration 99% (v/v%). According to the operation procedures, it takes 5 min to establish a constant concentration of

N_2 at the outlet. When the O_2 is stable, the gas could be used. For example for a target O_2 concentration of 2%: the N_2 concentration is adjusted to 98%; the top and bottom valves of the soybean tanks are kept open; then connect the vent hose of MSS to the top valve and at the same time observe the O_2 concentration on the recorder. When the concentration is stable within 2%±0.3%, remove the vent hose, and quickly close the top and bottom valves of the tanks. If there is no change for 10 min in gas concentration, we can assume that gas filling has been completed, otherwise, repeat the process, until the concentration of the gas in the tanks are stable.

The tested groups with different O_2 concentrations were of 4%, 6% and 8%.

RESULTS AND DISCUSSION

Chroma analyses of soybean stored at low-oxygen

Generally, color is considered as one of the parameters to determine quality changes of stored soybeans. In addition, the color of the oil affects the fat quality (Li, 2006). Figures 1 to 6 show the change in color of soybean at different temperature and oxygen storage conditions.

Figures 1 to 6 show that storage temperature, storage time and oxygen concentration had no significant effect on the chromaticity of soybeans. The lightness (L) was between 67.5 and 68.5, chromaticity (a) was -1 and -1.8, saturation (b) was 15 and 17.5.
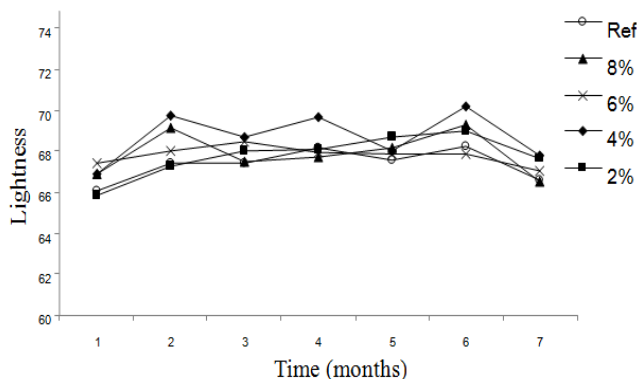

Fig. 1- The lightness (L) of the soybeans under different oxygen concentration conditions at 20°C.
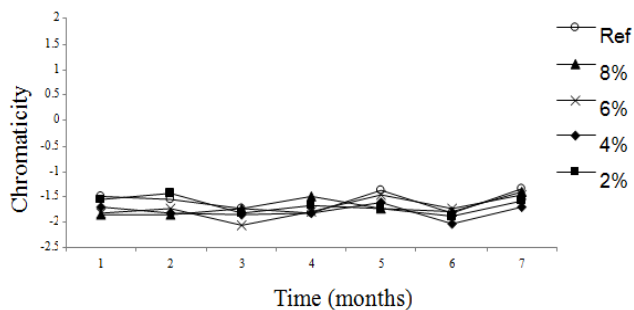

Fig. 2- The chromaticity (a) of the soybeans under different oxygen concentration conditions at 20°C.
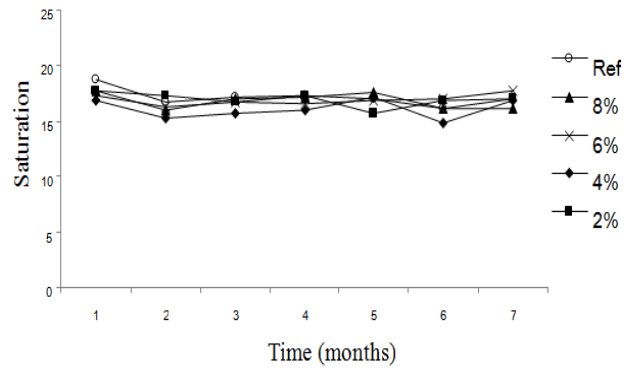
Fig. 3- The saturation (b) of the soybeans under different oxygen concentration conditions at 20°C.
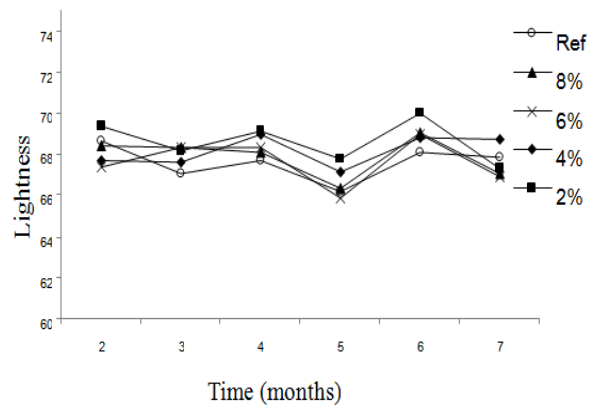

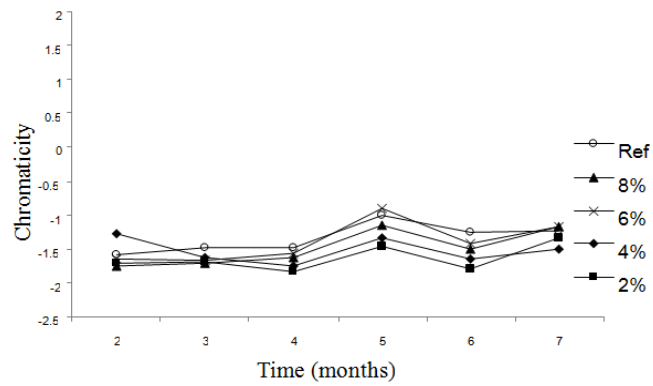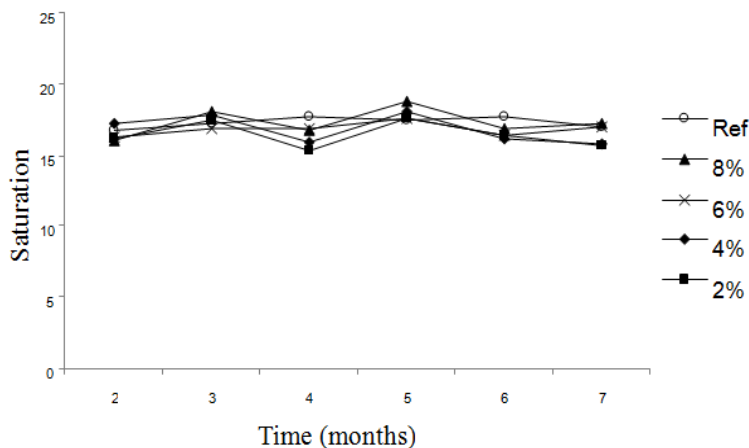Fig. 4- The lightness (L) of the soybeans under different oxygen concentration conditions at 30°C.

Fig. 5- The chromaticity (a) of the soybeans under different oxygen concentration conditions at 30°C.

Fig. 6- The saturation (b) of the soybeans under different oxygen concentration conditions at 30°C.

Acid value analyses of soybean stored at low-oxygen

Acid value is a very sensitive index to decide the soybean oil quality; it directly influences the soybean oil grade. The higher acid value indicates free fatty acid development caused by the activity of oil hydrolysis. Figures 7 and 8 show the effects of different temperatures and low-oxygen storage conditions at different storage times on the changes of acid values.

From results in Fig. 7, we can conclude that at 20°C and low-oxygen storage conditions, the acid value of soybeans have no significant changes in the first 4 months, but from the fifth month, the changes in the acid values are significant. Between 21% to 4% oxygen concentrations, the acid value increased as the oxygen level increased. However, the acid value at 2% oxygen concentration is higher than 4%. The result indicated that low oxygen was adverse for soybean storage and 4% low oxygen concentration is more favorable for preserving soybean acid value.
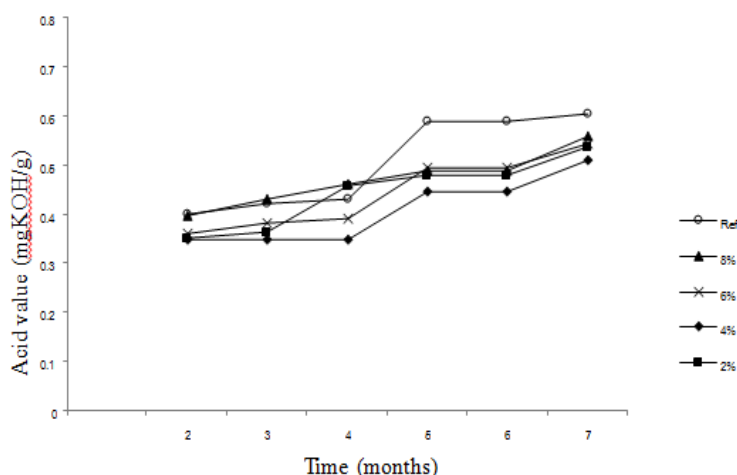

Fig. 7- The acid value of the soybeans under different oxygen concentration conditions at 20°C.

Results in Fig. 8 show that at 30°C, the trend in acid value change was in agreement with that at 20°C. At relatively high temperature conditions, low oxygen better prevented change in acid value. The acid value was in agreement with that at lower temperature (20°C) conditions.
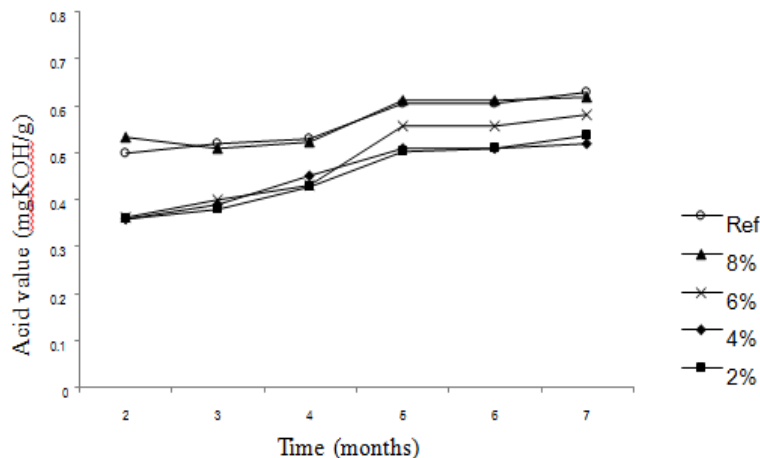

Fig. 8- The acid value of the soybeans under different oxygen concentration conditions at 30°C.

Storage at 4% oxygen appears appropriate for maintaining soybean quality, because on the one hand, the soybean acid value change could be delayed, on the other hand, in term of the efficiency of making nitrogen from the MS, 4% low oxygen concentration is relatively easy to achieve and the efficiency is also the highest. At high temperatures, reducing oxygen concentration appears an option to inhibit the change in the soybean acid value.

Peroxide value analyses of soybean stored at low-oxygen
Peroxide value is an index to measure how much unsaturated fatty acid was oxygenized and how much the double bond was oxidized. The small molecules, such as aldehydes, ketones and acids produced by hydrogen peroxide decomposition have strong pungent smell on the product. If the small molecules are further oxygenized, the produced level 2 dioxide is hard to be metabolized in the human body. Determining the degree of the fat oxidation is very important (Li, 2006). Figures 9 and 10 show the changes in peroxide value of soybeans at low-oxygen and storage time.

From Figures 9 and 10, we could see that the effect of storage time on the peroxide value is not significant. Low temperature and low oxygen environment helped reducing fat oxidation. The lower the oxygen concentration, the lower was the peroxide value. The results suggested that the low oxygen affected the soybean to be a stable environment; it restricted soybean respiration, slowed synthesis and metabolism of soybeans, and delayed the rise of peroxide value. To prevent oxidation of the soybean products and the soybean oil, quality preservation of the soybean in storage should be ensured. In addition to the drying after harvest, low temperature and low oxygen storage is needed to prevent fat oxidation.
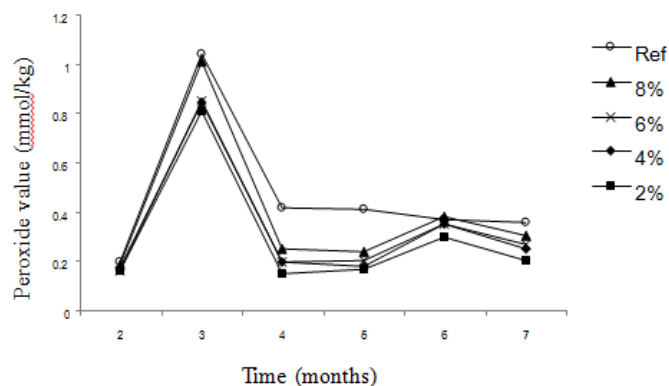
Fig. 9- The peroxide value of the soybeans under different oxygen concentration conditions at 20°C.
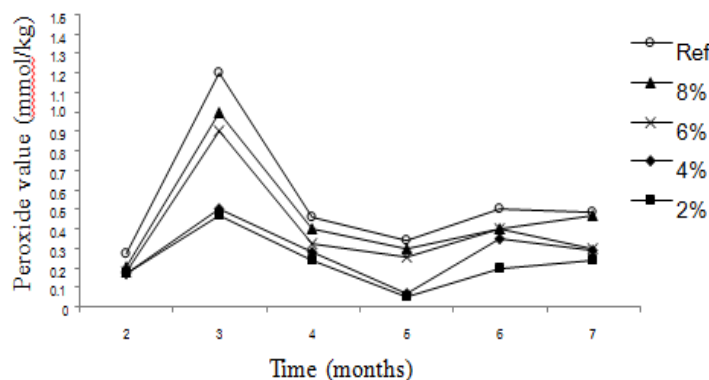

Fig. 10- The peroxide value of the soybeans under different oxygen concentration conditions at 30°C.

Urea enzyme activity of soybean stored at low-oxygen

The raw soybean not only is rich in protein, fat, carbohydrate and nutrition ingredients, but also contains a variety of anti-nutritional factors which could be damaged by heat. Anti-nutritional factor is a floorboard of the substances which was produced from plant metabolism and had an anti-nutrition effect on animals and in different mechanism, such as anti-trypsin factor, anti-vitamin factor and so on. Among them, the effect of anti-trypsin factor on animals is predominant. Anti-trypsin factor, which can disturb the function of the protein digestive enzymes in the small intestine, plays the role of protein decomposing enzyme in the inhibitory substance. These anti-nutritional factors not only affect the palatability, but also affect the nutrition value of animal feed, the digestion and absorption of the substances and some physical processes in the body, and accordingly present some threat to animal health. The enzyme called urea is one of the several natural enzymes contained in the soybean meal. Urea enzyme is not the anti-nutritional factor, but its content in the soybean meal is proportional to the content of anti-trypsin factor. Because of the activity of the urea enzyme is easy to determine, compared to anti-trypsin factor, the method of determining the activity of urea enzyme, Kauai, was usually adopted to evaluate the quality of the soybean. Under the

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low-oxygen storage conditions, the change of the urea enzyme activity of soybeans with the change of the storage time was shown in Figures 11 and 12.

These results indicated that with the extension of the storage time, the activity of the soybean urea enzyme has a trend of decreasing. There were no significant differences between different storage temperatures and low oxygen levels. Accordingly, low temperature and low oxygen were not enough to inhibit the activity of urea enzyme.
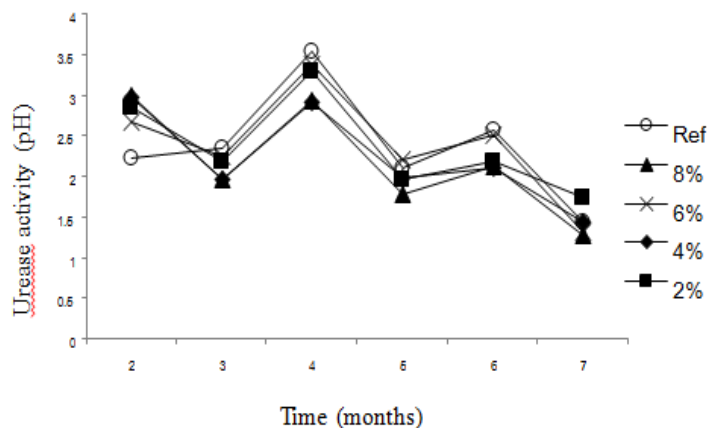

Fig. 11- The urease activity of the soybeans Urea enzyme under different oxygen concentration conditions at 20°C.
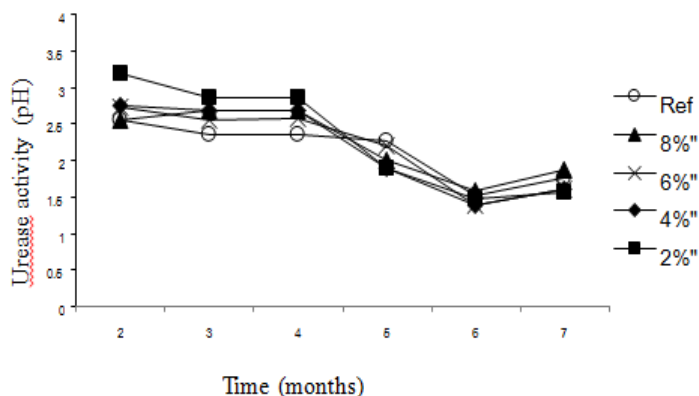

Fig. 12- The urease activity of the soybeans Urea enzyme under different oxygen concentration conditions at 30°C.

Total protein content analyses of soybean stored at low-oxygen
Under the low-oxygen storage conditions, the peroxidizing degree of soybeans with the change of the storage time was shown in Figures 13 and 14.

The results show that, at low-oxygen, the total protein content is maintained between 38.5% - 39.5%, and didn't change by storage time at different oxygen concentrations.
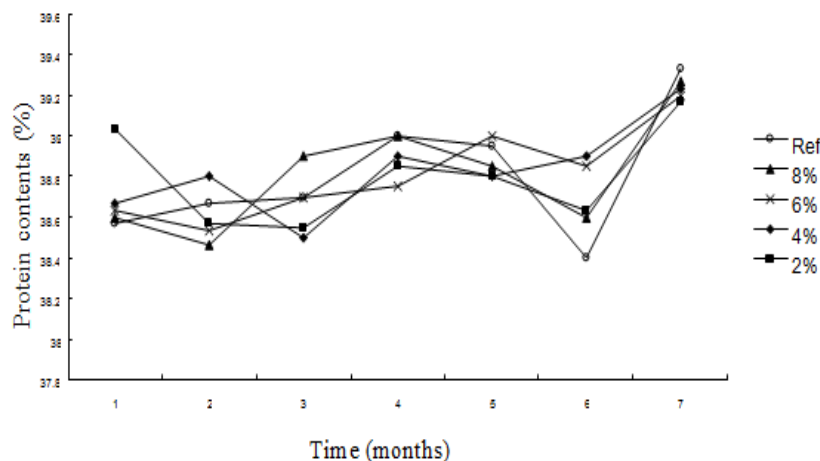
Fig. 13- The crude protein content of the soybeans under different low-oxygen concentration conditions at 20°C.
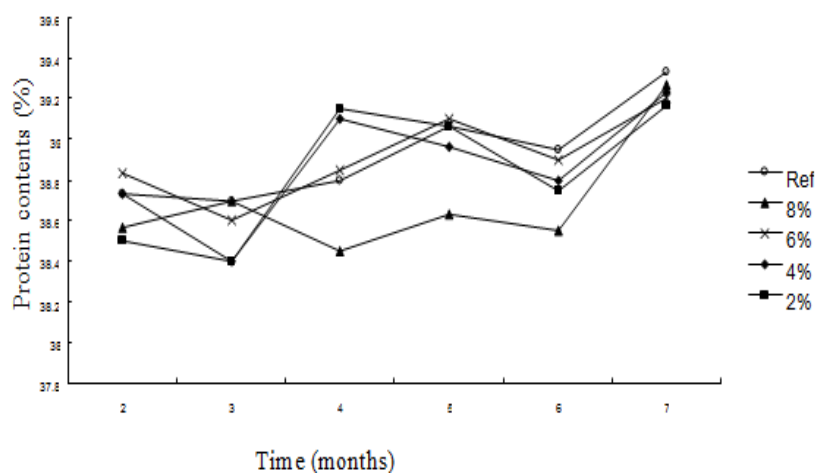

Fig. 14- The crude protein content of the soybeans under different low-oxygen concentration conditions at 30°C.

Oil content analyses of soybean stored at low-oxygen

One of the main usages of stored soybean is producing oils and fats (phospholipids). After a period of storage, the amount of oil extracted, is an important parameter to evaluate storage technology. Under the low-oxygen storage conditions, the change of oil content of soybean with the change of storage time was shown in Figures 15 and 16.

The results show that, the changes of the crude fat content with time are not significant and they remained between 20% and 21%. Therefore, the conditions of low-oxygen have no significant effect on the content of crude fat in the process of soybean storage.
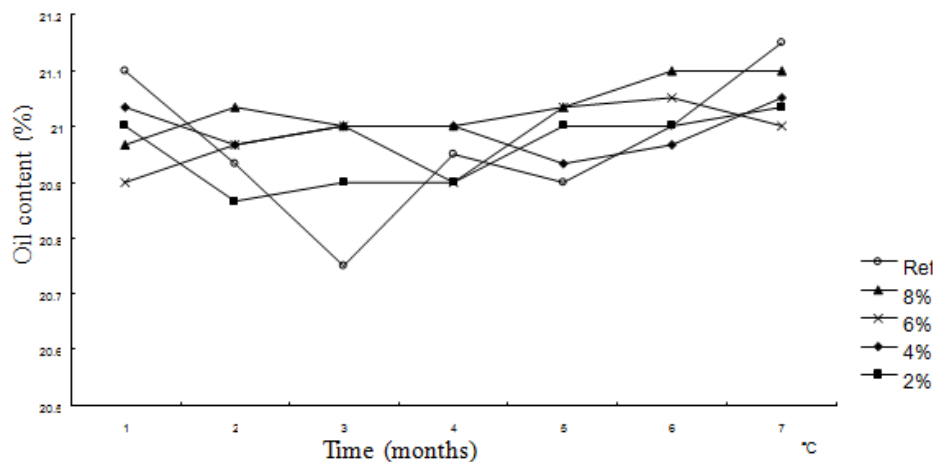
Fig. 15- The oil content of the soybeans under different low-oxygen concentration conditions at 20°C.

Fig. 16- The oil content of the soybeans under different low-oxygen concentration conditions at 30°C.

Moisture content of soybean stored at low-oxygen

Under the low-oxygen storage conditions, the changes in moisture content of soybean with storage time are shown in Figures 17 and 18.

The results show that storage time, did not significantly influenced the changes in moisture content. During the nitrogen purge process, controlling the relative humidity of the gas was sufficient to avoid water loss of soybean.

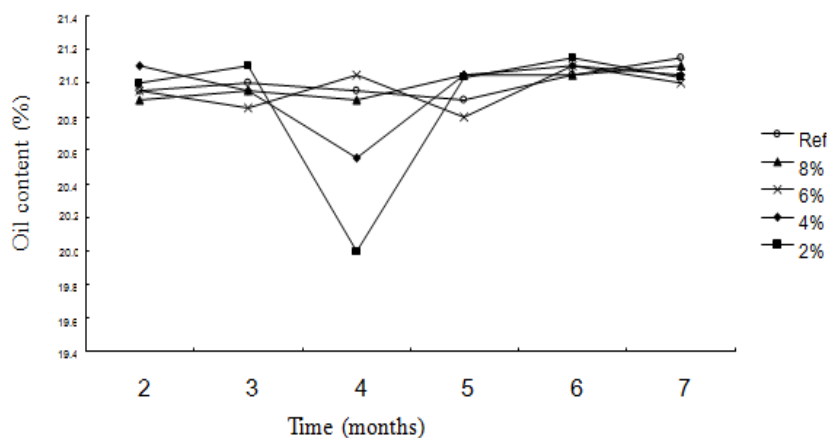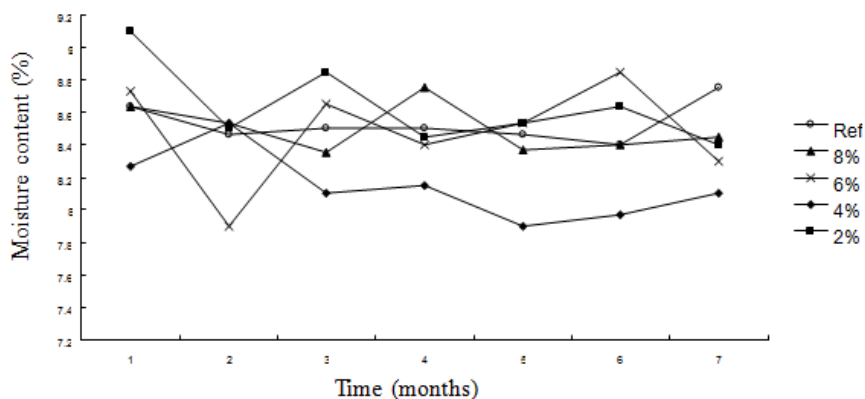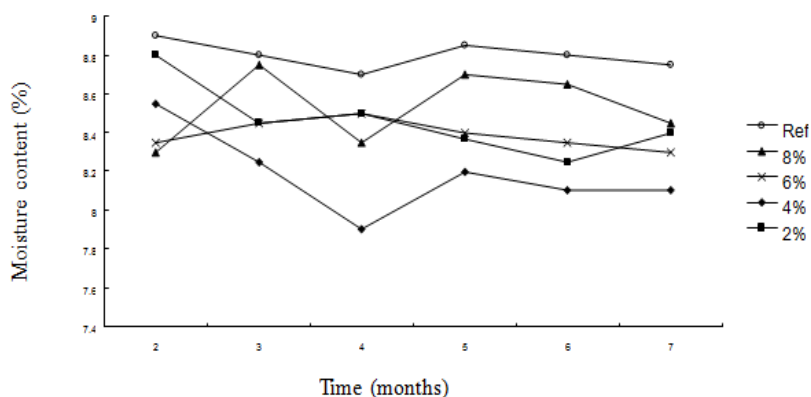Fig. 17- The moisture content of the soybeans under different low-oxygen concentration conditions at 20°C.

Fig. 18-The moisture content of the soybeans under different low-oxygen concentration conditions at 30°C.

CONCLUSIONS

Quality of soybean stored at different temperatures and low oxygen concentrations was compared with that stored under conventional storage condition. Results showed that the soybean chroma, oil content, total protein and moisture content did not change significantly as storage time prolonged. Acid value, peroxide value and the activity of the urea enzyme had a trend to be influenced as oxygen content increase; especially at high temperature conditions, low oxygen could play the important role on mitigating the negative changes in the soybean quality. Since the moisture of the tested soybean was low, the influence of low oxygen to ensure soybean quality was not significant. In the future, the effect of low oxygen technology on the quality of soybean stored at high moisture will be studied.

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QUALITY PRESERVATION OF STORED RICE USING MODIFIED ATMOSPHERES IN PORTUGAL

Maria Otilia Carvalho[1]*, Antonio Barbosa[1], Graça Barros[1], Ana Magro[1], Cornel Adler[2], Shlomo Navarro[3], Jordi Riudavets[4], Blaine Timlick[5]

[1]IPM on Stored Products/BIOTROP Centre, IICT, Tapada da Ajuda, Lisbon, Portugal [2] Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection, Berlin, Germany
[3] Food Technology International Consultancy, Bet Yehoshua, 40591, Israel
[4]IRTA, Ctra. Cabrils Km 2, Cabrils, Barcelona, Spain
[5] Canadian Grain Commission, Winnipeg; Canada
*Corresponding author's e-mail: *motiliac@iict.pt*

ABSTRACT

Portugal is the largest consumer of white rice in Europe, and consequently a large number of farmers and industries are associated with rice production, transportation and processing.

During five years, studies were conducted in six on-farm stores, and three rice industries in Portugal in order to identify the most important noxious agents (insects and fungi associated to stored rice) and implement IPM strategies, such as sanitation, tools for risk assessment, sampling programs, and non-chemical alternatives like ventilation/refrigeration and modified atmospheres as sustainable technologies to replace conventional chemical treatments.

The implementation of these strategies and the dissemination of the results, illustrates an advancement in stored rice protection in Portugal. Currently, many of the rice processing companies apply monitoring programs to assist in decision-making and in the use of strategies such as modified atmospheres (MA) on stored rice.

MA based CO_2 was tested to control *Sitophilus zeamais* and *S. oryzae* in bulk stored milled rice. The trials were conducted in a silo containing 40 tonnes of polished rice and four hermetic big bags of 1 tonne capacity; two with paddy and two with polished rice. The composition of the atmosphere was 90-95% CO_2 and 0.7-2.1% O_2. Three trials were carried out using different temperature and different treatment times: stored rice in the silo at 30ºC for 26 days (first trial) and at 34ºC for 10 days, (second trial) and in big bags at 22ºC during 26 days (third trial).

The exposure of eggs and adults of *Sitophilus* spp. to modified atmospheres showed mortality close to 100% and no F1 emergency was recorded after each treatment. This was the first time that a Portuguese rice mill used modified atmospheres.

This paper presents some of the most preeminent strategies and results on stored rice in Portugal.

Key words: stored rice, insect, fungi, IPM, traps, refrigeration, modified atmospheres

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for over half of the world's population and is grown on approximately 146 million hectares, more than 10 percent of the total available land. In the tropics, rice is the primary source of human nutrition, and is one of the cheapest sources of food energy and protein. Rice production is a seasonal crop in Europe. In Portugal, rice is grown on 18500 ha and the average per capita consumption is the highest in Europe, around 15 kg per year and person (Magro et al., 2006), and consequently around 2000 farmers and eight industries are associated with rice production, transportation and processing. Rice is stored as paddy in on-farm structures or in co-operatives in horizontal warehouses or vertical silos, until the end of winter when the remaining paddy is transported to processing facilities (Carvalho et al., 2004, 2010; Pires et al., 2008; Passarinho et al., 2008. In Europe, among the pest species of stored paddy *Sitophilus oryzae* (L.), *Sitophilus zeamais* Motschulsky and *Rhyzopertha dominica* (F.) are the main weevils present in rice (Trematerra 2009; Lucas and Riudavets, 2000; Pascual-Villalobos et al., 2006). In Portugal, the maize weevil *S. zeamais*, is the key-pest of stored rice, followed by *S. oryzae* (Barbosa et al., 2011). A common practice to control hidden infestation in stored paddy is the use of chemical fumigants to prevent insect development. The development of insect resistance to insecticides and consumer concern over the use of pesticides in food has resulted in the search for alternative methods of insect control. Consumers today expect a food product that is pesticide free or with much reduced residue levels (Carvalho et al., 2012). This is a general tendency that industry finds difficult to conform with because insecticides are often necessary to prevent economic damage. In addition, in many countries insects have been developing resistance to contact insecticides and to the fumigant phosphine. The most common non-chemical alternative identified in the rice storage and processing industry was using aeration to reduce the temperature of stored paddy rice (Barbosa et al., 2011; Barbosa et al., 2011). During these studies, the rice mills, for the first time, applied modified atmospheres as alternative control methods. The gas used (CO_2) is comparatively safe and environmentally friendly and showed to be effective against key pests on both paddy and polished rice (Carvalho et al., 2012). The implementation of these strategies was reflected in the significant decrease of the number of rejected units of polished rice from 111 tonnes of packaged polish rice before the project (2006) to 7 tonnes until the end of the project (January 2009) and continued decreasing to reach only 500 kg in June 2009, which may be interpreted as an increase in consumer satisfaction (Barbosa et al., 2011).

This paper pretends to be a compilation of the strategies and results along these studies.

MATERIAL AND METHODS

Monitoring of storage to perform risk assessment
a. Field studies
Experiments were carried out in rice fields in Tejo and Sado valleys, two main rice producer regions in Portugal. The objective of this study was to clarify the origin of stored rice pests. Pests were looked for in rice fields of Sado and Tejo valleys, during the maturation of rice until harvest (during August until the end of September - beginning of October). Different types of traps were used: Moericke, light, adhesive and cromotropic traps. Samples of rice panicles were taken and incubated at the laboratory (Mateus et al., 2008).

b. From on-farm storage to the rice mill

After harvest, paddy is cleaned and dried to 13-14% moisture. The majority of paddy is initially stored on farms and is periodically delivered to the mills over the course of the storage period (October to March). At the rice mills, paddy is stored prior to processing. Both, rice farms and the rice mills can have two types of storage, horizontal warehouses and silos, and have the equipment for cleaning, weighing, drying and aeration.

Insects: Studies were conducted in three rice fields, nine on-farm warehouses with stored paddy, and storage units and mills of three rice plants, in order to determine the insect species associated with stored rice and their abundance, distributed through two of the three main rice production regions: Mondego, Tejo and Sado valleys.

For rice in bulk, Storgard WB Probe II traps, without lures, were used and for structures, Storgard Dome traps containing standard attractant oil with or without pheromone (for *Tribolium* spp or for *Sitophilus* spp.) were placed on the floor below rice mill equipments and silos for grain drying. The traps were observed weekly and insects were counted and identified (Pires et al*.,* 2008; Passarinho et al., 2008). Visual inspection and samples of paddy, brown and polished rice were taken to collect psocids and fungi (Kucerova et al., 2006, 2007; Magro et al., 2006).

Fungi: One gram of rice was placed on Potato Dextrose Agar (PDA) medium. For each sample, three replicates were made. These grains were incubated during a week to available fungal growth. Isolation of the colonies was made to obtain pure cultures. The identification was carried out using identification keys (Carmichael et al., 1980; Domsch et al., 1980; Onions et al., 1981; International Mycological Institute, 1991;; Hanlin, 1997; Malloch, 1997; Pitt & Hocking, 1997; Barnett & Hunter, 1998).

c. Monitoring environmental conditions

Data loggers were used to measure the temperature and relative humidity of the cereal and of the environmental conditions of the rice plant. The grain moisture was measured mainly using two methods: by heating and by the Trime GW sensor device, that measures the values of grain temperature and moisture, in real time, using TDR technology. The rice temperature was monitored at several locations using temperature probes and data loggers placed on the surface and at several depths of rice in bulk in warehouses and silos.

d. The susceptibility of different varieties of rice produced in portugal to the attack of Sitophilus zeamais

The grain samples of 20gr, of four rice varieties (Gládio, Albatros, Thaibonnet and Eurosis) under three types of post-harvest treatment (polished, brown and paddy) were artificially infested with 20 *S. zeamais* adults and kept in laboratory conditions at 27° C and 70% relative humidity. Ten replications of each treatment were experimented including ten replications for control. At the end, the following resistance parameters were evaluated: Dobie index, mean developmental time, percentages of weight loss and damaged grains (Antunes, 2011).

e. Examining the use of modified atmospheres to control Sitophilus zeamais and S. oryzae on stored rice

The trials were conducted in a silo containing 40 tonnes of polished rice and in four hermetic big bags of 1 tonne capacity each: two with paddy and two with polished rice. Gas was supplied through a battery of food grade CO_2 contained in 13

cylinders of 30 kg eachThe composition of the atmosphere ranged 90-95% CO_2 and 0.7-2.1%O_2. Three trials were carried out using different temperature and different treatment times: stored rice in the silo with 29.6±0.1ºC during 26 days (first trial) and 34.1±0.2ºC during 10 days, (second trial) and in big bags at 22ºC during 26 days (third trial).

To evaluate the efficacy of the treatment, metal cages with 16 g of infested rice were placed at bottom, middle, top and surface of the polished rice in the silo. Four replications of infested brown and polished rice containing one-week-old of *S. zeamais* adults or eggs of *S. zeamais* were incubated at laboratory at the same temperature as in the silo, served as control (Carvalho et al., 2012).

RESULTS AND DISCUSSION

Monitoring of storage to perform risk assessment
 a. Field studies
Traps placed in the fields did not catch insects associated to storage and no insects emerged from the incubated panicles. Nevertheless, traps placed outside the stores, which were empty by that time, and next to the fields, caught *Sitophilus* sp. (Coleoptera, Curculionidae), *Cryptolestes* sp. (Coleoptera, Laemophloeidae) and *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), indicating that the storage environment may constitute a source of pests infestation. Measures must be taken in order to avoid rice infestation between harvest and storage, and to avoid insect entrance into the stores while rice is stored there (Mateus et al., 2008).

 b. From on-farm storage to the rice mill
The list of insect species collected from harvest until package is shown in Table 1.

In on-farm storage moisture content was the major factor that affects paddy storage. Given that the relative humidity determined from this study (during paddy storage) ranged from 75% to 85%, this may explain the presence of *Cryptophagidae* and *Mycetophagidae* spp as the main insect species that are fungus-feeders. Mycotoxin producing fungi were also detected. At the rice mills the main insects caught were commodity feeders and the key-pest in Portugal is *Sitophilus zeamais* Motschulsky followed by *S. oryzae* (L.) (Coleoptera, Curculionidae), *Tribolium castaneum* (Herbst) (Coleoptera, Tenebrionidae) and Cryptolestes *ferrugineus* (Stevens) (Arthur et al., 2007, Barbosa et al 2011, Carvalho et al., 2004, 2005; Magro et al., 2006; Pires et al., 2008).

At the rice mill, the main insects caught were commodity feeders, especially *Sitophilus* spp. (mainly *S. zeamais* followed by *S. oryzae*). Predators and parasitoids were recorded but most of them were occasional. During trials, when *Sitophilus* spp and *Cryptolestes ferrugineus* populations were high, their associated parasitoids, *Anisopteromalus calandrae* and *Cephalonomia waterstoni,* were also reported with some abundance. Fungus–feeding insects were also collected and identified: *Ahasverus advena*, *Coninomus* spp., *Cryptophagus* spp., *Typhae stercorea* and *Litargus balteatus* but at much lower populations when compared with commodity-feeders.

Psocids records from Portuguese rice stores and comparison with worldwide psocids occurrence in stored rice and other cereals are given. Five psocid species (Psocoptera, Liposcelididae) were recorded as new stored product species for Portugal found in stored rice. Three of them (*Liposcelis entomophila* (Enderlein)*, L. rufa* Broadhead*, L. tricolor* Badonnel) are new species for Portugal fauna as well (Kucerova et al., 2006, 2007).

Table 1. List of insect species of stored products caught in stored rice and rice mills

| FH | Species | On-farm storage | Rice plant | | | Facility |
| | | | Rice | | | |
		Paddy	Brown	Polished	
	Anobiidae					
C	*Stegobium paniceum* (L.)	+				
	Anthicidae					
C	*Anthicus floralis* (L.)	+		+		
C	*Anthicus quadriguttatus* Rossius	+		+		
	Bostrichidae					
C	*Rhyzopertha dominica* (F.)	+				+
	Carabidae					
P	*Harpalus rufipes* (Degeer)	+				
	Laemostenus complanatus (Dejean)	+				
	Cryptophagidae					
F	*Cryptophagus cellaris* (Scopoli)	+				
F	*Cryptophagus saginatus* Sturm	+				
F	*Cryptophagus perrisi* Brisson	+				
	Cucujidae					
C	*Cryptolestes turcicus* (Grouvelle)	+				
C	*C. ferrugineus* (Stephens)		+	+		+
	Curculionidae					
Ck	*Sitophilus oryzae* (L.)	+	+	+		+
Ck	*S. zeamais* Motschulsky.	+	+	+		+
	Lathridiidae					
F	*Coninomus constrictus* (Gyllenhal)	+				
F	*C. nodifer* (Westwood)	+		+		
F	*C. bifasciatus* (Reitter)	+				
	Mycetophagidae					
F	*Litargus balteatus* LeConte	+				
F	*Typhaea stercorea* (L.)	+		+		+
	Nitidulidae					
C	*Carpophilus dimidiatus* (F.)	+				
	Ptinidae					
S	*Ptinus raptor* Sturm	+				
	Silvanidae					
C,F	*Ashaverus advena* (Waltl)	+				
C	*Monotoma* sp.	+				
C	*Oryzaephilus surinamensis* (L.)	+		+		

FH	Species	On-farm storage	Paddy	Brown	Polished	Facility
	Staphylinidae					
P	*Aleochara sparsa* Heer			+		
P	*Leptacinus linearis* (Grav.)	+		+		+
	Tenebrionidae					
C,P	*Tribolium castaneum* (Herbst)	+		+		+
C,P	*T. confusum* Duval					+
C,P	*Gnathocerus cornutus* (F.)	+				+
F	*Alphitobius ovatus* (Herbst.)	+				
F	*Alphitobius piceus* Olivier	+				
	Pyralidae					
C	*Ephestia kuehniella* Zeller	+				
C	*Plodia interpunctella* (Hubner)	+				
	Gelechiidae					
C	*Sitotroga cerealella* (Olivier)	+				
	Pteromalidae					
Pc	*Lariophagus distinguendus* (Förster)	+				
Pc	*Anisopteromalus calandrae* (Howitz)			+		
	Braconidae					
Pl	Braconinae *sp.*	+				+
	Bethylidae					
Pc	*Cephalonomia waterstoni* Gahan	+		+		+
	Anthocoridae					
P	*Lyctocoris campestris* F.					+
P	*Xylocoris flavipes*(Reuter)	+				+
	Acaridae					
C	*Glycyphagus domesticus* (De Geer)	+				+
P	*Cheyletus* spp	+		+		+
F	Psocoptera	+	+	+	+	+

C=Commodity -feeder; Ck=Key-pest; F=fungus-feeder; P=predator; Pc=Coleoptera parasitoid ;
Pl=Lepidoptera parasitoid

Insect infestations are likely to occur relatively soon after conditions become appropriate. The insect pest infestations (especially *Sitophilus* spp.) create damage and consequently allow for more fungal production. Those managing rice should consider the damaged grain, from these two factors, seriously as it results in direct economic loss (Carvalho et al., 2004, 2005).

Several fungi were isolated, mainly *Aspergillus* sp., *Penicillium* sp., *Fusarium* sp., *Alternaria* sp. and *Trichothecium* sp. Some of them are known to be mycotoxin producers. This information about fungal mycoflora is essential to describe the status quo and to establish programs to prevent mycotoxin formation if necessary.

c. Risk assessment

The insects' populations fluctuations followed the temperature oscillations inside the rice plant while the stored rice can be at risk all over the year because temperatures are always above the lower developmental threshold of some 13°C-18°C depending species (Barbosa et al., 2011; Fields, 1992). Hidden infestation of *S. zeamais* can surpass all the process until package.

Studies on the susceptibility of different varieties of rice produced in Portugal showed that grain temperature of stored rice was different among varieties and among paddy and brown rice which may confer different susceptibility to grain weevil attack (Pires et al., 2008). Laboratory experiments demonstrated that the physical characteristics were not directly related with the number of emerged insects by female and with the biological cycle. The chemical composition of the rice appears to influence rice susceptibility to *S. zeamais* attack. Brown rice varieties showed the highest susceptibility, followed by polished and finally paddy. The polished rice varieties more resistant were Eurosis and Gladio and the more susceptible was Thaibonnet (Antunes, 2011).

d. Examining the use of modified atmospheres to control Sitophilus zeamais and S. oryzae on stored rice

Figures 1 and 2 show that the exposure of eggs and adults of *Sitophilus* spp. to modified atmospheres showed mortality close to 100% and no F_1 emergency was recorded after each treatment.
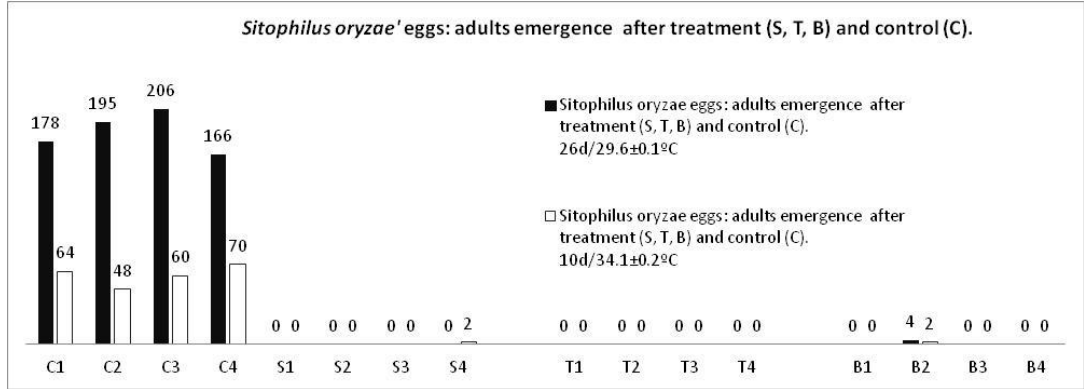

Fig. 1- *Sitophilus oryzae* eggs: adults emergence after treatment with CO_2 (S, T, B) and control (C) during 26 days with the rice temperature of 29.6±0.1°C and during 9 days under 34.1±0.2°C. (S= surface, T= Top, B= Button)

The treatment suppressed eggs, early larvae and adults of *Sitophilus* spp. The exposure time to the gas depended on grain temperature. Increasing rice temperature could lead to

decrease in exposure time to the gas and could thus suppress development of insect pests (Barbosa et al., 2011, Carvalho et al., 2012).
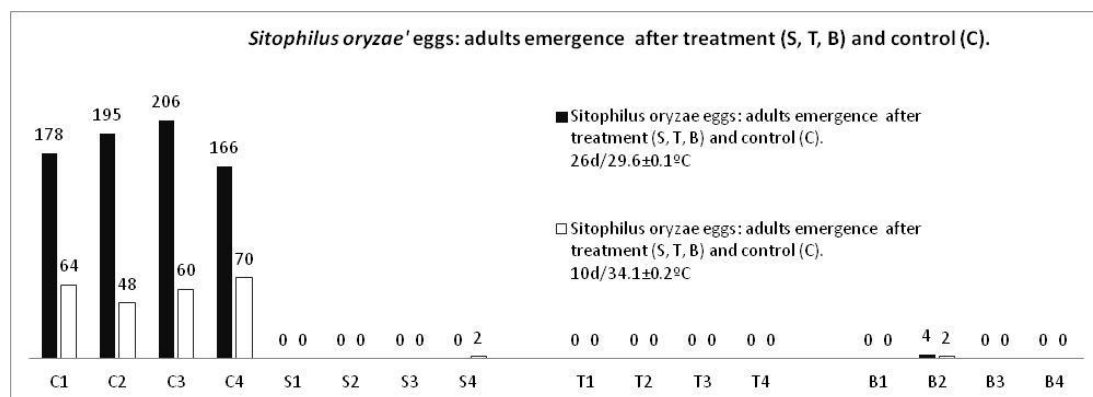

Fig. 2- *Sitophilus zeamais:* adult mortality after treatment with CO_2(S, T, B) and control (C) during 26 days with the rice temperature of 29.6±0.1ºC and during 9 days under 34.1±0.2ºC. (S= surface, T= Top, B= Button)

CONCLUSIONS

The implementation of monitoring programs to identify the noxious agents sounds fundamental for risk assessment and to help to decision-making. Together with the application of alternative control methods more environmental friendly and innocuous to consumer to protect the stored rice, applied by the rice mills in Portugal was reflected in the increase rice quality and the significant decrease of the number of rejected package units of polished rice, which may be interpreted as an increase in consumer satisfaction (Carvalho et al., 2011, 2012). These strategies can be classified as social, economical, and environmentally sustainable.

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EFFECT OF POST-HARVEST CARBON DIOXIDE APPLICATION ON STORAGE PESTS AND FRUIT QUALITY OF DRIED FIGS

Uygun Aksoy[1*], Fatih Sen[1] and K. Betul Meyvaci[1]

[1]Ege University, Faculty of Agriculture, Department of Horticulture, 35100 Izmir, Turkey
*Corresponding author's e-mail: uygun.aksoy@gmail.com

ABSTRACT

In Turkey, methyl bromide (MB), a fumigant used widely in controlling storage pests of dried figs was banned by January 1, 2007 leading to continuous search for alternatives especially for the dried fruit sector. The present experiments were designed to test the effects of carbon dioxide (CO_2) at atmospheric pressure (94 % CO_2 for 7 days in gas-tight cubes) and under high pressure (2, 3 and 5 h at 20 bars and 98% CO_2) on major storage pests (*Ephestia cautella* and *Carpoglyphus lactis*) of dried figs. CO_2 concentrations, temperature (°C) and relative humidity (%) values were monitored during treatments. Comparisons were made with control fruit treated with MB (60 g.m^{-3} for 24 h). Dried fig quality was assessed after the treatments and after 2.5 months of storage at ambient conditions. All tested CO_2 treatments controlled both of the tested pest species and had no negative effect on dried fig quality. Among tested treatments, high pressure application of CO_2 required high investment cost however was effective even at very short exposure periods as 2 h. CO_2 application in gas-tight cubes was recommended as a low-cost alternative despite its longer exposure requirement. CO_2 fumigation is allowed in organic certification systems and thus can be recommended for organic dried figs and other fruit and nuts that have a high demand in the organic market.

Key words: *Ficus carica*, controlled atmosphere, *Ephestia cautella*, *Carpoglyphus lactis*, fruit quality

INTRODUCTION

For many decades, Turkey has been the major dried fig producer and trader known also for its supreme dried fruit quality. However, pests may create significant problems and damage fruits if not controlled before and during storage. Major storage pests of dried figs are reported as *Ephestia cautella* Walk., *Plodia interpunctella* Hbn. (Lepidoptera: Pyralidae)*, Oryzaephilus surinamensis* L. (Coleoptera: Silvanidae)*,* and *Carpophilus hemipterus* L. (Coleoptera: Nitidulidae) in decreasing order of importance for Turkey and Greece (Turanlı, 2003; Eliopoulos and Athanassiou, 2004) whereas *Carpophilus* spp. is the main pest in California (Smilanick, 1979). *Carpoglyphus lactis* (L.) (Acari: Carpoglyphidae) may also create significant problems in stored dried figs. The pest damage occurs by feeding and by contaminating through droppings, webbing and other residues. There are tolerance limits foreseen in the dried figs market standards for each quality class (Anonymous, 2006).

Methyl bromide was the unique fumigant for disinfestation of storage pests with its wide spectrum of activity and relatively low-cost (Fields and White, 2002). However, it was banned in developed countries since 2005 and since 2007 in Turkey under the directive of the

Montreal Protocol on Substances that Deplete Ozone Layer (Schneider et al., 2003) stimulating the research work on seeking environmentally sound, user-friendly, effective and economic alternatives to methyl bromide. There are many research works performed to test fumigants, non-chemical, and chemical prevention methods against various pests in different commodities (Fields and White, 2002; Schneider et al., 2003; Aksoy et al., 2004).

Although controlled atmospheres (CA) have been considered as alternative to MB fumigation, at normal ambient temperatures, they have the limitation of long exposure times required for complete mortality (Navarro and Jay, 1987). However, the required exposure period may be comparable to phosphine fumigation (Navarro and Donahaye, 1990). The efficiency of CO_2 depends on the gas concentration, exposure period, temperature, moisture content of the product, pest species and life stage (Jay, 1984). Applying modified atmosphere under high pressure (20-25 bars) conditions are shown to shorten the required exposure period however its use is limited to high-value crops because of high investment in equipment and operation costs (Adler et al., 2000).

This study was performed as a part of the project that seeks MB alternatives suitable for the dried figs sector by testing application of CO_2 at atmospheric or high pressure conditions for the disinfestation of *Ephestia cautella* and *Carpoglyphus lactis,* and determination of their effects on dried figs quality.

MATERIALS AND METHODS

The CO_2 treatments at atmospheric or high pressure conditions were carried out on sun-dried fruit of Sarılop (= Calimyrna) fig variety. Each variable had a control group of MB treated (60 g.m^{-3} for 24 h) fruit to compare impact of treatments on dried fruit quality. The atmospheric CO_2 was maintained for 7 days in a flexible gas-tight PVC storage unit (Volcani Cube™ or GrainPro Cocoon®) with a volume of 36 m^3 (capacity of 15 tonnes of dried figs). CO_2 and O_2 concentrations were daily monitored using a thermal conductivity detector (CO_2 analyzer Model 20-600, Gow-Mac Inst, USA), and an electrochemical detector (O_2 analyzer Model OxyCheck 2, David Bishop Ins., UK) at three different levels ($0.8 \pm 0.4\%$ O_2, $94 \pm 3\%$ CO_2) of the PVC storage unit. Carbon dioxide treatment under high pressure was performed at commercial scale in two pressurized tanks (BuseGastek Company, Badhöningen, Germany), each of 20 m^3 capacity, by using exposures of 2, 3 and 5 h at 25 bar pressure.

The fruits treated with CO_2 or MB in 25 kg boxes (37 x 53x 31 cm) were further stored at ambient conditions for 2.5 months. Six boxes were randomly taken from each treated lot and 5 kg of composite samples were used per replicate. Water activity, surface color, moisture content, total soluble solids and sugaring were analyzed to assess fruit quality. Quality parameters were checked in 4 replicates immediately after treatment and at the end of storage.

Test organisms comprised different life stages (0-24, 24-48, 48-72 h old eggs, larvae, pupae and adults) of *E. cautella* and mixed stages of *C. lactis*. Test species were placed, prior to sealing, at various locations of the cube in 100 mL perforated plastic containers possessing food. After each treatment, test insects were further transferred to the laboratory and kept at 25°C and 65% r.h. Mortality of the active stages was determined 14 days after the end of trials. Eggs and pupae mortality were determined as a failure of hatch 10 days after the end of each exposure period.

Water activity was measured by a water activity meter (TH 500, Novosina, Pfaeffikon, Switzerland) at 25°C. Moisture content was based on nominal moisture content and calculated as the percentage of water lost after 24 h of drying in a vacuum oven (VD23, Binder, Germany) at 70°C. Total Soluble Solids were quantified using a refractometer (ATC-1, Atago,

Japan) and expressed as %. Sugaring was assessed on a 1–5 scale, each class describing the fruit surface area covered by white sugar crystals (Aksoy et al., 2004).

The experiments were conducted as completely randomized design with four replicates. Significant differences among groups were determined using Duncan's multiple range tests at $P \leq 0.05$. All computation and statistical analyses were done using SPSS (SPSS, Inc., Chicago, IL, USA) package version 19.0.
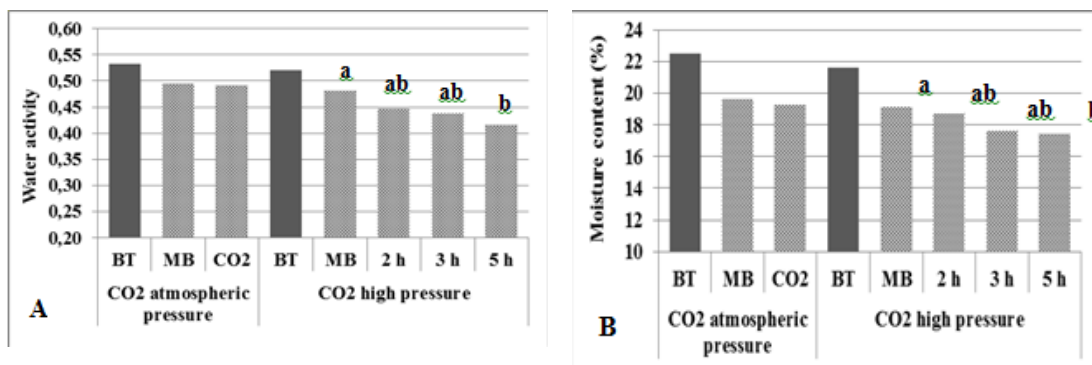

Fig. 1- Effect of 94% CO_2 at atmospheric pressure for 7 days, at 25 bar pressure for 2, 3 ad 5 h, and MB treatment on water activity (A) and moisture content (B) values after 2.5 months of storage. BT=before treatment; Significant differences of exposure to CO_2 at high pressure at p< 0.05 level were indicated by different letters at top of histograms.

RESULTS AND DISCUSSION

All tested variables of CO_2 and MB provided complete mortality of all stages of *E. cautella* and *C. lactis* including eggs and pupae. At high CO_2 conditions high insect mortality could be obtained in comparatively short exposure times (Navarro and Donahaye 1990).

Water activity and moisture contents of dried figs were affected only at CO_2 application under high pressure (Fig. 2A, B). Exposure to 5 h was significantly different than 2 and 3 h which were grouped together with MB treated figs. The water activity (a_w) of dried fig was relatively low thus most chemical and biochemical reactions, as well as microbiological growth, were inhibited at low a_w (Rahman and Labuza, 1999).

CO_2 applied at atmospheric pressure exerted no negative effects on color of dried figs after 2.5 months of storage and were statistically similar to the MB treated fruits. On the other hand CO_2 application for exposures varying between 2 to 5 h under high pressure lowered L*, b* and C* values significantly. Exposure to CO_2 for 5 h under high pressure resulted higher reduction in b* and C* values (Fig. 2).
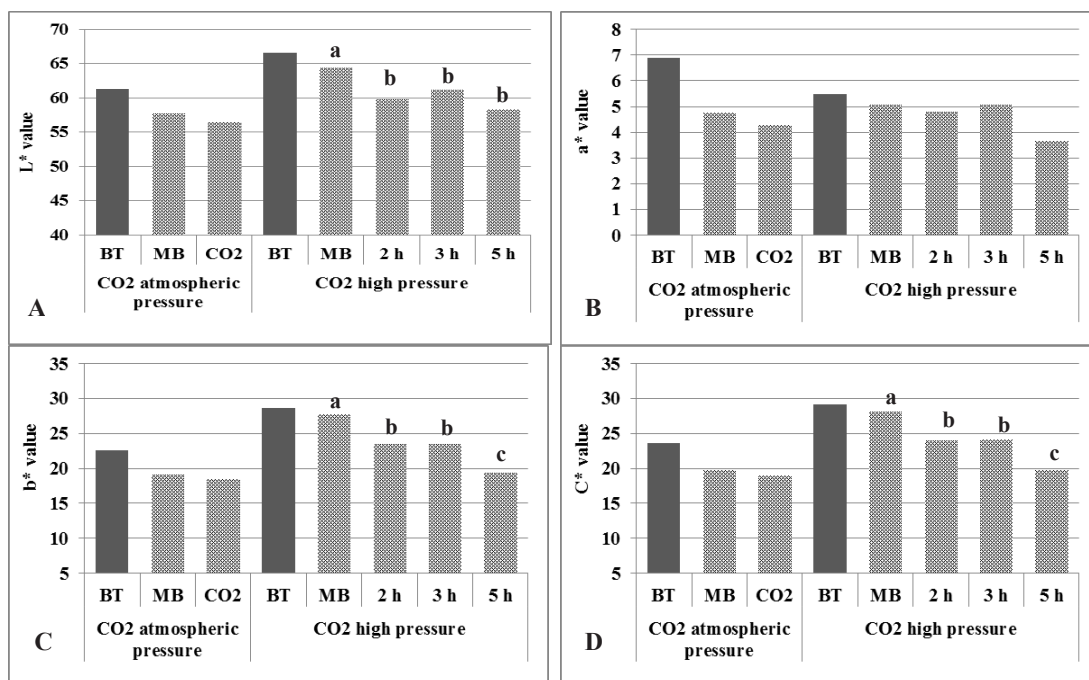
Fig. 2- Effect of 94% CO_2 at atmospheric pressure for 7 days, at 25 bar pressure for 2, 3 ad 5 h, and MB treatment on fruit colour L* (A), a* (B), b* (C) and C* (D) values after 2.5 months of storage. BT=before treatment; Significant differences of exposure to CO_2 at high pressure at p< 0.05 level are indicated by different letters at top of histograms.

The reduction in colour values show slightly darker and dull colour of dried figs treated with CO_2 at high pressure. This effect was found significant in quantitative analysis but not in visual evaluation. CO_2 had no significant effect on a* and h^o values. Color is accepted as an important quality attribute for Turkish figs which are known for their light colour. Darkening of the fruit colour and sugaring are the major quality attributes affected by the storage conditions. According to Meyvacı et al. (2003), fig fruits darken even if stored in packages containing CO_2 (as passive modified atmosphere packaging) at atmospheric pressure and ambient temperature for 7.5 months.

The tested variables did not have statistically significant effect on TSS content (Fig. 3 A). According to Fennema, (1976), dried figs lose their quality in storage and the rate of deterioration is dependent on the duration of storage and the prevailing temperature and relative humidity conditions. Atmospheric CO_2 application did not have any effect on sugaring (Fig 3 B). At 25 bar pressure sugaring was enhanced with increased exposure period (Fig. 3B). During 2.5 month storage, sugaring, a natural process under ambient conditions occurred on fruits exposed to all treatments. Incidence and severity of sugaring increase with storage temperature and time (Mitcham et al., 2012). Although sugaring has no risk in respect to food safety, in some cases consumers may consider it as a serious defect.
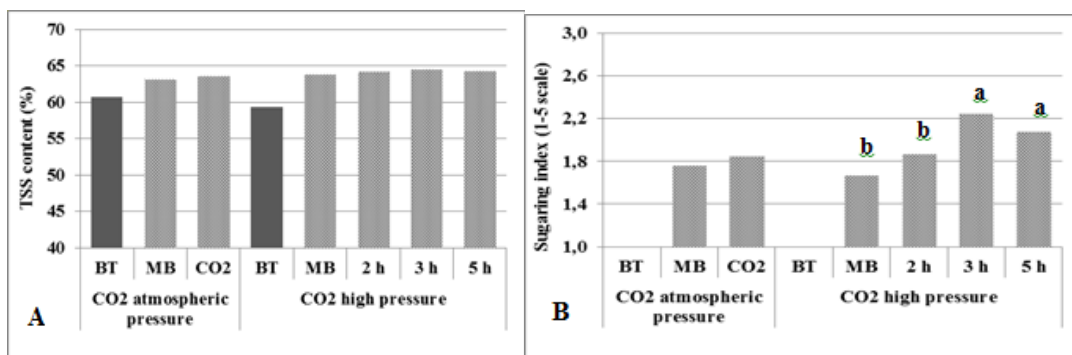
Fig. 3- Effect of 94% CO_2 at atmospheric pressure for 7 days, at 25 bar pressure for 2, 3 ad 5 h, and MB treatment on TSS content (A) and sugaring index (B) values after 2.5 months of storage. BT=before treatment; Significant differences of exposure to CO_2 at high pressure at $p< 0.05$ level are indicated by different letters at top of histograms.

Evaluation of the results obtained in the present research reveals that CO_2 at atmospheric pressure can be utilized to maintain dried figs quality in storage. In addition, it serves as short term fumigation of dried figs. The gas-tight Volcani Cube™ can be suitable during early season when the storage capacity can be a limiting factor in the processing plants of the Turkish dry figs sector. Since most of the crop is processed and exported until Christmas, a good management plan is required to supply the processing lines with good quality of figs.

High pressure CO_2 applications are expensive as alternatives to MB for dried fruits disinfestation but can be accepted as a viable alternative when short exposures are crucial. CO_2 may have an additional advantage in case of organic production since it is allowed in Turkish and other national regulations governing organic production.

ACKNOWLEDGMENT

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LABORATORY FUMIGATION OF WHEAT FLOUR WITH SULFURYL FLUORIDE – PENETRATION AND FLUORIDE RESIDUES

Gabriele Flingelli* and Dagmar W. Klementz

Julius Kühn-Institut, Federal Research Centre for Cultivated Plants,
Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection,
Königin-Luise-Straße 19, D 14195 Berlin, Germany

*Corresponding author's e-mail: *Gabriele.Flingelli@jki.bund.de*

ABSTRACT

Research on sulfuryl fluoride (SF) in the recent past has been focused on its potential in applications, which were covered by methyl bromide formerly. The fumigation of flour mills is one of the areas of SF use. A lot of publications are focusing the efficacy of SF against stored product pests. However, there are only few data on its behaviour on flour. The present work wants to add some more information about the property of SF to penetrate flour and, in consequence of the SF break down, the fluoride residues. Two different experimental set ups were chosen. In a larger scale experiment different amounts of wheat flour, densely packed in a glass column were exposed to SF. The glass column was connected to a SF reservoir of 2.5 m^3. Heights of 110 cm, 30 cm and 15 cm of flour were investigated. In the headspace and on the bottom of the flour the SF concentration was recorded during an exposure of 50 h. In a second experimental set up the SF was pumped through flour in a circuit array, changes in the SF concentration were observed. For both, an initial concentration of 50 gm^{-3} was chosen. The concentrations were determined with FTIR-spectroscopy methods. After aeration, the fluoride content of the flour samples was determined using a fluoride-selective electrode. The time – concentration curves during the fumigation of flour indicate a decline in the initial concentrations. In case of the first set up, the concentration on the bottom of the flour column increased in a time period, depending on the depth of the flour. It leveled off within a certain time but remained less than the head space concentration on the top. The fluoride contents of the fumigated samples ranged from 7 mg kg^{-1} to 31 mg kg^{-1}. The results are discussed.

Key words: fumigation, sulfuryl fluoride, fluoride residue, flour, fluoride-selective electrode.

INTRODUCTION

Sulfuryl fluoride (SF) is used worldwide and is in Germany in stored product protection currently registered for the control of insect pests. Along to the phase out of methyl bromide (MeBr), which includes the ban of MeBr in Germany since 2005, the pest control companies as well as the millers have adapted to alternatives like heat treatment or SF fumigation. Moreover some millers decided to invest in fixed installed fumigation-systems (Böye and Mück, 2011). Apart from tree nuts as commodity only, the use of SF relates to empty rooms,

before storage of products. All other stored products are excluded from treatment. The authorization includes empty silo bins, rooms, stores and storehouses as well as empty mills. Conditions are 128 g m^{-3} maximum concentration and 1500 g h m^{-3} maximum dosage with no more than three applications a year and 4500 g h m^{-3} a year in total. As a further application SF is registered for mills with the reduction to only one treatment per year and with 128 g m^{-3} maximum concentration and 1500 g h m^{-3} maximum dosage. This meets the situation of mills, which have to disrupt the running process for the time of the SF fumigation. Restrictions take care, that no food or feed comes into contact with the fumigant: grain, stored in the mill has to be enclosed gastight before the treatment. Silos and tubing systems have to be emptied before the treatment. It has to be avoided, that all grain - exposed to the fumigant because of technical reasons - comes into the further processing or delivery-chain as food or feed. Finally, the presence of grain or milling products during the treatment has to be excluded.

Mills consist of many process units like grain cleaning, grain preparation, roller mills, sifter and purifier, flour collection, separation of flour (different types) and other milling products, packaging and storage. There are several transporting-tubes in between. It takes time and causes production loss to empty all these units but it is essential. It might concern, if flour or other milling products have been forgotten and remained unintended in the mill during fumigation.

There are only few studies, which describe the penetration of SF into flour (Bell et al., 2003), the residues of SF itself (Sriranjini and Rajendran, 2008) and of fluoride (Guogan et al., 1999, Scheffrahn et al., 1989). The penetration study of Bell et al. (2003) aimed at a sufficient gas concentration in various depths of flour, because of the insect pests, which may be hidden in the flour. However, there was evidence, that SF penetrates thick flour layers in a short time.

MATERIALS AND METHODS

All trials[1] were carried out on wheat flour type 405 ("Diamant"; the type number corresponds to the ash content: 405 g per 100 kg) using SF by Dow Agroscience (Vikane^{TM}, 99.8%). There were two different fumigation set ups. In the larger scale experiment, a glass column of 130 cm height and 20 cm diameter with closed bottom was used. There was a perforated plastic tube fitted across the whole diameter in the inner of the glass column near to the bottom. The ends of the perforated tube were connected in a closed set up. The gas in the inner of the perforated tube was continuously pumped through. The flow was recorded using a flow controller. The SF concentration was measured using an infrared spectrometer (Gasmet Cr-1000, Temet Instruments, Finland). In order to prevent flour getting into the spectrometer, a filter was used in the gas line from the glass column to the spectrometer. A dessicator top fixed on the top of the glass column was sealed gastight with vacuum grease. The headspace of the glass column was connected to a 2.5 m^3 fumigation chamber in a closed set up. This was carried out by means of a gas wash-bottle head and teflon gas tubes. The gas was pumped through in this second loop continuously. The fumigation chamber was filled with SF to a concentration of 50 g m^{-3} directly from the gas cylinder. There was a second infrared spectrometer (Gasmet Cx-1000, Gasmet Technologies, Finland) between the glass cylinder

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[1] Sulfuryl fluoride is a toxic, colourless and odourless gas, the handling requires extreme caution. The TLV is 10 mg m^{-3}. The described experiments were conducted in the Berlin fumigation laboratory with safety equipment and by certified employees.

and the chamber for determining the concentration in the chamber and in the headspace of the glass column.

The cylinder was filled with the flour in different heights. To make sure that there were no empty holes or cavities, the cylinder was put on a laboratory shaker, originally for sieving (Fritsch analysette 3) and fixed during the shaking procedure. It has been shaken until no drop in the height of the flour was noticed. Three different heights were investigated, 110 cm, 30 cm and 15 cm. The corresponding amounts of flour were 26 kg, 15 kg and 7 kg, respectively. During fumigation the gas from the chamber was pumped continuously over the headspace of the flour. The gas concentrations on the top and on the bottom of the flour were observed simultaneously for 50 h at room temperature. Spectra were recorded in intervals of 20 – 60 seconds and the concentration was calculated with the software Calcmet vers.11 (Ansyco GmbH, Germany).

In the second, smaller experiment, a plexiglas tube of 5 cm diameter and 30 cm length was filled with flour. The tube was connected at both ends to teflon tubes in a circuit array. To prevent flour pouring in the tubes, a piece of filter paper in the plexiglas tube and an additional filter were used. A fitted 2.5 l bottle with ground glass stopper and septum served for the injection of the SF. The gas was pumped through the flour in the upright positioned tube. A concentration of 50 g m^{-3} was chosen. The change in concentration in the gas stream during 50 h was observed by means of FTIR spectrometry, as described above.

After the fumigation and aeration the content of the glass column, or the plexiglas tube respectively, was removed and mixed. Per condition the samples were taken out of the mixed flour. The flour-samples were analyzed for residual fluoride by a potentiometric method with a combined ion-selective electrode (Mettler Toledo, perfectION™ comb F$^-$) according to an official method (BVL, 2012).

RESULTS

During the fumigation of different layers and consequently different amounts of flour in the glass column, an initial concentration of 50 g m^{-3} SF was provided in the headspace. As shown in Figure 1, in all cases a decrease of the headspace concentration of about 10% in 48 h took place. The SF concentration at the bottom of the flour layers (30 cm and 110 cm) increased and got to a constant level depending on the thickness. The SF concentration at 15 cm was not recorded. In comparison to the 30 cm thick layer, the SF concentration increased at the bottom of the 110 cm layer slowly and got near to a level of about 8% of the headspace concentration in about 15 h. The concentration at the bottom of the 30 cm layer increased faster and showed a constant level of 36% after about 5 h. In both cases, the concentration remained at a level lower than the headspace concentration during the observed exposure time.

The trials with flour in the plexiglas tube in the circuit array were accompanied with some difficulties in keeping up the system without fluctuations in pressure. Reliable results were achieved using about 100 g flour in the tube in upright position with a gas flow from the bottom to the top. As presented in Figure 2, the initial gas concentration decreased continuously. Only 24% of the SF has been found after 50 h. However, there was a slight concentration drop in the empty system (without flour).
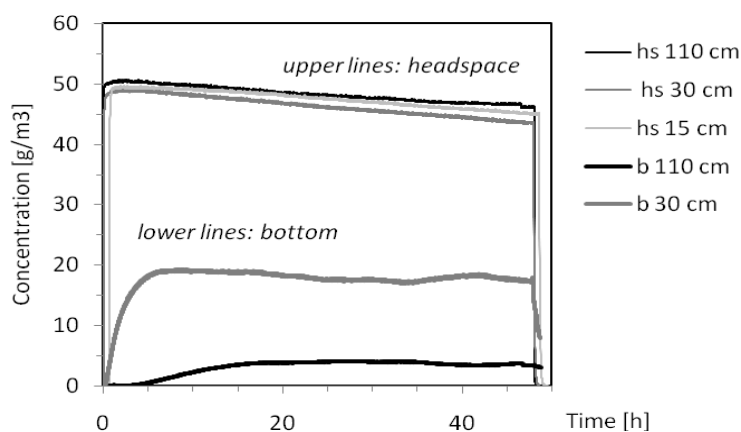
Fig. 1- Sulfuryl fluoride concentration by time depending on the height of flour in the glass column. Fumigation at room temperature.
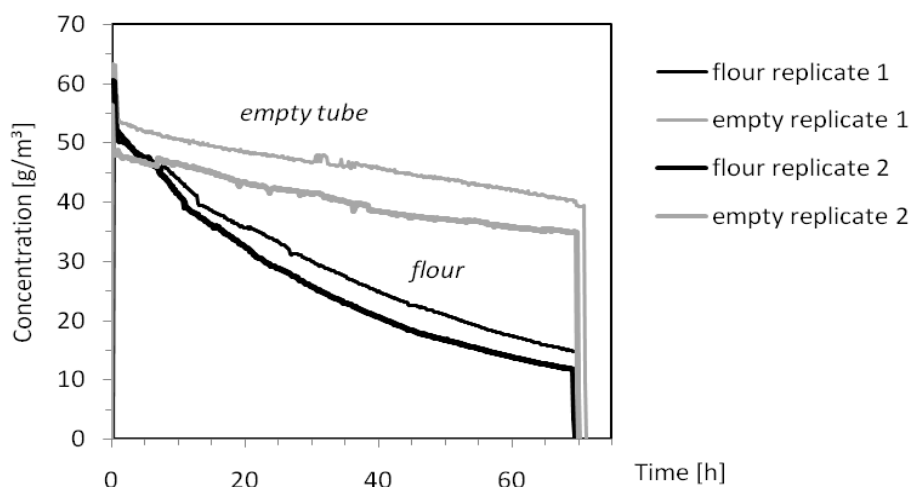
Fig. 2- Sulfuryl fluoride concentration by time under recirculation. Fumigation at room temperature.

The residual fluoride contents are listed in the table. The highest amount of fluoride was found in the 30 cm thick layer, followed by the 15 cm layer sample. In the large amount of flour (110 cm layer) 7.3 mg kg^{-1} fluoride was determined. The second experiment resulted in 17.1 mg kg^{-1} and 8.3 mg kg^{-1} fluoride in both replicates of the fumigation trials. The total dosage of SF was a bit higher in the first replicate.

Table 1. Fluoride residues [mg kg^{-1}] following fumigation of flour with sulfuryl fluoride in different experimental set up

Fluoride content in flour following fumigation by gravity penetration

Penetration in glass column	Thickness of flour	Dosage [g h m^{-3}]	Fluoride [mg kg^{-1}]
110 cm (26 kg)	110 cm	2317	7.3 ± 0.2
30 cm (7 kg)	30 cm	2231	31.1 ± 0.2
15 cm (4 kg)	15 cm	2303	25.3 ± 0.4
Flour exposed to sulfuryl fluoride under recirculation			
100 g	Replicate 1	2008	17.1 ± 0.1
	Replicate 2	1795	8.3 ± 0.03

DISCUSSION

The penetration of flour by sulfuryl fluoride, the sorption and the formation of fluoride is complex. In contrast to Bell et al. (2003), who investigated 30 cm layers and assumed equal concentrations in any depth of flour for appropriate long times, the present study found that the concentration of SF at the bottom of the flour layer did not reach an equilibrium to the head space concentration. The diffusion of gases is driven by the concentration gradient. During its diffusion through the flour, there are other processes likely, i.e. sorption and desorption as well as the break down into fluoride and other compounds. An attempt to explain the findings could be the part of the sulfuryl fluoride-breakdown as the predominant process during its dispersion through the flour. This hypothesis is supported by the results of the second experimental set up, the continual decrease in concentration in the flour streamed by the sulfuryl fluoride.

Fluoride residues have been found, as expected, following the sulfuryl fluoride exposure experiments. Sulfuryl fluoride is known to form residual fluoride on various commodities. It is caused by the break down either by a potential hydrolysis of the molecule or, as formerly described by Meikle (1964) on flour, by reaction with proteins and amino acids. Scheffrahn et al. (1989) found fluoride on flour ranging between 60 – 450 mg kg^{-1} depending on the fumigant concentration (36 – 360 g m^{-3}) in 20 h exposure. According to this background, it has to be found out which maximum residue amounts (and the causing conditions) are principally possible. The present study tried to give an idea of a worst case scenario, an unintended fumigation of milling products in a mill. The potential contribution to the daily fluoride intake and the significance to a risk of overexposure due to this pathway have to be discussed.

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FATE OF ^{32}P LABELED PHOSPHINE IN GRAIN AND GRAIN FRACTIONS

Yonglin Ren[1,2,3], Megan Jansen[1,2], Manjree Agarwal[1,2], Rob Emery[1,3] James Newman[1,2] and Hui Cheng[1,2]

[1] Cooperative Research Centre for National Plant Biosecurity, Australia
[2] Stored Grain Research Laboratory, School of Biological Sciences, Murdoch University, Murdoch, WA 6150, Australia
[3] Department of Agriculture and Food, Western Australia, 3 Baron Hay Court South Perth WA 6151, Australia
*Corresponding author's e-mail: *y.ren@murdoch.edu.au*

ABSTRACT

Murdoch University Stored Grain Research Laboratory investigated the fate of ^{32}P labelled phosphine in grain and grain fractions. Radioactively-labelled phosphine is a useful and sensitive tool to study residues of phosphine in grains and their fractions. This research will assist industry to improve phosphine fumigation practice. Five representative grains (wheat, barley, oats, canola and lupins) were fumigated with ^{32}P labelled phosphine at 700 ppm and 25±2°C for two weeks exposure. Two types of residues were formed in grains from phosphine fumigation – phosphine residue and phosphine converted non-volatile residues. After one week exposure to atmosphere, the levels of ^{32}P radioactive residue in the grains and their fractions were analysed. The results show that more than 85% of absorbed phosphine which was converted to non-volatile compounds cannot be removed by ventilation. Furthermore, 80-90% of non-volatile ^{32}P residues were present in water and acid soluble extractions. Radiation imagery shows that more than 80% of ^{32}P residues were located or distributed in the embryo, testa, pericarp and husk of wheat, barley, canola, lupins and oats. However, 90% of absorbed phosphine will be desorbed after one week exposure to the atmosphere.

Key words: fumigant, ^{32}P labelled phosphine, residue, grain.

INTRODUCTION

Phosphine (PH$_3$) has been used as a fumigant for many years. The world grain industry relies heavily on phosphine for control of insect pests during storage. However, we know very little of its physical and chemical behaviour or its residues in treated and re-treated grain (Desmarchelier and Ren 1999). Phosphine has been scheduled for a toxicological and residue Re-Evaluation Review in 2015 by the FAO Codex Alimentarius organisation that sets internationally agreed maximum residue limits (MRLs) for pesticides. Despite the international importance of phosphine there is limited information on the physical and chemical behaviour of phosphine in grain and its degradation. It is extremely important that the Australian grain industry has access to information on the fate of phosphine in grains so

that MRL breaches do not occur as these could jeopardise market access (Desmarchelier and Ren 1999; Ren and Desmarchelier 1998).

Therefore, it is necessary to understand phosphine residues in bulk grain to establish better procedures for fumigation and multi-fumigation (Ren et al. 2012). This will guide industry in the conduct of good phosphine fumigation practice, such as the time of exposure and aeration, as well as application methods. This could potentially support re-labelling phosphine in the future when higher dosages may be required to deal with increased resistance.

Murdoch Stored Grain Research Laboratory has investigated the fate of ^{32}P-labelled phosphine in grain and grain fractions. Radioactively-labelled phosphine is a useful and sensitive tool for studying residues of phosphine in grains and their fractions. This paper reports on the uptake of ^{32}P-labelled phosphine on grains of the three major food groups: cereals, legumes and oilseeds.

MATERIALS AND METHODS

Reagents and apparatus

Scintillation vials (20 mL) and scintillation fluid were obtained from Edwards Instruments Company. ^{32}P was purchased as high specific activity sodium orthophosphate in dilute (c. 0.015M) hydrochloric acid from the Australian Atomic Energy Commission (AAEC), Lucas Heights, NSW. The specific activity was 20 mCi (740 MBq) and concentration was 2-10 Ci mg^{-1} (74-370 Bq mg^{-1}). All reagents were analytical grade, unless otherwise specified. Magnesium powder and orthophosphoric acid (>85%) were purchased from Ajax Chemicals, Australia.

The purity of synthetic ^{32}P labelled phosphine was determined on a GOW-MAC (Model 40-001) gas density detector (GOW-MAC Instrument Co., Madison, NJ), after separation on a 1 m × 5 mm (i.d.) Porapak Q 100/120 mesh (Alltech Associates, Sydney, Australia, Cat. No. 2702) at 105°C and carrier (N$_2$) flow of 150 mL min^{-1}. The reference gas was tetrafluoroethane (> 99.9 % pure).

A model LS 200 Beckman (Beckman Instrument Co., USA) liquid scintillation analyser was used for scintillation counting, operating at the appropriate wavelength for the radioisotope.

A model of FLA-5000 Fluorescent Image Analyzer (Fuji Photo Film Co. Ltd. Japan) was used for scanning radiation images of extracted and sectioned commodities.

Synthesis of ^{32}P- labelled phosphine

A modified method for laboratory phosphine production outlined in Reichmuth (1994) was used to produce the ^{32}P labelled phosphine used in this study.

Commodities conditioning and fumigant dosing

Five representative commodities (hard wheat, soft wheat, barley, oats, lupins and canola) were used. Grain samples (380-400 g) were placed into a sealed jar (500 mL) and allowed to equilibrate at 25°C and 65% relative humidity (r.h.). After a period of 6 weeks the commodities were removed and moisture content and equilibrium relative humidity checked.

The conditioned grains (10 g for each variety) were separately placed in beakers (20 mL), and all samples were placed in a desiccator (1.5 L), equipped with a septum. ^{32}P-labelled phosphine (3 mL and 35% purity balanced with CO$_2$) was injected into the desiccator by gastight syringe to give an initial concentration of 400 ppm, v/v. Grain samples were

fumigated for the typical industry maximum exposure period of 14 days at 25±2°C. Following initial dosing, the fumigant was circulated in the desiccator by a magnetic stir bar. After 14 days exposure, the desiccator was opened and the samples transferred to a fume hood where they were aired for 7 days at 25±2°C.

Measurement of total ^{32}P in whole grain kernels

This study involved the dynamic monitoring of ^{32}P degradation and reduction during aeration from day 0 to day 7. These results include ^{32}P labelled phosphine and non volatile residues. Fumigated grain samples (3 g) were collected at day 0, and then 1, 3 and 7 of aeration days after initial opening, and placed into a bottle (7 mL) containing 3 mL of AgNO$_3$ extracted for 7 days. The grains were then ground and 10 mL of distilled water was added after which 3 mL of the mixture was transferred to a 20 mL scintillation vial containing 5 mL scintillation fluid. Unfumigated samples of each commodity were used as controls and 4 replicates of each sample were prepared and stored at 25°C in the dark for 7 days prior to counting. These samples were counted three times to reduce error.

Determination of radioactivity in the extractions

The method of fractionating nutrients relies on the sequential solubilising of one fraction while leaving the residue as the substrate for the next extraction. The procedures were carried out to determine the fate of applied ^{32}P as described by Ren and Mahon (2007). Untreated grains were used as controls. All samples were prepared for duplicate testing.

   (a)   A weighed sample of the commodity (5 g) was removed and placed in a stainless steel mortar. The sample was crushed (not finely ground) and transferred to a Soxhlet extraction thimble.

   (b)   The extraction thimble containing the 5 g sample was placed into the Soxhlet apparatus and the sample extracted with 30 mL chloroform overnight. Following extraction, the solvent (containing lipid, fat-soluble vitamin, pigments etc) was transferred into a 25 mL volumetric flask for analysis of ^{32}P in total lipid. The thimble was placed in a fume hood to allow complete evaporation of residual chloroform and the sample was transferred to a 25 mL centrifuge tube.

   (c)   The sample (remaining from step b) was resuspended in 10 mL distilled water, vortexed and allowed to soak at 25°C for 3 hours. It was centrifuged for 10 minutes at 3500 r.p.m and the supernatant transferred to a 25 mL volumetric flask. The pellet was resuspended twice in 5 mL distilled water with a 15 min soak between each washing. Centrifuged washings were collected in the 25 mL volumetric flask for analysis of ^{32}P in this solution, which contained sugars, amino acids, inorganic acids and sulfide. The pellet was retained (for step d).

   (d)   The pellet (from step c) was resuspended in 10 mL 2N HCl, vortexed and allowed to soak at 25°C for 1 hour. It was centrifuged for 10 minutes at 3500 r.p.m and the supernatant transferred to a 25 mL volumetric flask. The pellet was resuspended twice in 5 mL distilled water with a 15 min soak between each washing. The centrifuged washings were collected in the 25 mL volumetric flask for analysis of ^{32}P in this solution which contained sugars, amino acids and sulfide. The pellet was retained (for step e).

   (e)   The remaining pellet was resuspended for three times with 20 mL 2N HCl in a 25 mL volumetric flask for subsequent analysis of residual ^{32}P.

Scintillation counting

Liquid samples (2 mL each, collected from steps b to e) were mixed with the scintillation fluid (5 mL) in a scintillation vial and placed in the dark. Precautions were taken to avoid complication due to photoluminescence. Four replicate samples were prepared and each was counted four times, and averaged. Quenching was determined on each fraction to allow correction for counting efficiency.

Radiation image

Commodity kernels treated with ^{32}P labelled phosphine were sectioned by cutting in half (crosswise and longitudinally). The sections were held by BLU TACK (Bostik Pty. Ltd. Australia) on the sample holder of the Fluorescent Image Analyzer for scanning of the radiation images.

Preparation of quenched standards

In scintillation counting, quench correction was carried out by calibrating a series of progressively quenched standards with reference to an external standard of ^{32}P labelled phosphine in AgNO$_3$ solution. Samples were replicated 4 times, and each was counted 4 times and averaged. All radioactive residue data were converted/calculated from scintillation counting data by calibrating with the quenching standard curve.

For calibration of ^{32}P decay during four weeks counting, the following method was used based on ^{32}P half-life decay (Eq. 1).

$$M_t = M_o \ (1/2)^{t/14.3} \qquad\qquad \textbf{Eq. 1.}$$

Where: $\mathbf{M_t}$ is amount of ^{32}P at analysis time
$\mathbf{M_o}$ is starting amount of ^{32}P
t is time (days)
14.3 is ^{32}P half-life time (day)

RESULTS AND DISCUSSION

^{32}P labelled phosphine standard

The quenching standard curve (Fig. 1) was not linear over the tested region. However, it was very well fitted to equation: Y=4E-11χ^2+2E-08 χ-2E-05 (R^2=1), where: Y is level of ^{32}P in mg and χ is counts of radioactivity. The counts increased with increasing dose of ^{32}P mixing with scintillation fluid. The standard error (SE) between replicates was < 10%. The levels of ^{32}P in grains and grain fractions were calculated on the basis of the counts, and calibrated periodically with Eq. 1.

Total uptake ^{32}P labelled phosphine and non-volatile ^{32}P by grains

The total uptake of radioactive ^{32}P on different grains at different time of aeration is shown in Fig. 2. The total radioactivity decreased with increasing periods of aeration due to desorption of ^{32}P labelled phosphine. The remaining radioactivity in the day seven aired grain samples was mainly due to non-volatile ^{32}P substances. After fumigation without aeration, the levels of radioactive residues were 5, 19-22 and 64 mg kg^{-1} in lupins, wheat/barley/canola and oats respectively. However, after 7 days aeration, radioactive residues remained at high levels 59.0, 65.2, 81.2 and 93.7% of non-volatile ^{32}P substances in canola, wheat/barley, lupins and

oats respectively. This portion of ^{32}P was permanent residue in treated grains. These results show that during exposure period, 59-93.7% absorbed phosphine is converted to non-volatile compounds which cannot be removed by ventilation.
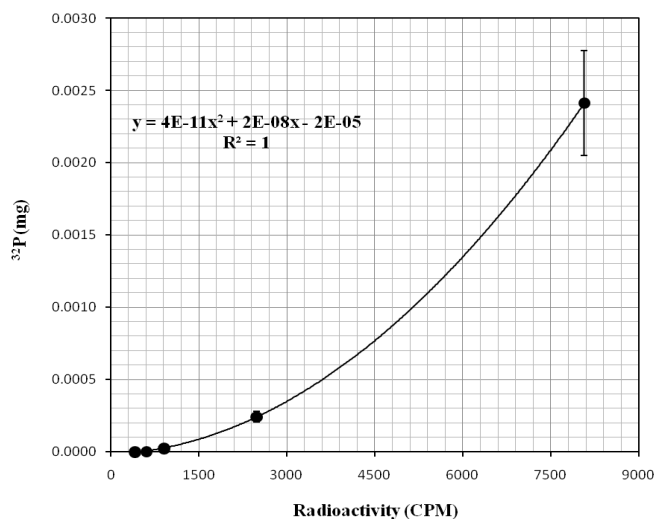
Fig. 1- Quenching standard curve plot from scintillation counting data (CPM) against series of progressively added ^{32}P labelled phosphine. Error bars indicate SD, n=9.

	Wheat	Barley	Lupine	Oats	Canola
Day 0	19.00	19.84	4.67	63.95	21.89
Day 1	14.26	14.73	4.10	60.50	14.95
Day 3	12.94	12.11	4.04	58.73	14.46
Day 7	12.37	12.94	3.79	59.93	12.91

Fig. 2- Total residue levels of ^{32}P (mg kg^{-1}) in grains at different times after aeration. Error bars indicate SD, n=9.

Uptake ^{32}P by grain fractions

The residues of ^{32}P in the extractions from treated grain samples are shown in Fig. 3. The amount of ^{32}P was calculated as mg ^{32}P equivalent to per kilogram of commodity (mg kg^{-1}). ^{32}P residues were found at high levels in organic acids, e.g., 29.2, 49.4, 35.1, 36.4 and 34.5% in wheat, barley, lupine, oats and canola. 66.3, 79.6, 66.9, 60.2 and 48.6% of total non-volatile ^{32}P residues in wheat, barley, lupins, oats and canola were present in water and acid soluble extractions (organic and amino acids, sugars, lignins and hemicelluloses). This could result in changes in grain and cereal nutrition and beer flavour. Further research will be conducted to determine how the phosphine affects grain qualities and beer flavour. In general, ^{32}P residues were at low levels in protopectins and non-cellulose polysaccharides, cellulose, lipids and hemicelluloses. The total amount of $^{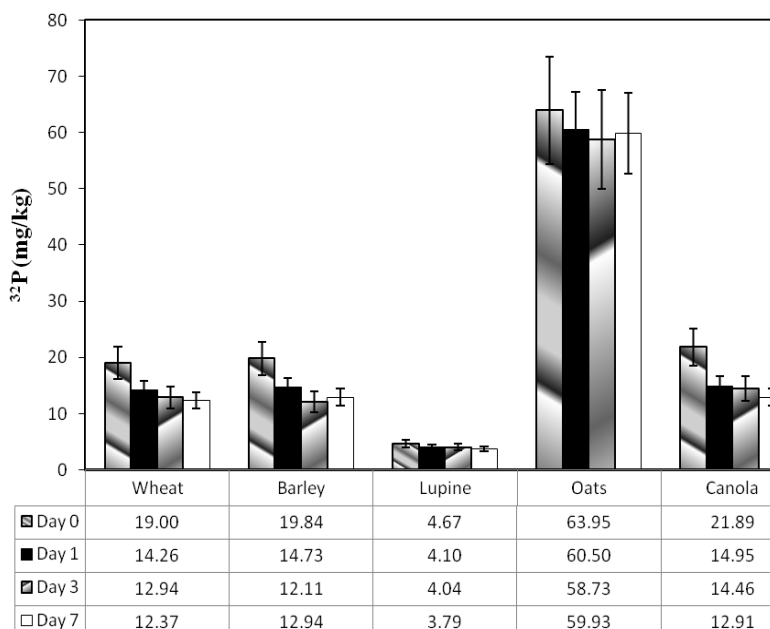32}$P after seven days aeration period was 15.1, 15.3, 7.5, 52.9 and 16.6 mg kg^{-1} in fractions of wheat, barley, lupine, oats and canola. These results are consistent with results from whole grain extractions (Figures 2 and 3).

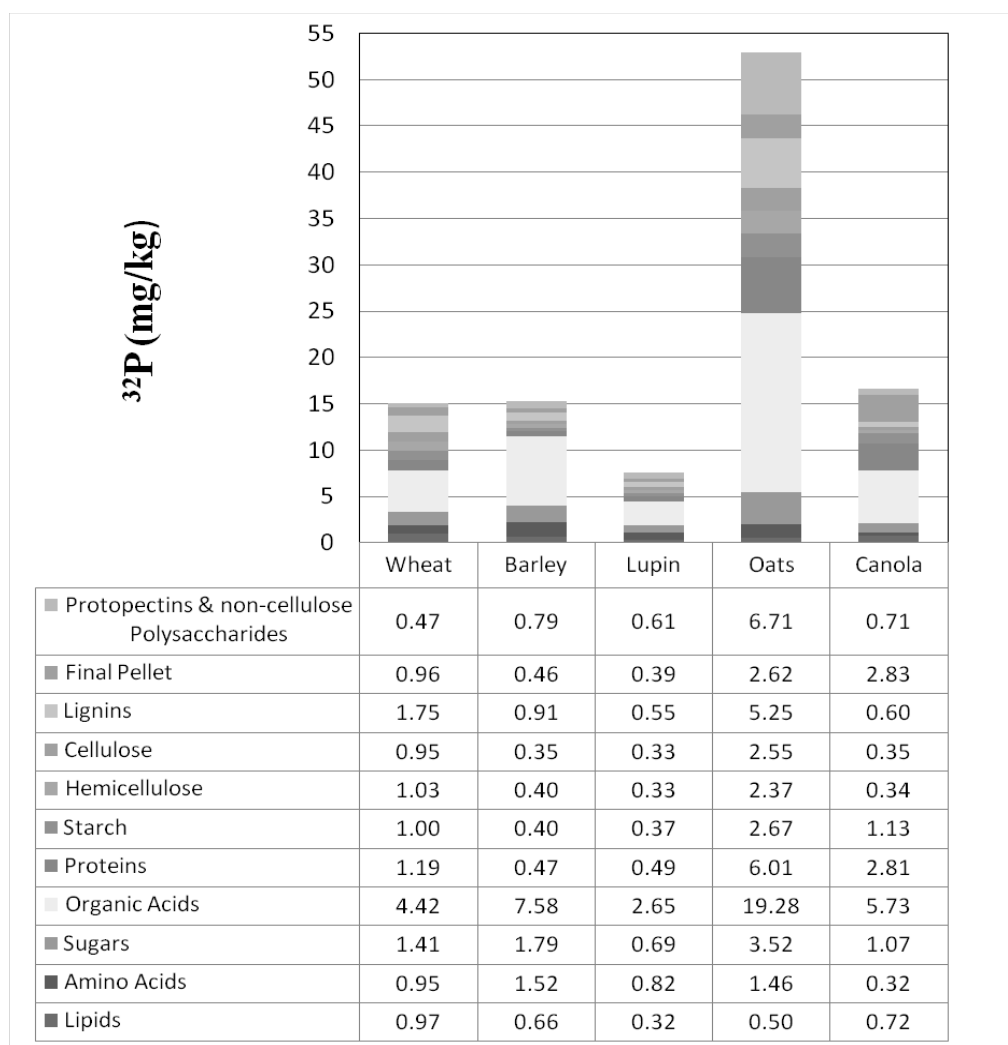|	Wheat	Barley	Lupin	Oats	Canola
▨ Protopectins & non-cellulose Polysaccharides	0.47	0.79	0.61	6.71	0.71
▨ Final Pellet	0.96	0.46	0.39	2.62	2.83
▨ Lignins	1.75	0.91	0.55	5.25	0.60
▨ Cellulose	0.95	0.35	0.33	2.55	0.35
▨ Hemicellulose	1.03	0.40	0.33	2.37	0.34
▨ Starch	1.00	0.40	0.37	2.67	1.13
▨ Proteins	1.19	0.47	0.49	6.01	2.81
▨ Organic Acids	4.42	7.58	2.65	19.28	5.73
▨ Sugars	1.41	1.79	0.69	3.52	1.07
▨ Amino Acids	0.95	1.52	0.82	1.46	0.32
▨ Lipids	0.97	0.66	0.32	0.50	0.72

Fig. 3- The levels of ^{32}P residues (mg kg^{-1}) in different extractions from grains fumigated with ^{32}P labelled phosphine.

Distribution of ^{32}P in grains

Radiation imagery shows that more than 80% of ^{32}P residues were located or distributed in the embryo, testa, pericarp and husk of wheat, barley, canola, lupins and oats (Fig. 4). This work will contribute to the cereal and brewing industries by ensuring that residues that could affect nutrition and flavour will be minimized.
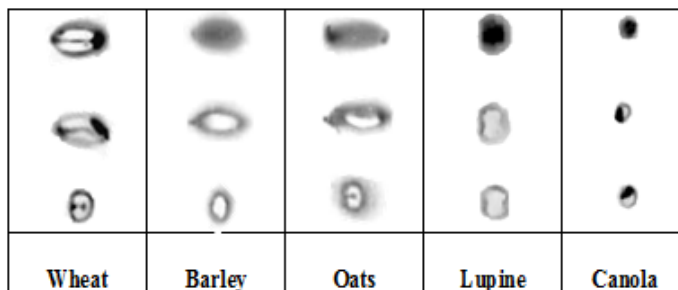

| Wheat | Barley | Oats | Lupine | Canola |

Fig. 4- Distribution and position of ^{32}P in the grains fumigated with ^{32}P labelled phosphine.

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ENVIRONMENTAL IMPACTS OF THREE DATE DISINFESTATION TECHNOLOGIES QUANTIFIED BY THEIR CARBON FOOTPRINT

Hagit Navarro*,

Food Technology International Consultancy Ltd., P.O. Box 3300, Beit-Yehoshua, 40591 Israel.
*Corresponding author's e-mail address: *HNavarro@ftic.info*

ABSTRACT

Nitdulid beetles and Phycitidae moths start their infestation in the date orchard, where pest management is insufficient and may continue their infestation in storage. Therefore, to eliminate the insects from the dates and to prevent further damage during storage, disinfestation of the dates should be carried out as they reach the packing stations. Methyl bromide has been very successful fumigant in removing the insects from the fruit, and kills the insects. As alternative to methyl bromide, in accordance with Montréal protocol, fumigation using Vapormate^{TM} and thermal disinfestation have been developed and implemented. Thermal disinfestation technology is based on the principle of transfer of hot air through a channel containing 3 tonnes of dates, where the dates are exposed on trays for 3 h to $50^{o}C$. Economic and environmental analyses were done by the carbon footprint assessment which is a measure of the exclusive total amount of carbon dioxide emissions that is directly and indirectly caused by an activity. The analysis was done for three disinfestation methods of dates; fumigation using methyl bromide, fumigation using Vapormate^{TM} and thermal disinfestation using LPG or solar energy. In each disinfestation method, the emissions were converted to carbon equivalents according to its Global Warming Potential values. Then these carbon equivalent values were converted to monetary values according to the update of the 2005 ExternE methodology. Results show that the most effective disinfestation technology both economically and environmentally is the use of solar energy based thermal disinfestation. The lowest carbon footprint values were obtained using solar energy. Thermal disinfestation method was found as a suitable alternative technology to methyl bromide in all biological, environmental and economical aspects. Currently the thermal disinfestation technology is successfully implemented on all major date cultivars including Medjool as an alternative to methyl bromide, in accordance with Montréal protocol in Israel.

Key words: Dates, Medjool, insect control, methyl bromide, ethyl formate, Vapormate^{TM}, thermal disinfestations, carbon footprint, solar energy.

INTRODUCTION

The dates industry in Israel serves as an important portion of Israel's agricultural export. The dry cultivars grown in Israel are Medjool, Deglet-Noor, Hadrawi, Halawi, Zahidi, Derhi and Ameri (Cohen, 2011). Most pests develop in the field, where pest management is insufficient, then brought to the warehouses where some Nitidulidae beetles and Phycitidae moths

continue to develop (Blumberg, 2008). Therefore, to prevent further damage during storage within the warehouses and to remove the insects from the dates, insect control should be carried out by disinfesting the dates as they reach the packing stations.

In accordance with Montréal protocol, Israel agreed to decrease the use of methyl bromide (MB) and search for alternatives (TEAP & MBTOC, 2003). After the successful implementation of the thermal disinfestation technology on the major cultivar, Medjool (Navarro, 2006), three field trials were carried out on other date cultivars which resulted in expanding and implementing the thermal disinfestation technology to other dry cultivars in Israel (Navarro, 2011). Thermal disinfestation technology is based on the principle of transfer of hot air through a channel containing 3 tonnes of dates, where the dates are exposed on trays/boxes for 3 h to 50°C (Navarro et al., 2003; 2004).

Along with the implementation of the thermal disinfestation technology, another method was registered in Israel – the use of the fumigant VAPORMATE^{TM} which consists of 16.7% ethyl formate in liquid CO_2. Its registration by the Israeli Plant Protection Services for the date's industry was accomplished in 2009 as an alternative to MB (Lendler, 2009). This fumigant is environmentally friendly and in use in the food industry.

The thermal disinfestation technology could be applied based either on Liquid Petroleum Gas (LPG), solar energy or combined energy. All of the above mentioned disinfestation methods have different impacts on the environment. Nevertheless, the choice of which method should be applied is influenced by its economic feasibility. A means of evaluating the benefit of technology application is by evaluating the costs which takes into account its impact on the environment (e.g., external costs). The present work aims at evaluating the environmental impacts of the disinfestation methods by their "carbon footprint" which is "a measure of the exclusive total amount of carbon dioxide emissions that is directly and indirectly caused by an activity or is accumulated over the life stages of a product" (Wiedmann and Minx, 2008).

The "carbon footprint" serves as a common denominator that allows comparing between similar products of different processes that are generated due to their contributions to understanding the impact of the product on global warming. This concept has become popular in recent years and is used for comparison. In contrast to Life Cycle Analysis (LCA) which compares the entire environmental impacts, it is actually more complicated analysis for different products that might skip the concept of the common denominator of a products' influence on the environment, since the carbon footprint is popular, has attractive marketing advantage, and thus has the potential to increase the green environmental awareness of consumers when greenhouse gases emission is the main reference on global warming (Weidema et al., 2008).

MATERIALS AND METHODS

The main differences resulting from different disinfestation methods depend on the way disinfestation is applied. Therefore, comparison of costs served as basis for the different disinfestation methods:

A. Fumigation using MB.
In this method, dates are placed into plastic enclosures of different sizes (200 or 400 kg vats or factory boxes that contain about 13 kg dates) in fumigation chambers where the gas is distributed and circulated during an exposure of 4 to 6 h. The MB fumigation is no longer in use in Israel after the approval of the thermal disinfestation technology by the Israel Ministry

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of Environmental Protection in 2010. In the present work MB was used as a reference (TEAP, 2009), to compare with alternative methods at a dosage of 30 g/m^3.

B. Fumigation using VAPORMATE^{TM}.

In 2008 the product VAPORMATE^{TM} was registered by the Israel Ministry of Agriculture for disinfestation of dates (Finkelman, 2010). This fumigant is particularly useful for fumigating vats containing 400-450 kg fruit or half vats (50 cm height) containing about 200-220 kg of fruit that are not suitable for the thermal disinfestations due to resistance of hot air flow through the bulk of dates. This method is a complete alternative to thermal disinfestation technology when dates are handled in vats. Technically, the application of the fumigant is as with MB in fumigation chambers. For disinfestation of dates VAPORMATE^{TM} is applied at 420 g m-3 for 12 h exposure time, at 30°C.

C. Thermal disinfestation using LPG, solar energy or combined as an energy source.

The common thermal disinfestation method is based on solar radiation, in case of a cloudy day it is backed up by additional energy. The disinfestation facility is built as a greenhouse structure which is covered with polyethylene sheets and has concrete floor.

Theoretical energy consumption calculations required for thermal disinfestation using different energy sources when compared to solar energy:
Calculations for disinfestation process of 1 tonne of dates were considered in two scenarios:
1- On cloudy days when active heating is required constantly, the heating unit is operated during the whole disinfestation process.
2- On sunny hot days, the heating unit is operated intermittently during the whole process.

The heating units were LPG based. To reach the target temperature of 50°C, pre-heating of 1-4 h is required. The calculations were based on pre-heating duration time, disinfestations exposure time to 50°C (3 h) in accordance with the facilities tested and their capacities. Calculations were based on the price of kg LPG in 2009 which was $US 0.026 and electricity price (0.34 $US per kW/h) needed to circulate the hot air through the dates by the electric fans (GEA, 2011).

Comparative environmental analysis of the disinfestation methods:
Environmental impacts resulting from the different disinfestation methods were quantified by converting the emissions resulting from the processes into metric tonnes of carbon dioxide equivalents (MTCE). Each calculation was determined by its Global Warming Potential factor (GWP) (Forster et al., 2007). According to the equation:
 [MTCE (Tg of gas) x (GWP) x (12/44)] (ICBE, 2000)
Where: Tg is Terra gram of gas and 12/44 by weight is 12/44^{ths} of carbon dioxide.

The data gathered in the present work is from emissions that resulted from:
   a.) Fumigation using MB- GWP factor is 5 for 100 years (Forster et al., 2007).
   b.) Fumigation using VAPORMATE^{TM}, since the product VAPORMATE^{TM} consist 83.3% carbon dioxide, the emissions are based on this factor. The rest is the active ingredient ethyl formate which hydrolyses to formic acid therefore has no impact on the environment. Therefore its GWP factor is 1.
   c.) Thermal disinfestation on cloudy days when heating units were operated constantly.

d.) Thermal disinfestation on hot and sunny days when heating units were operated intermittently.

For the above conditions described in items c and d, the GWP factor is 3.65 (Forster et al., 2007) derived from the use of LPG which consists of 50% propane and 50% butane (EPPO, 2011). The amount of carbon was quantified by the calories needed for the disinfestation process for 1 tonne of dates.

External environmental cost calculations were based on the update values in the monitories of ExternE methodology for 2005 which shows a value of 19 €/tonne of carbon dioxide emitted. This methodology is based on a published FUND model by the environmental protection air quality division in cooperation with the department of economics and the matched price tonnes of carbon dioxide, according to the costs of prevention (cost of saving emissions according to international standards) and per capita compared to the EU-designated GDP (Gross Domestic Product) on € 14.83 (Kadmi et al., 2008). The environmental report published by the Israel Electric Corporation revealed that in 2009 the specific emission of carbon dioxide per kW h-1 was 707 g (Israel Electric Corporation, 2009).

RESULTS

Fumigation using MB
Methyl bromide 2009 cost in Israel was 11.84 $US/kg. Israel ministry of Environmental Protection recommends a dose of 300 g/tonne MB to fumigate dates. Although a dosage of 30 g m-3 is sufficient to control date insects, in practice 300 g/tonne is calculated because of the volume factor that varies often, the fumigation chamber is not full with commodity. Therefore, the direct cost of MB fumigation resulted in 3.55 $US/tonne fora full 95 m^3 fumigation chamber that contains 14 tonnes of fruits.

Fumigation using VAPORMATE^{TM}
VAPORMATE^{TM} 2009 cost in Israel was 31.57 $US/kg. The manufacturer recommendation was to use a dose of 420 g m-3, equivalent to 2850 g/tonne. Therefore, the direct cost of VAPORMATE^{TM} fumigation was 90 $US/tonne fora full 95 m^3 fumigation chamber that contains 14 tonnes of fruits.

Thermal disinfestation
Table 1 represents the energy and direct costs of different energy sources for thermal disinfestation based on the above data and on the caloric value (12.73 kW) obtained from 1 kg of LPG (propane butane ratio 1:1). Two disinfestation facilities were investigated; "Tzemach" facility is able to disinfest in a single batch maximum 21,840 kg of dates (28 pallets). In "Tzemach" there were two heating units which consume 33 kg LPG/h with capacity of 418.68 kW. Therefore, 7.5 kg LPG were needed to disinfest 1 tonne dates (95.6 kW/tonne). During the disinfestation process both heating units and axial fans (Termotecnica Pericoli, Italy) consumed electricity of 1.1 kW/h. There were 7 fans and two heating unit fans (4 and 6 kW/h each). Therefore, for a 5 h disinfestation process 88.5 kW/h was needed, this accounted for 4.05 kW/h per tonne. Considering the price of kW/h in Israel ($US 0.14) and LPG price ($US 0.026) continuous heating costed 3.05 $US/tonne.

Table 1. Energy (kW) needed to thermal disinfest a tonne of dates and their direct costs based on different energy sources.

Type of thermal disinfestation	kW	Direct cost ($US/tonne)
LPG based, constantly heating ("Tzemach")	99.52	3.05
Combined solar & LPG, alternately heating ("Tzemach")	29.58	0.91
LPG based, constantly heating ("Timura")	40.84	1.89
Combined solar & LPG, alternately heating ("Timura")	21.75	1.39
Solar based ("Timura")	1.99	0.67

In another scenario, on hot sunny days the energy consumption at "Tzemach" facility stands on 30%. Therefore, when the heating units were operated intermittently the cost was 0.91 $US/tonne.

"Timura" facility contained a single batch, maximum 60 tonnes dates. Therefore, 3 kg LPG were needed to disinfest 1 tonne dates (38.19 kW/h/tonne). In "Timura" there was one main heating unit fan (5 kW) with 20 fans (0.7457 kW/h each) for each channel. Since this facility was much bigger than that of Tzemach, 4 h preheating were needed to reach the target temperature of 50°C. Therefore, the disinfestation process in "Timura" required 8 h in total where the energy consumption was 2.65 kW/h/tonne derived from electricity and 38.19 kW/h/tonne derived from LPG consumption. Therefore, when installation heaters were continuously in operation, the total disinfestation cost was 1.89 $US/tonne.

On hot sunny days when the heating units were operated intermittently, the energy costs were for moving air by fans using 21.75 kW/h/tonne (1.39 $US/tonne).

The least energy needed, hence, the cheapest thermal disinfestation method was solar based disinfestation in "Timura" which needs 1.99kW and costs 0.67 $US/tonne derived only from the fans.

Table 2 summarizes the external costs derived from their carbon equivalent emissions for disinfestation of 1 tonne of dates in two thermal disinfestation facilities compared to fumigation with MB and VAPORMATE^{TM}. It is clear that the larger the amount of carbon equivalent was emitted (e.g., the carbon footprint), the higher was the external cost. The cheapest total cost was obtained for thermal disinfestation derived from solar energy (0.67 $US/tonne with $0.38*10^{-3}$ carbon equivalents/tonne) and surprisingly fumigation with MB gave carbon emission ($0.40*10^{-3}$ carbon equivalents/tonne) close to solar energy system but at a higher total cost (3.553 $US/tonne).

DISCUSSION

Results indicated that the most effective disinfestation technology both economically and environmentally was the use of solar energy. Similarly, in the analyzed thermal disinfestation method, the lowest carbon footprint ($0.38*10^{-3}$ carbon equivalents/tonne), was obtained using solar energy. Thermal disinfestation method was found as an appropriate alternative technology to MB in all senses; biologically to achieve complete disinfestation including the egg stage, environmentally and economically (Navarro, 2011).

Table 2. External and total costs, carbon equivalent (tonne) emitted from each disinfestation method for disinfesting dates.

Type of disinfestation	Carbon equivalent (tonne) / tonne dates disinfestation	External Cost ($US/tonne)	Total cost ($US/tonne)
Thermal-LPG based, constantly heating ("Tzemach")	$8.05*10^{-3}$	0.157	3.207
Thermal-Combined solar & LPG, alternately heating ("Tzemach")	$2.41*10^{-3}$	0.047	0.957
Thermal-LPG based, constantly heating ("Timura")	$3.45*10^{-3}$	0.067	1.957
Thermal-Combiend solar & LPG, intermittently heating ("Timura")	$1.85*10^{-3}$	0.036	1.426
Thermal-Solar based ("Timura")	$0.38*10^{-3}$	0.007	0.677
Fumigation using MB at 300 g/tonne	$0.40*10^{-3}$	0.008	3.553
Fumigation using VAPORMATE^{TM} at 2850 g/tonne	$0.64*10^{-3}$	0.012	90.012

The carbon footprint includes activities of individuals, populations, governments, companies, organizations, processes, and various industry sectors. Analyzed products include goods and services. In any case, all direct (on-site, internal) and indirect emissions (off-site, external, embodied, upstream and downstream) need to be taken into account (Weidema *et al.,* 2008). To simplify the calculations and to provide a clear solution, the stable/constant variants in this work, since they were the same for each disinfestation method, were not taken into account (e.g., 1- Check in and check out of dates from the disinfestation facility, 2- management, general and professional supervision on the process and 3- packing containers of dates (i.e., costs of Medjool trays), and 4- factory boxes (used in handling and disinfestation of other date varieties than Medjool and vats).

In boxes where the airflow through the bulk of dates was above 1.4 m/s the target temperature of $50^{o}C$ was achieved within 3 h from the start of the heating of the chamber, and at higher airflow rates same results were achieved within 1 to 2 h (Navarro, 2011). Although actions were made to prevent energy loss by achieving adequate sealing and improving circulation with fans to achieve homogenous air distribution, Table 1 shows that solar energy absorbed by "Timura" facility is greater than "Tzemach" facility, where the disinfestation process lasted longer time.

According to Montréal protocol an alternative would be implemented and accepted if it is proven to be economically feasible. The above suggested alternatives to MB have been approved and are in use in Israel. In spite of the high price of VAPORMATE^{TM}, due to shipping and handling, efforts are being made currently to reduce its price (personal information). Although VAPORMATE^{TM} according to Montréal protocol is environmentally user friendly, there is no consideration on its impact on the environment as an alternative to MB. It seems that when the thermal disinfestation alternative is improperly used, it has a much harmful impact on the environment than MB or VAPORMATE^{TM}. However, one must not be mistaken by the carbon footprint only, since the carbon equivalent emitted by the MB fumigation has much more harmful impact on the environment, since the damage caused to the ozone layer is irreversible compared to the pure carbon emitted to the environment (Walter, 2009).

CONCLUSIONS

Thermal disinfestation method of dates was found as a suitable alternative technology to the methyl bromide fumigation both from environmental and economical aspects. To reduce the carbon footprint impact of thermal disinfestation process, solar energy absorbing ability of the disinfestation installations must be maximized by providing a transparent cover of the unit, sealing the disinfestation facility, and by providing appropriate circulation of the hot air at recommended rates.

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OCCUPATIONAL SAFETY IN IMPORT CONTAINERS CONTAINING FUMIGANTS, OTHER GASES AND VOLATILE SUBSTANCES: PRACTICAL EXPERIENCES

Otto Mück[1]* and Alexander Stock[2]

[1] EWCS GmbH, Südweststraße 19, 28237 Bremen, Germany
[2] PPD/EWS N.V., Postbus 44, 2170 Antwerpen, Belgium
*Corresponding author's e-mail: *o.mueck@ewcs-gmbh.de*

ABSTRACT

In transit fumigation of freight containers is a convenient means of preventing damage by insect pests and their accidental introduction to other countries. On the other side, containers containing hazardous substances including fumigants have become an important occupational safety hazard because most of these containers have not been labeled according to the regulations in force. In 2011, members of the PPD/EWS group performed a total of 123,349 measurements of import containers in different European countries. A total of 17 substances were measured on a regular basis including common fumigants and the most frequently encountered industrial gases and vapours. The results were communicated to the customers in immediately generated on-line gas measuring reports. For serial measurements the preferred technology applied was Selective Ionisation Flow Tube Mass Spectrophotometry plus sensors for low molecular weight gases and lower explosive limit (LEL). Handheld technology such as reactive tubes plus gas pumps, photo ionisation detectors (PID) and others were used. Of the 123,439 containers measured, 13% were rejected because the concentrations of hazardous substances inside were above the respective occupational exposure limits (OEL). In containers with food the proportion of rejected containers was 15 %. The substances found most frequently in concentrations above OEL (in order of importance) were carbon monoxide, 1,2-dichloroethane, formaldehyde, toluene, benzene, and carbon dioxide. Phosphine came in seventh place, methyl bromide in tenth and sulphuryl fluoride in fourteenth place. The origins of most hazardous containers were countries from South East Asia. The applied system of risk assessment and indexation combined with the use of high performance measuring instruments, and state of the art ventilation and gas recapture procedures allows hazardous containers to be handled in a safe and economic way.

Key words: Fumigants, import containers, occupational safety, risk assessment, toxic gasses, gas measurement, methyl bromide, phosphine

INTRODUCTION

In transit fumigation of freight containers is being practiced in order to prevent damage by insect pests. Examples are prevention of the accidental spread of wood destroying insects according to the ISPM 15 standard of the International Plant Protection Convention (IPPC) and quarantine as well as pre-shipment fumigations against post-harvest pest insects. Phosphine is a widespread fumigant for post-harvest treatments of dry commodities such as food and feed grain or cocoa, for example while the use of methyl bromide has been phased out to a large extent due to the Montreal Protocol stipulations on protection of the stratospheric ozone layer. Within the framework of ISPM 15 methyl bromide is still commonly used to fumigate pallets and other packaging made of wood. Fumigation of freight containers should be performed according to an international regulatory framework issued by the International Maritime Organisation (IMO). However, in practice a large portion (presumably about 99 % of all fumigated containers) is not properly labelled (Knol-de Vos, 2002). Some reasons might be prevention of additional cost and possible delays imposed by importers.

In addition, different scientific and practical studies published more recently (e.g. Baur et al., 2006 and Luyts & Mück, 2011) indicate that other hazardous gases and vapours even exceed fumigants in number and importance with regard to occupational safety at the time of opening and unloading import containers from overseas. Practical gas measurements performed by PPD/EWS group companies in different European countries in 2011 confirm these findings.

MEASURING STRATEGY AND EQUIPMENT USED

Risk evaluation with regard to residual gasses or vapours in import containers is performed using Risk Indexes. Measuring frequencies of hazardous substances are determined in relation with a Risk Index per product stream that is based on the cargo description (i.e. articles in the container, supplier and country of origin). As a 100 % safety level would require measuring 100 % of the incoming containers, an acceptable risk indexation system is used in order to reduce the amounts of measurements needed to realize reliable risk control without taking irresponsible safety risks. For practical reasons a three categories risk system is targeted (No Risk / Low Risk / High Risk).

There are two measurement types for hazardous substances: serial and ad-hoc. Serial measurement means large amounts of containers on the same place at the same time. Ad-hoc means few containers at a time. For serial measurement the technologies applied are SIFT (Selective Ionisation Flow Tube Mass Spectrophotometers) plus sensors for gasses with a low molecular weight and LEL (lower explosive limit). Handheld technology used for ad-hoc situations includes PID (Photo Ionisation Detectors) for VOC's (volatile organic chemicals), Infrared devices for sulfuryl fluoride, and sensors for light substances. Colorimetrical test tubes are mainly used to check and exclude possible interferences.

RESULTS OF MEASUREMENTS

In 2011, members of the PPD/EWS group performed a total of 123,349 measurements of import containers in several European countries (Austria, Belgium, Denmark, Germany, Netherlands, and Spain). From these measurements, 13 % exceeded limit values such as Occupational Exposure Limits (OELs). Depending on the contents of the containers, there

were even higher levels of excess of limit values such as food commodities (15 %), electronics (17 %), consumables such as garden furniture, tools and pet equipment (19 %), and shoes with 42 % of excess of limits valid according to the respective occupational safety legislation in force.

The substances most frequently measured and detected in levels higher than legal limits are given in table 1, in order of importance. Apart from VOC's and fumigants, a number of gases with small molecules play an important role. Some sources of these substances have been reliably identified while others sometimes remain questionable up to now. Apart from the frequency of detection of the respective substances in levels above agreed limit values the highest concentration found is given as compared to limits in force in the Netherlands where the highest number of measurements were performed.

Table 2 provides an overview of substances found compared to selected product groups in freight containers. Certainly, this table is far from being complete, but it is good start for understanding hazards that were hardly perceived by anybody ten years ago.

Compared to 2010, the overall picture was similar. By that time the total number of rejected containers was 11 % (N = 42,888 container measurements) compared to 13 % in 2011. Food containers accounted for 20 % (15 % in 2011).

Table 1. Selection of substances detected in freight containers in quantities higher than official limit values (N = 123,349 measurements)

Substance	Frequency of value transgressions	Highest concentration detected	Workplace Exposure Limits (Netherlands)	Transgression Factor
Carbon monoxide	5,150	1,000 ppm	25 ppm	40
1,2 Dichlorethane	4,746	152 ppm	1,7 ppm	89
Formaldehyde	4,625	38 ppm	0,1 ppm	380
Toluene	3,435	693 ppm	40 ppm	17
Benzene	3,288	131 ppm	1 ppm	131
Carbon dioxide	3,020	28,674 ppm	5.000 ppm	5.7
Phosphine	1,856	329 ppm	0.1 ppm	3,290
Xylene	1,034	676 ppm	48 ppm	14
Styrene	963	189 ppm	20 ppm	9.5
Methyl bromide	492	82 ppm	0.25 ppm	328
Ammonia	333	361 ppm	20 ppm	18
Low oxygen	278	11 % (lowest)	20.9 %	-
LEL (explosion)	182	59 %	10 %	-
Sulphuryl fluoride	87	15 ppm	3 ppm	5
Chloropicrin	9	26 ppm	0.1 ppm	260
Hydrogen cyanide	2	3 ppm	0.9 ppm	3.3

ANALYSIS OF THE RESULTS AND CONCLUSIONS

The findings of measurement performed during the past two years have provided a sound database of hazardous substance to be found in freight containers. Important insights include the ones listed below:

- Registered fumigants are less frequently found than industrial chemicals such as solvents and others.
- Fumigated containers from overseas (mainly East Asia, South East Asia and India) are very rarely labelled as fumigated.

- Many containers come with a cocktail of several different hazardous substances which may act in a synergistic way to damage human health (e.g. through sensitization).

Table 2. Relationship between hazardous substances detected in freight containers and selected product groups (x = substance found)

Substance	Shoes	Electronics	Wood	Consumables	Textiles	Food	Packaging
Carbon monoxide	X	X	X	X	X	X	X
1,2 Dichlorethane	X	X		X	X		X
Formaldehyde	X	X	X	X	X		X
Toluene	X	X		X	X		X
Benzene	X	X	X		X		
Carbon dioxide	X	X		X		X	
Phosphine			X	X	X	X	X
Xylene	X	X		X			
Styrene	X	X		X			
Methyl bromide	X	X	X	X	X	X	X
Ammonia	X	X		X			
Low oxygene	X				X	X	
LEL		X					
Sulphuryl fluoride	X		X	X			X
Chloropicrine	X	X	X				
Hydrogen cyanide		X					

Fumigated containers constitute a small proportion of hazardous containers. Containers treated with fumigants registered in the European Union (phosphine and sulphuryl fluoride) plus methyl bromide account for slightly less than 2 % of all hazardous containers. Methyl bromide rejection frequencies show a remarkable decreasing tendency with 302 in 2010 and 492 in 2011. In relative numbers this means a reduction from 0.7 % in 2010 to 0.4 % in 2011. Is this perhaps an indicator of (partly) successful methyl bromide substitution as a consequence of the Montreal Protocol? On the other side it is obvious that sulphuryl fluoride (SF) has not taken the place of methyl bromide in container fumigation. One of the reasons is the higher cost of SF compared to methyl bromide and the fact that SF is still not listed in the ISPM 15 standard.

An important conclusion is that standard gas free measurements for single components (performed with gas hand pumps plus measuring tubes) are in most cases not sufficient to protect workers and consumers from hazards due to gases and vapours included in containers or emanating from the goods packed in them. These measurements and formally correct gas free certificates may even generate a false feeling of safety and provoke additional hazards.

Apart from the substances highlighted in this contribution there are others that may appear more or less frequently depending on the cargo and origin of the containers. The SIFT measuring device disposes of a library of about 400 substances so that full scans can provide a broad overview during orientation measurements. This can be completed by using other

measuring equipment of high performance. Once the range of substances to be expected has been scaled down, routine measurements can be performed in a few minutes time. Using this approach, occupational safety can be raised to a very high level as large numbers of containers can easily be measured in comparatively short periods of time.

Measures taken to protect workers from hazards caused by inhaling toxic substances include different ventilation methods ranging from simple natural ventilation (opening of container doors for a certain period prior to gas testing) to forced ventilation with special equipment including active carbon filters if required until the concentration has fallen to safe levels. Taking care of hazardous containers in a professional way should be in everybody's mind to protect workers' as well as consumers' health. Using gas detecting devices is only one step to towards this goal.

A long term challenge remains: to influence production procedures of goods in their countries of origin and the way freight is packaged and treated against pest organisms in order to minimize hazards for workers and consumers. Alternatives are available for practically all hazardous substances such as solvent-free glues for shoes, treatments of agricultural commodities based on oxygen depletion or heat treatment of wooden pallets instead of methyl bromide fumigation. It is up to the consumers, importers and government agencies to impose safer procedures and to be willing to pay a little bit more for safe and sustainable production of goods.

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EFFECT ON GERMINATION AND MILLING QUALITY OF PADDY RICE STORED BY VARIOUS HERMETIC OPTIONS

C Cabardo*, E Secretario and M Gummert

Postharvest Unit, International Rice Research Institute
DAPO Box 7777, Metro Manila, Philippines
*Corresponding author's e-mail: *c.cabardo@irri.org*

ABSTRACT

Hermetic storage involves storing crops in a low-oxygen, high-carbon-dioxide atmosphere modified through the respiration of the crop, insects or fungi. In recent years, a number of 50-kg capacity hermetic storage bags have been released in the market as well as locally produced hermetic containers. It has become a problem to distinguish which of these are hermetic and which are not.

The study was conducted to evaluate the effect of various hermetic storage options on the germination and milling characteristics of paddy rice. Hermetic storage options evaluated were five types of hermetic bags: the IRRI Super Bag, GrainPro SGB IIZ, Kantong Semar, Pioneer and Purdue Improved Cowpea Storage (PICS) Bag. Small metal silos were also evaluated.

Paddy rice was dried at $43^{\circ}C$ using a recirculating dryer and then stored using the various hermetic storage options. Paddy rice stored in ordinary woven sacks served as the control. Oxygen content and carbon dioxide content inside the containers were monitored weekly. Samples were taken after 3 and 6 months of storage and tested for moisture content (m.c.), germination, insect count, discoloration, milling recovery, head rice recovery and whiteness. Results showed that various hermetic options had different effects on the germination and milling quality of paddy rice. This means that, based on the parameters tested, some options exhibited hermetic characteristics while some did not.

Key Words: paddy rice, hermetic storage, moisture content, germination, live insect count, discoloration, milling recovery, head rice recovery, whiteness

INTRODUCTION

From pre-Neolithic times, people in the Middle East and Europe have kept grain in holes dug in the ground as a form of airtight storage (Hall et al., 1956; Sigaut, 1980). In recent years, airtight storage has been developed both to control insects in dry grain and to prevent mold growth in high-moisture grain (Hyde and Burrell, 1982). Airtight or hermetic storage (HS) is based on the principle of generation of an oxygen-depleted, carbon dioxide-enriched interstitial atmosphere caused by the respiration of the living organisms in the ecological system of a sealed storage (Villers et al., 2008). Controlling the moisture content and insect growth in stored paddy rice maintains high germination in seeds and high milling recovery and head rice recovery in milled rice (Gummert et al., 2006).

Recent technological advances in plastic manufacturing have led to the development of large commercial PVC liners with airtight seals that provide the required durability for climate and gas permeability, and the physical properties to enable HS for extended periods of time (Rickman and Aquino, 2004). In 2004, the International Rice Research Institute (IRRI) in

collaboration with GrainPro, Inc. developed a 50-kg capacity hermetic bag called the "IRRI Super Bag" that fits inside a traditional storage bag or jute sack meant to be used by seed growers and subsistence farmers. Years of evaluation, dissemination and promotion of the IRRI Super Bag prompted other suppliers to come up with their own versions of hermetic bags and other hermetic containers as well. In 2011, IRRI obtained samples of different types of hermetic bags and sent them to a gas transmission laboratory and testing center for evaluation of oxygen permeability. Results of the test for oxygen transmission rate (OTR) of the hermetic bags in $ml/m^2 \cdot d$ were as follows: IRRI Super Bag (39), GrainPro SGB IIZ (0.6), Kantong Semar (1.9) and Pioneer (1470). The study was conducted to confirm the oxygen permeability report and to evaluate the effect of various hermetic storage options on the germination and milling characteristics of paddy rice.

MATERIALS AND METHODS

Paddy rice of the same variety (NSIC Rc148) and harvested from the same field was dried at $43^{o}C$ using a recirculating dryer. Dried paddy rice was stored in various hermetic units: IRRI Super Bags (IRRI SB), GrainPro SGB IIZ bags (ZIP), Kantong Semar bags (KS), Pioneer bags (PIO), Purdue Improved Cowpea Storage bags (PICS) and 100-kg capacity household metal silos (SILO) that are airtight and maintain the quality of stored products (Muhindi, 2008; AGST FAO, 2008). Dried paddy rice was also stored in ordinary woven sacks that served as the control (CTRL). The paddy rice was infested with stored insect pests at different growth stages with a ratio of 10 insects/kg before sealing. Oxygen (O_2) content and carbon dioxide (CO_2) content inside the hermetic options were monitored daily for 14 days and then weekly using Bacharach CD 98 plus multi-gas analyzer. Samples weighing 500 g from the various hermetic options were taken initially (INTL) and then after 3 months and 6 months of storage. These samples were evaluated for moisture content (m.c.), live insect count (LIVE INS), germination (GR), 500 grain weight (500 GW), discoloration (DSCLR), whiteness (WHT), milling recovery (MR) and head rice recovery (HRR). Samples were set up using a randomized complete block design (RCBD) and sample means were analyzed using analysis of variance at the 95% level of significance by RCropStat version 2.11.1 statistical software developed by the IRRI Crop Research Informatics Laboratory Unit.

RESULTS AND DISCUSSION

Oxygen (O_2) Content

From day 1 up to day 13 of storage, all hermetic options exhibited O_2 content >15% but, at day 22, IRRI SB, ZIP, KS and PICS exhibited O_2 content <10%. At day 56 of storage, there was a noticeable upward spike in the O_2 content of all hermetic options except SILO, which maintained its O_2 content >15% (Fig. 1A). This spike may be attributed to a combination of low live insect count and a high point in the oxygen that permeated through the plastic film of the hermetic bags from the outside atmosphere. At 105 days after storage and thereafter, IRRI SB, ZIP, KS and PICS exhibited O_2 content <10% while O_2 content of PIO was 10-14% and SILO 16-19%. The pattern in Fig. 1A is typical for hermetic storage systems where oxygen levels drop fast until most insects are killed and respiration processes are minimized. Oxygen leaking through the plastic film then leads to an increase of oxygen levels that favors insect development, which in turn reduces oxygen levels again after some time.

Carbon Dioxide (CO_2) Content

From day 1 up to day 29, IRRI SB, ZIP, KS and PICS showed an upward trend in CO_2 content (Fig. 1B). This may be attributed to the insects steadily consuming the oxygen inside

the hermetic bags. From day 35 to day 82 of storage, CO_2 content of previously mentioned hermetic options declined steadily (Fig. 1B), which may be attributed to a corresponding insect population decline inside the bags and also CO_2 leaking out of the plastic film through osmosis. After 29 days of storage and thereafter, IRRI SB, ZIP and KS exhibited similar CO_2 trends with readings from 9.0% to 13.5% at the higher end while, at the lower end, PIO and SILO displayed similar trends with readings of 1.4-5.4%. The CO_2 trend of PICS was in the middle with readings of 3.4-9.2%.
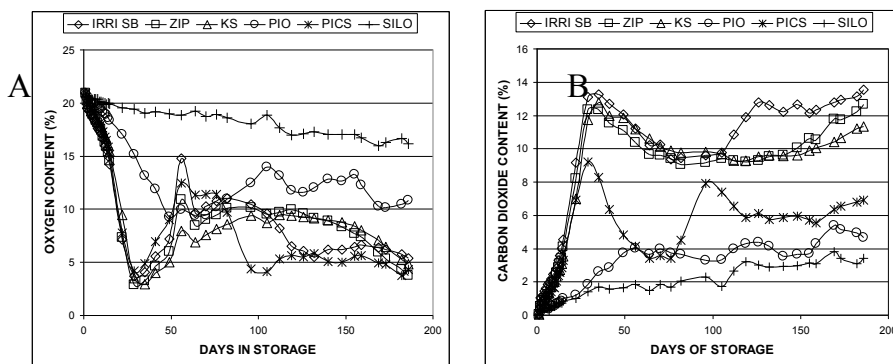

Fig. 1- Oxygen content (A) and carbon dioxide content (B) trends of inside atmosphere of various hermetic options during 6 months of storage

Moisture Content (m.c.)
At 3 months of storage, the MC of ZIP (12.0%) was significantly lower than the MC of INTL and all the other hermetic options (Table 1) while, at 6 months, there was a significant difference between the MC of KS (12.2%) and SILO (12.9%) (Table 2).

Live Insect Count (LIVE INS)
At 3 months, the CTRL displayed significantly higher LIVE INS compared with the other hermetic options (Table 1). At 6 months, the LIVE INS of SILO and CTRL were higher than with the other hermetic options although only SILO was significantly different (Table 2). Note also that, at 6 months, all hermetic bags except PIO exhibited LIVE INS < 0. Fixed structures like metal containers are difficult to seal properly. Also, they do not adjust their volume to atmospheric pressure changes and therefore under and overpressures can lead to leakages.

Germination (GR)
At 3 months, CTRL (67.3%) had the lowest GR but only ZIP (84.3%) was significantly higher than CTRL among the other hermetic options (Table 1). At 6 months, SILO (54.0%) had the lowest GR, which was significantly different from INTL, PICS, PIO and ZIP (Table 2). The low GR of CTRL and SILO may be due to increased insect damage of grains compared with the other hermetic options.

500 Grain Weight (500 GW)
At 3 months, CTRL (12.2 g) had the lowest 500 GW, which was significantly different from INTL, ZIP, KS, PIO and SILO, which all had 500 GW >13 g (Table 1). At 6 months, CTRL (10.4 g) again displayed the lowest 500 GW, significantly lower than that of INTL, IRRI SB, KS and PICS (Table 2). Although the MC at sampling was similar to that of the other treatments, open storage leads to moisture fluctuations and therefore to increased respiration,

causing higher dry matter loss. Insect damage to grains can also be credited for the decrease in grain weight.

Discoloration (DSCLR) and Whiteness (WHT)
At 3 months and 6 months of storage, analysis of variance of sample means exhibited no significant difference between treatments (Tables 1 and 2). This means that the various hermetic options had no effect on the DSCLR and WHT of the paddy rice stored.

Milling Recovery (MR)
At 3 months, CTRL (64.0%) showed significantly lower MR than the rest of the hermetic options (Table 1). At 6 months, all the hermetic options, with the exception of PIO (64.4%), had significantly lower MR than INTL (66.9%) (Table 2).

Head Rice Recovery (HRR)
At 3 months, IRRI SB (84.2%) had the lowest HRR, significantly different from the HRR of KS (86.3%) and INTL (89.0%) (Table 1). At 6 months, HRR of INTL (89.0%) was significantly higher than that of all the other various hermetic options (Table 2).

Table 1. Germination and milling characteristics of paddy stored in various hermetic options after 3 months of storage[*, **]

TRMT	m.c. (%)	LIVE INS	GR (%)	500 GW (g)	DSCLR	WHT	MR (%)	HRR (%)
INTL	12.8 a	5.0 c	89.0 a	13.3 a	13.9	40.6	66.9 a	89.0 a
CTRL	13.0 a	121.3 a	67.3 b	12.2 b	14.9	42.1	64.0 c	85.2 bc
IRRI SB	13.0 a	19.7 c	83.0 ab	13.0 ab	16.1	41.7	65.7 ab	84.2 c
ZIP	12.0 b	3.3 c	84.3 a	13.1 a	15.3	41.8	65.7 ab	85.5 bc
KS	13.0 a	4.4 c	79.3 ab	13.2 a	11.9	41.7	65.4 b	86.3 b
PIO	12.7 a	41.0 bc	77.3 ab	13.0 a	12.9	41.5	66.0 ab	85.8 bc
PICS	13.0 a	4.3 c	79.7 ab	12.7 ab	13.7	42.1	65.6 ab	85.7 bc
SILO	13.0 a	84.0 ab	75.0 ab	13.2 a	13.0	41.4	65.4 b	85.8 bc

* Means within a column with the same letter are not significantly different (p<0.05)
**TRMT=treatment, m.c.=moisture content, LIVE INS=live insect count, GR=germination rate, 500 GW=500 grain weight, DSCLR=discoloration, WHT=whiteness, MR=milling recovery, HRR=head rice recovery

Table 2. Germination and milling characteristics of paddy stored in various hermetic options after 6 months of storage[*, **]

TRMT	m.c. (%)	LIVE INS	GR (%)	500 GW (g)	DSCLR	WHT	MR (%)	HRR (%)
INTL	12.8 ab	5.0 b	89.0 a	13.3 a	13.9	40.6	66.9 a	89.0 a
CTRL	12.3 ab	105.0 ab	72.0 ab	10.4 b	12.9	41.4	62.0 b	84.4 b
IRRI SB	12.5 ab	0.0 b	74.3 ab	12.8 a	10.8	41.3	63.9 b	84.7 b
ZIP	12.5 ab	0.7 b	81.7 a	12.3 ab	11.6	41.4	63.8 b	83.5 b
KS	12.2 b	0.7 b	80.0 ab	12.6 a	12.2	41.2	63.2 b	85.2 b
PIO	12.5 ab	18.7 b	83.0 a	12.3 ab	10.9	41.4	64.4 ab	85.5 b
PICS	12.4 ab	0.3 b	86.3 a	12.7 a	12.0	40.9	63.8 b	84.8 b
SILO	12.9 a	159.3 a	54.0 b	12.4 ab	13.3	41.8	63.1 b	85.4 b

* Means within a column with the same letter are not significantly different (p<0.05)
**TRMT=treatment, m.c.=moisture content, LIVE INS=live insect count, GR=germination rate, 500 GW=500 grain weight, DSCLR=discoloration, WHT=whiteness, MR=milling recovery, HRR=head rice recovery

CONCLUSIONS

The following conclusions were drawn from using various hermetic options to store paddy rice for 6 months:

- The oxygen content trend for 6 months confirms the lab test report that PIO has significantly higher oxygen permeability than IRRI SB, ZIP and KS.
- Hermetic bags are still permeable to oxygen from the outside atmosphere but different bags have different permeability.
- Some hermetic bags not only prevent oxygen from penetrating inside the stored paddy rice but also prevent carbon dioxide from leaking out of the bags, evidenced by high CO_2 content readings (Fig. 1B).
- Metal silos are the least hermetic among the other options evidenced by the oxygen content not going below 15% and very high live insect count after 6 months of storage.
- Insect activity in all hermetic options except metal silos declined significantly.
- Germination rate, grain weight, milling recovery and head rice recovery were lowest when no hermetic options were used to store paddy rice due to the presence of insects in the stored paddy rice.
- The various hermetic options had no effect on the discoloration and whiteness of the stored paddy rice.

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SESSION 3

Methyl bromide alternatives

Chairpersons:
Jonathan Banks, Australia
Robert Ryan, Australia
Irfan Tunc, Turkey

METHYL BROMIDE ALTERNATIVES

Patrick Ducom*

CAPTSYSTEMES – 20, rue de Lormont Village - 33310 Lormont, FRANCE.
*Corresponding author's e-mail: *ducom.patrick@free.fr*

ABSTRACT

Methyl bromide (MeBr) is the fumigant of choice for almost all commercial and quarantine fumigations for disinfestation. Since 1992, it is regulated as a significant ozone depleting substance and it is phased out since 2005 with several exemptions like quarantine and preshipment, critical uses and article 5 countries. The main characteristics of MeBr are excellent effectiveness on insects, mites and nematodes, whatever the temperature if it is above 5°C. The exposure time is between 2 to 24 h, and the dosage changes with the temperature. As direct alternatives, only two fumigants remain, phosphine for all stored products and sulfuryl fluoride (SF) for some stored products and structural fumigations. For commercial fumigations, there are two ways to find alternatives: keep the same technique with a curative disinfestation or change completely the way of the treatment. The same (in-kind) techniques are fumigants, included phosphine gas, controlled atmospheres including high pressure CO_2, and also contact insecticides, heat and cold, irradiation and some other techniques. The other way is to completely change the production, for example by introducing a better IPM. For quarantine fumigations, it is much more complicated because all the standards used for disinfestation come from lot of studies carried out in many countries, often long ago, giving international rules, based on the probit 9 (99.9968% efficacy). The change to another curative disinfestation like heat, cold or other fumigants needs new studies to prove the quarantine efficacy which means money and time. Systems approaches capitalise on cumulative pest mortality from multiple control components to achieve quarantine security in an exported commodity. In conclusion, many techniques exist for commercial and quarantine MeBr fumigations but they require changes in habits and many times have economic implications. Nevertheless, the ban of MeBr is a fantastic opportunity to find new ways from old or new techniques.

INTRODUCTION

Methyl bromide (MB) is a very old fumigant, discovered in 1932. It was used for almost all the fields where disinfestation was a necessity, for commodities, wood and wooden products and quarantine on an infinite number of products. It has very particular properties, since it can kill all types of insects, mites, nematodes, etc. at any stage, very quickly, in 24h maximum, whatever the temperature; only the dosage varies with the temperature.

Unfortunately, in 1992, it was identified as an ozone depleting substance and it has been banned for use on stored commodities since January 2005, except for some uses under the CUN[1] rules and Quarantine and Preshipment. Alternatives to MB are to be found for all uses of MB. But, it is impossible to find a universal alternative like MB. That is why, for each family of product, or even for each species for quarantine purposes, an alternative has to be found or searched for. In fact, many compounds or techniques were available, but they were not in use because they were too specific, too expensive or led one to change the disinfestation method. Since MB permitted any disinfestation quickly, efficiently and cheaply, the incentive to do research was blocked. The decision of the parties at the Montreal protocol allowed one to have a new look at what existed other than MB and gave the signal for a lot of research which has often led to progress in quality on products, residues, human safety, etc. Every four years, the MBTOC[2] has requested there be an assessment report update on all the alternatives to methyl bromide and each year, it has the duty to evaluate the CUNs in light of the available alternatives and other criteria like registration, economics, etc…These assessments (1994, 1998, 2002, 2006 and 2010) are the basis for this paper and they can be found on the website at this address: http://ozone.unep.org/teap/Reports/MBTOC/index.shtml.

HOW METHYL BROMIDE WAS DISCOVERED AND FIRST USED?

MB was discovered by Mr Le Goupil, who was searching for an alternative to carbon disulfide (CS2) and hydrogen cyanide (HCN) to decrease fire risks. These fumigants were used under partial vacuum (Mallet system) for quick treatments in harbours. He carried out experiments with MB among some other compounds. He found that MB was an insecticide by itself, in a vacuum with an exposure time of 1.5h and different dosages. Even if the insects seemed to be alive just after the exposure time for the lowest dosage used, $25g/m^3$, they die very quickly after some hours. He presented its efficacy at the "Academie d'Agriculture" in 1932. Two years later, the harbours of Bordeaux, Le Havre and Marseille, were fumigating quite a lot of their imported products with MB. At that time, MB was not considered very toxic for humans, except in massive doses.

MB is a very good fumigant for many reasons: it is fast-acting; in 1.5 h to 2 hours or 4 hours in a vacuum, in 24 h at normal atmospheric pressure, the temperature does not change the exposure time, only the dosage is adapted to this temperature, a bigger dosage at low temperatures and conversely at high temperatures. It is very cheap. Its efficacy is very well known, since it has been used for almost 80 years and the CTP gives a very good criterion for efficacy.

Nevertheless, it also has some adverse effects. It leaves some bromide residue with acceptable MRL, but residue is present and sometimes exceeds the MRL. It has some negative effects on fruits, vegetables and seeds, which are manageable if precautions are taken. Its human toxicity is at a high level, acute and chronic, and it can be carcinogenetic.

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[1] CUNs : Critical Uses Nominations, but these require a country to demonstrate, year after year, the need for MB for some production.

[2] The Methyl Bromide Technical Options Committee (MBTOC) is the technical body for MB of the Technical and Economical Assessment Panel (TEAP) which advises the Parties to the Montreal Protocol.

Last, but not least, it was identified as an ozone-depleting substance and should be banned for all uses in the future.

These negative aspects have led companies to use some "alternatives", in fact, phosphine instead of MB, but just when the time and temperature were not a constraint and mainly for dried fruits and nuts, herbs and beverages.

For post harvest, MB was used as an insecticide and acaricide for all commodities, structures and, for quarantine, for wooden pallets, logs and many fruits and vegetables. The need for alternatives is huge, because one alternative can work on one product, or for one situation, but not for other ones.

HOW IS AN ALTERNATIVE TO METHYL BROMIDE DEFINED?

What is a real alternative to MB? It is difficult to answer this question since we can see the problem from different viewpoints.

The first viewpoint is linked to exposure time and temperature. Strictly speaking, an alternative to MB is a treatment, which kills pests in one shot and in a very short time whatever the temperature. Only heat and CO2 under pressure can compete with it, also methyl iodide in the future if it is registered and irradiation if available. Less strictly speaking, it is a treatment, which kills pests in one shot, but exposure time will depend on the temperature and it may be long. Typically, these alternatives are phosphine and CA.

There is another way, i.e., to completely change the production process. For example, for shipment, to avoid the necessity to have to carry out a quick treatment just before shipment, all the conditions of pest control should be changed. That has to be designed in the long term and may cost a lot.

The second view is a problem of efficacy, mainly for quarantine: Some pests are only killed by MB, or at least until now. Alternatives have to be found through research and the difficulty comes from the fact that lot of niches have to be studied.

The parties to the Montreal Protocol have defined what is an "official" alternative. *MBTOC assumes that an alternative (Refer Decision IX/6 1(a)(ii)) demonstrated in one region of the world would be technically applicable in another unless there were obvious constraints to the contrary e.g., a very different climate or pest complex. Additionally, it is recognised that regulatory requirements or other specific constraints may make an alternative available in one country but unavailable in another specific country or region. When evaluating CUNs, MBTOC accounts for the specific circumstances of each Party.*

This definition is not really only about technique, but it is necessary to have such a definition to evaluate the CUNs.

MBTOC has identified some remaining uses without technically effective alternatives: disinfestation of high-moisture fresh dates, cheese and cured pork products infested in storage in the USA, immovable museum artefacts (especially when attacked by fungi in some circumstances). Obviously, the MBTOC concept takes into account much larger considerations than just the technical points presented above. In these cases, alternatives exist but are not possible to use for the specific circumstances.

In fact, almost all uses of MB have an actual or theoretical alternative. There are just a few examples where there is still no alternative. Below are two examples.

Ditylenchus dipsaci Filipjev is a quarantine pest living in part inside alfalfa seeds, and only MB may kill it. Some countries do not use disinfestation, but that means that any lot recognized as infested is withdrawn. It costs a lot. That is the fact now in France where MB, like in all the EU, is banned for all uses and the loss for the seed companies is very high.

Cocoa beans are stored long before shipment in very big warehouses on the harbour in tropical conditions, like in Abidjan, Cote d'Ivoire. There is no way to control 100% of the infestations in these conditions. Usually, phosphine fumigation is carried out some days before shipment. If the ship comes sooner than scheduled, and if, just before loading, the stock is recognised as infested, the only means to export cocoa beans is to use MB fumigation.

ALTERNATIVES FOR COMMODITIES

There are many viable alternatives to methyl bromide fumigation for commodities, including: Integrated Pest Management (IPM), fumigants (phosphine, sulfuryl fluoride), contact insecticides, temperature manipulation (heating and cooling), controlled atmospheres (low oxygen and fumigation with carbon dioxide), and some other means less effective ones, which cannot be called alternatives like *Bacillus thuringiensis*, insect growth regulators, viruses, biological controls, etc.

IPM is a broad, rational, ecological approach to solutions of pest problems by combining, either concurrently or sequentially, biological, physical and chemical tools to ensure pest control while ensuring the protection of the environment, a maintaining of profitability and fulfilment of consumer demand for decreased or no pesticide use. An IPM program has to provide effective for pest prevention, based on an accurate pest monitoring system and provide training for industry staff for the tools employed to maintain an acceptable level of control. In the context of phasing out methyl bromide, IPM should be defined as a means of minimising chemical use, but also incorporating full site or curative treatments, involving fumigation or other processes as part of the program. IPM should be considered a required pre-requisite for any means to control insects. It is not an alternative to MB by itself.

Phosphine is the only fumigant, as methyl bromide was, which is widely registered for disinfestation of durable commodities. It is, in fact, the only available alternative extensively in use, for most commodities: cereals and legumes, dried fruit and nuts, beverage, herbs and spices, etc. It is a cheap fumigant, leaving no residue after desorption. It has replaced MB long before its regulation by the parties of the Montreal Protocol each time when temperature and time were not a constraint.

Its action against pests tends to be much slower than methyl bromide, with long exposures required, particularly under low temperatures. This is due to the mechanisms of phosphine action, which requires active oxygen metabolism and mitochondrial activity to allow, through respiration, the toxicity of phosphine. These mechanisms were well described by Kuang et al. (2008). That is why phosphine is usually not recommended at temperatures below 10ºC, or even 15ºC in some countries for stored product insects. Depending upon the temperature, fumigations with phosphine require from three to fifteen days for full effectiveness, this is in contrast to the 24-hour period used for methyl bromide over a wide range of temperatures.

Solid formulations produce *in situ,* by reaction of atmospheric moisture, the phosphine gas. They can be made of aluminium phosphide, or less commonly, magnesium phosphide in

several presentations such as tablets, bags and pellets, which are widely available and have been in use for over 50 years. Phosphine generated from metallic phosphides is produced slowly and that is another negative aspect of these formulations as an alternative to MB.

Two types of phosphine gas production have been developed to overcome its slow release. The first type are the cylinder-based formulations containing phosphine mixed with carbon dioxide at 2% (Eco$_2$Fume®), nitrogen at 1.7% (Frisin®) or pure phosphine (Vaporphos®) developed in recent years. The pure phosphine in cylinders has to be mixed with nitrogen to reduce the phosphine concentration (Horn Diluphos System®).

The second type are the generators working with formulations of metal phosphides and water to produce phosphine at a very high rate (phosphine generators from Degesch, Liang Mao Technology Development Co and UPL). With these devices, it is necessary to introduce the phosphine into a recirculation system, which is designed to give a perfect, even concentration of phosphine. Because the fumigant is rapidly available, it has been possible to shorten to some extent the exposure time whilst still maintaining fully effective disinfestation. In addition, phosphine is produced whatever the temperature and the humidity.

With these new quick production processes can we say that phosphine may replace MB? Not exactly, because temperature remains a constraint, which controls the exposure time. Even at 30°C, the biology requires 3 days to kill all stages. In conclusion, phosphine is an alternative to MB only if time and temperature are not a constraint.

Sulfuryl fluoride is an excellent example of the potential risks, which can happen when an alternative seems at first sight very good, but some hidden aspects suddenly appear.

Sulfuryl fluoride was developed in the late 1950's in the USA as a structural fumigant, mainly for termite control. Sulfuryl fluoride is highly toxic to post-embryonic stages of insects, but the eggs of many moths and beetles are difficult to control, especially at lower temperatures (Ciesla and Ducom, 2010). Early research indicated that the lower activity on eggs is primarily due to slow penetration through the chorion and eggshell.

The U.S. Environmental Protection Agency (EPA) first registered the agricultural use of sulfuryl fluoride in 2004 as an insecticide. Two main usages were registered, structural fumigation and commodity fumigations for cereal grain, beans, dried fruit, tree nuts, cocoa beans, etc. Temporary tolerances were established in 2001 for residues of fluoride resulting from the post-harvest treatment with SF. The tolerances are measured and expressed as ppm of fluoride. In EU, very few tolerances were established to allow a SF fumigation. The tolerance for nuts (25 ppm) is too low to allow treatment of chestnuts in normal fumigation practice. For cereals and cereal products, 2 ppm is usually not enough to allow a fumigation, even too low for inadvertent contact during a structural fumigation.

Very recently, on May 1 2012, the EPA opened a second comment period on the proposed order to revoke residue tolerances for sulfuryl fluoride (SF) on food and cancel associated uses. The Agency found that when residues on food products are combined with fluoridated drinking water and toothpaste, aggregate exposure levels are too high. The tolerances could be soon withdrawn and the use of SF reviewed.

A typical application rate is 40 to 60 g m^{-3} for a fixed duration of 24 hours with CTP's not over 1500 gh/m^3, essentially for reasons of residue.

Controlled or modified atmospheres is based on carbon dioxide and nitrogen. Low oxygen atmospheres, typically created by adding nitrogen to a fumigation enclosure, require that there be a maximum of 1% oxygen for effective action. Carbon dioxide atmospheres are typically applied at about 60% CO2 in the air. At this level, there is about 8% oxygen present,

normally enough to support most stored product pests indefinitely. CO_2 is thus regarded as having a toxic effect on insect pests and not acting just as an inert gas that reduces the oxygen level to below that supporting life. Most species are completely controlled by exposures of 1 - 3 weeks at 25 – 30°C.

In conclusion, CAs are useful alternatives if time is not a problem, but require a high level of investment cost. But, like for phosphine, CAs are a real alternative to MB only if time and temperature are not a constraint

The latest method combines carbon dioxide with high pressure of 20 to 40 bars and controls all stages and species of insects within less than three hours. It requires a gas-tight chamber, which can withstand pressure of this kind and may require a high capital investment. This method is really an effective alternative to MB, since it acts in several hours, whatever the temperature.

Heat treatment, if possible, is a real alternative to MB. Most stored-product insects are killed within hours after exposure to temperatures of 50°C or more (Fields, 1992), and, at lower temperatures, mortality can be related to the time that the insects are exposed. High temperature treatments are used for disinfestations of dried fruits and nuts and grains. Recently, heat treatment has been adapted to the treatment of dates in Israel by Navarro *et al* (2004). The treatment of 2 hours at 50°C resulted in 92% disinfestation of key pests; at 50 – 55°C, 100% mortality was observed. Navarro and co-workers noted that in their samples, the pests emigrated from the dates during the treatment.

Cold, at around 5°C, is typically used to prevent damage, multiplication and reinvasion of pests. Is not an alternative. However, freezing, with temperatures at around -20°C, is a real alternative to MB, but the treatment time is long, typically, one week, to allow the product to be frozen in the centre of a big bag, for example.

These treatments may be useful in specific instances, such as small museum objects or small quantities of cereals where a mild non-chemical disinfestation is required. Under these circumstances, they can present an alternative to methyl bromide use.

Dupuis et al (2006) examined the use of cold to kill all life stages of the bean weevil (*Acanthoscelides obtectus* (Say)) in beans immediately after harvest. The beans are used for both seeds and human food. They found that a temperature of -22°C has to be reached in the centre of the bean mass to ensure disinfestation.

Contact insecticides are used only on cereal grains. They work through residual deposits on the grain. These compounds are alternatives to phosphine more than for MB.

ALTERNATIVES FOR STRUCTURES

Structural fumigation is a pest management technique that provides broad-spectrum control and the ability of the fumigant gas to kill the pests that are not on the surface and cannot be readily contacted by other types of pesticide applications. The ability to penetrate through packaging materials, walls, and other areas to hidden infestations is particularly valuable in structural fumigation. That is why MB was used and an alternative to MB has to be a gas or heat.

Since the 1900's, pest control in mills and other structures were based on occasionally full site treatments. Contact insecticides were not yet discovered and to decrease insect pest populations, the only solution was a full site treatment. Fumigation was the most widely used technique, but in some countries such as the USA, heat was also used. The only fumigant

used was hydrogen cyanide (HCN). MB came much later as an alternative to HCN. There were some reasons to change: it was easier to apply than to manage the impregnated cardboard HCN formulations, there was much better penetration in all parts of the machinery and equipment even if it was full of accumulations of flour and milling residues. For example, in France, mills were fumigated with HCN until the 70's, in Switzerland until the beginning of 2000 and still in use in many eastern European countries. Nevertheless, its use should be reconsidered positively because of its efficacy against the major flour mill insects, *Tribolium* spp. A CTP of ~5 g h m^{-3} gives a LD90, at all stages, at 20°C instead 1000 g h m^{-3} or more for SF (Rambeau et al, 2000). Gas introduction should be revised.

Now in, pest control is essentially based on IPM. IPM should be considered a required pre-requisite for any means to control insects by an accurate pest monitoring system, associated with contact insecticides and full-site treatment. The presence of food and other materials in these facilities dictates the type of treatment, which may be utilised. Some fumigants, for instance, have no food tolerances set or may adversely react with non-food materials in the structure.

Sulfuryl fluoride is non-corrosive, an important characteristic for a fumigant, especially in settings where sensitive equipment and electronic devices are present. Sulfuryl fluoride is highly toxic to post-embryonic stages of insects, but the eggs of many moths and beetles, especially eggs of *Tribolium castaneum* (Herbst), are difficult to control, especially at lower temperatures. Efficacy of treatment for eggs was significantly enhanced by increasing the temperature from 25 to 30°C and a complete control of eggs of most species was obtained by a concentration time product of 1000 g h m^{-3} at 25°C and about 700-800 g h m^{-3} at 30°C.

A concern is the MRL for fluoride residues arising from SF treatments on cereal grains, flour and other derivatives. In some countries, like in EU, MRLs are uniform for all grain and grain products at 2 ppm fluoride. As a result, the flour present in the milling machinery has to be withdrawn by turning the mill one hour. That is an extra cost. In addition, if flour bins are built inside a building, SF may penetrate through the concrete or some leaks and the F^{-} residues will be very quickly higher than the 2 ppm limit. Klementz et al (2008) found, in these situations, residues between 3 to 10 ppm. But it is just impossible for the miller to empty these locations. This situation could also happen in the US if the MRL for cereals and cereal products were significantly decreased or removed.

At last, it should be mentioned that SF has been identified as a greenhouse gas. It is a strong absorber of IR radiation and has a long atmospheric lifetime (on the order of 30-40 years). It has a very high Global Warming Potential, GWP of ~4000, CO2=1. But it is not a current problem since its abundance in the atmosphere is very low, 1.5 ppt (1.5×10^{-6} ppm) in comparison with 390 ppm for CO2 (Andersen, 2009).

Heat treatment of structures is an age-old technology for managing insects associated with food factories and especially flour mills. In general, heat treatment involves raising the ambient temperature of a food-processing facility to 50-60°C and holding these temperatures for 24 h to kill all life stages of stored-product insects. Some companies extend this time to at least 36 h. Flour is a good insulator, and before heat treatments, flour mill staff do extensive cleaning to remove flour that can serve as refuge for insects to escape the heat. It is a useful action to avoid future infestation.

Mortality in insects at high temperatures depends on both the temperature and time of exposure. Insects are killed by desiccation and protein denaturation.

Heating the building may be carried out by different techniques. With forced air gas or steam heaters, the building is placed under positive pressure during the heat treatment. The heaters are placed outside the building. It allows heat to reach gaps in the building and equipment much better than static heaters. Heating may be conducted with many static electric heaters placed inside the building on different floors and different places. For both techniques, several fans should be placed on different floors to redistribute heat and to uniformly heat the building. During heat treatments, fans should be moved to eliminate cool spots-areas where the temperature is less than 50°C.

In addition to food-processing facilities, heat treatment can also be used in empty storage structures (bins, silos), warehouses, feed mills, and bakeries. It is an environmentally benign method for managing insects.

In conclusion, heat treatment is very effective as full-site disinfestation, except in crevices or in the basements which are difficult to heat to the required temperature.

Contact insecticides are part of IPM, used when monitoring shows an increase in pest population, and between the full-site treatments. They are used in two ways. The first, by spraying the surfaces with long-lasting insecticides such as pirimiphos methyl, chlorpirifos methyl, deltamethrin, etc. The second way, by fogging in the volumes with quick-acting insecticides, like pyrethroids or dichlorvos. Dichlorvos was banned in EU in 2008, and since then, we do not have effective foggings, because it is the only contact insecticide with a high vapour pressure, able to kill insects anywhere. There is no alternative to dichlorvos.

ALTERNATIVES FOR QUARANTINE

Quarantine pests are listed pests. These pests are potentially of economic importance to the area endangered thereby and not yet present there (Quarantine pest, e.g. list A1 for EPPO, European and Mediterranean Plant Protection Organization), or present, but not widely distributed and being officially controlled (Regulated non-quarantine pest, list A2 for EPPO).

A quarantine pest is not a common pest. In many countries, there is a confusion between unacceptable pests, which are qualified as quarantine pests whereas they are only common pests, even if they are of commercial importance. For example, for cereals or rice, pests are common, but they are often called quarantine pests. The difference is important for disinfestation: the target efficacy for control of a quarantine pest should be better than 99.997% mortality (probit 9). For "commercial" pests, most of the time, a 99% kill of all stages is recognised as sufficient.

Methyl bromide is the treatment of choice for many reasons, particularly because it is effective against all life stages of a wide range of pest organisms, fast-acting and recognised by almost all countries as a quarantine treatment. All other alternatives are more or less specific to a particular species. It is a field in continuous progress. MB used to be the unique compound used for treatment. Already, many alternatives have been discovered, but it is very difficult now to be able to search for all needs. Alternatives include many chemical, physical or even logistic systems.

The development of postharvest quarantine treatments can be both expensive and time-consuming. It is necessary to determine the most tolerant stage of the pest to the treatment by laboratory studies, then on a full scale, with a mortality of $> 99.997\%$.

Wood Packing Materials

Heat according to ISPM 15 is currently the only recognized treatment, apart from methyl bromide. Potential alternatives have been submitted to the IPPC and are under evaluation. These include SF, SF + methyl isothiocyanate (MITC) mixture, hydrogen cyanide, phosphine, methyl iodide and ethanedinitrile (EDN). Their efficacy against the main species of insects: of *Agrilus planipennis* (Emerald Ash Borer), *Anoplophora glabripennis* (Asian longhorned beetle) and *Bursaphelenchus xylophilus* (Pinewood nematode, PWN) is evaluated by the IPPC with the target of probit 9. Until now, any of these compounds was accepted.

MITC is a very effective compound used for soil disinfection and is also available mixed withethyl formate. The mixture is registered only in Japan for wood-boring insects. HCN is a very effective gas, but sorption is important and decreases the actual concentration.

Methyl iodide is very close to MB except that it does not deplete the ozone layer, since it is rapidly photo-decomposed by UV-light and remains for only a few days in the atmosphere. It seems to possess a lethality similar to MB. Some research has begun on its efficacy on certain wood-boring insects.

EDN has the widest spectrum of efficacy among the new fumigants. It is a gas at room temperature, with an almond-like odour. It is more toxic than MB; it can be used for treating soil, insect pests, weeds and diseases**.** It cannot work in dry conditions. The molecule belongs to Linde under the name of Sterigas^{TM} . It is very promising for wood treatment.

Wood and Logs

Logs, timber and wooden materials are infested by pests of quarantine significance. SF, SF + MITC mixture, hydrogen cyanide, phosphine, methyl iodide and EDN are identified as potential alternatives to MB.

Phosphine is used for the in-transit fumigation of New Zealand export *Pinus radiata* logs destined for China. It is now routinely used as a quarantine and pre-shipment measure and has partially replaced methyl bromide for this purpose. The current dosage specification requires at least 200 ppm v/v of phosphine to be maintained for 10 days. Due to sorption of the gas by the logs, top-up of phosphine is required 5 days into the voyage to prevent the concentration falling below 200 ppm. In-transit tests have shown an even gas distribution throughout the loaded ship holds. High concentrations of CO_2 also occur within the ship holds during the fumigation period that may increase the insecticidal action of the fumigant.

Perishables

This is a very complicated domain, since every species has its own pest and a particular sensitivity to the treatment. The main quarantine treatments apart from MB are physical, cold for a long time at low temperatures like for the Mediterranean fruit fly and others, or heat like for mangoes or papayas.

Many systems exist with combinations of various individual treatments, but each one is specific. For example, CATTS (Controlled Atmosphere/Temperature Treatment System) is a combination of short duration high temperatures under low oxygen, elevated carbon dioxide atmospheric environment.

It is strange that phosphine, traditionally used for stored products with a long exposure time at temperature >10°C or 15°C, shows very good efficacy at 0°C for fruit pests. Since the arrival of ability to inject phosphine, in cylinders or generators, it has become possible to get

an instantaneous high concentration of phosphine without ammonia, which may taint or damage the fruits. For example, it is the case with high moisture content dates and chestnuts. A preliminary trial to fumigate fresh fruits (apple, nectarines, pears, grapes and plums) at -0.5°C to +1°C using the Horn Diluphos system indicated that 36-72 h fumigation with 1500 ppm phosphine showed complete mortality of all insects tested at all developmental stages (eggs and larvae) without causing damage to the fruit (Horn et al. 2005). These results are not official ones, and phosphine is not yet an approved alternative to MB for perishables.

Ethyl formate in mixture with CO2 (Vapormate®) seems to have good properties for fruit and vegetables. Nevertheless, due to high concentrations often needed to kill all insects, some phytotoxic effects may occur. Nevertheless, the high moisture content Deglet Noor dates can be treated with $420g/m^3$, 12-hour exposure time, without any injury with 100% *Carpophilus* mortality. In this case, it is a real alternative to MB. It is registered in Israel for this usage.

COS, carbonyl sulphide, has been tried for perishables and flowers. It is very fast-acting, at above 15°C, mainly on surface insects, but it may be phytotoxic on certain species.

Gamma irradiation has been shown to be an efficient method to treat some fruits. Generally, doses from 0.05 to 0.2 kGy are sufficient for quarantine security. The advantage of irradiation is that the treatment is fast, non phytotoxic, residue-free and fruit can be treated in the final packaging. But the capital costs of irradiation are high, irradiation can render the pest stages sterile rather than dead, and, lastly, it has poor consumer acceptance in some markets.

Other non chemical methods are used. For example, the system approach for fruit flies, includes at least two independent measures which may be applied throughout various stages of the process, specifically during the growing period and harvest; post-harvest and transportation; and entry and distribution within the importing country.

The alternatives to MB for quarantine treatment is a broad very complicated field, because each plant species has its own pest and a sensibility to the stress caused by the treatment. In addition, if it is a chemical, it has to be registered, with residue tolerances. GRAS compound, like EF, or combination treatments with CA and temperature variation have the most promising future, if they can work at probit 9.

CONCLUSION

The banning of MB was considered unacceptable at one time. It was used everywhere as the fumigant of choice, effective on almost all pests and fast-acting whatever the temperature. We know that a universal alternative does not exist, which means that a huge amount of alternatives have to be adopted or found. The existing alternatives were not used at all, or very little for different reasons, mainly for longer exposure times in function of the temperature, but also for efficacy reasons in the quarantine sector. Researchers and companies, since 1992, have made a lot of effort to find new fumigants able to replace MB, like ethyl formate, carbonyl sulphide, ethanedinitrile or methyl iodide. Phosphine, an old fumigant used for dry commodities at temperature >15°C, was surprisingly tried on fresh fruit at very low temperatures. Controlled atmospheres and heat were modernised in their implementation.

Durable commodities have a lot of existing alternatives, and techniques were improved to better use them. Phosphine is a good example of the efforts made by researchers for a better understanding and by pesticide companies to produce it as a gas with different systems,

creating a safer and quicker fumigation. Controlled atmospheres are now in common practice with shorter exposure times than before.

Structure fumigation has two true good alternatives, SF and heat, but their implementation is more complicated than MB and the cost is much higher. That leads one to manage the treatments more carefully than before and could lead to better results.

Quarantine remains a very difficult problem. Quarantine gathers together a lot of products of very different natures: wood, seeds, fruits, vegetables, leaves, plants, etc. For each one, the pests are different. Efficacy and non phytotoxicity are the target with one more important constraint, the mortality rate must be higher than 99.997%. In addition, the alternative has to be accepted by the import country. At last, if the alternative is a chemical compound, it has to be registered and as the market is very small and the cost is getting higher and higher, there is no easy solution.

Many niches without alternatives to MB remain, mainly in the quarantine sector and that is an opportunity for research. Nevertheless, with the financial crisis, research may miss this opportunity. The choice for the decision-makers is very difficult: keep MB, that costs nothing in the short term and disregard the ozone layer, or ban MB without research, that costs almost nothing in the short term and disregard quarantine pests? Neither is the valid solution; we need to take this opportunity to start good research in all of the fields covered by MB but it will have to be financed immediately.

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COMPARISON OF EFFICACY OF METHYL BROMIDE AND SULFURYL FLUORIDE FUMIGATIONS IN CANADIAN PASTA PLANTS

Paul G. Fields

Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd., Winnipeg, MB R3T 2M9, Canada
*Corresponding author's e-mail: *paul.fields@agr.gc.ca*

ABSTRACT

We conducted trials in three pasta manufacturing facilities in Canada: three trials with sulfuryl fluoride (SF, ProFume®) and five trials with methyl bromide (MB). The efficacy of treatments was estimated using bioassays and trapping. For the bioassays, just before the treatments, vials containing adults and eggs of the red flour beetle (*Tribolium castaneum*) were placed throughout the plants. For trapping, pheromone traps were placed throughout the plant before and after treatments. Both MB and SF treatments were effective in killing 100% of adult *T. castaneum* in bioassay vials. All eggs were killed in the MB fumigations. In the SF treatments, egg mortality ranged from 69 to 81%. Some of the egg survival could be due to doing partial fumigations and leaky sections of the plant. Insects caught in the traps rose after the MB fumigations in Plants #1 and 3. In Plant #1, insects were trapped immediately after MB fumigation, but insects trapped did not rise to pre-treatment levels within the 20 weeks of sampling. After the SF fumigation, insects trapped rose to 100% of pre-treatment levels in 12 weeks in Plants #1 and 3. Comparing the SF with the MB fumigations is difficult, because pest pressures change from year to year, weather conditions change from year to year, two of the three SF fumigations did not fumigate the entire plant, pheromone trapping did not start before MB fumigations in Plant #3, and Plant #2 had insect counts too low to estimate efficacy. Plants #1 and 3 replaced a MB fumigation with the SF, and did not have to redo the fumigation with MB. Plant #2 did not conduct a full plant SF treatment. In Plant #3, additional pest control measures, fogging with dichlorvos and extra sanitation, were needed after the SF fumigation, that were not needed after the MB fumigation.

Key words: SF, MB, pasta, red flour beetle, *Tribolium castaneum*

INTRODUCTION

Methyl bromide (MB) (Banks, 2002; Fields and White, 2002) is a very effective broad spectrum fumigant. It is used around the world to control a wide variety of pests (pathogens, nematodes, weeds and insects) in diverse substrates (soil, food, museum artefacts, buildings, equipment and aircraft). It is the major tool to control insects in food processing facilities, such as flour mills, pasta production plants and breakfast cereal plants.

In 1992, methyl bromide was recognized as a significant ozone depletor and was to be phased out in 2005 for developed countries and 2015 for developing countries. Given that methyl bromide is such a widely used fumigant, critical use exemptions (CUE; MBTOC,

2007) for very specific uses of methyl bromide was allowed after these dates. CUE have been granted for some pasta plants in Canada from 2005-2011.

Sulfuryl fluoride (SF or SO_2F_2) has been proposed as a replacement for methyl bromide in the fumigation of flour mills and other structures (Bell et al., 1996; Banks, 2002). Sulfuryl fluoride was originally registered for termite control in 1961, under the trade name Vikane®. Since 1995, Dow AgroSciences has been expanding the use pattern of sulfuryl fluoride for use in flour mills, under the trade name ProFume® (Schneider and Hartsell, 1999). Currently it is registered in USA, Canada, across Europe, Mexico and Australia.

MATERIALS AND METHODS

Treatments

Plant #1 had a MB treatment in May 2007, May 2008 and September 2008 and a partial SF treatment in October 2007. As SF can not come into contact with food or food ingredients, not all of the plant was treated. The regrind area, which takes pasta and grinds it into semolina, is separate from the production area in the middle of one of the warehouses. It was sealed and treated with SF. Plant #2 had a MB treatment in June 2007 that treated the processing, warehouse, packing and semolina receiving areas. October 2007, they had a SF treatment of just the semolina receiving area. This area is adjacent to the processing area, doors and vents leading to the processing area and the outside were sealed before the fumigation. Plant #3 did a MB treatment June 2007. They did an SF fumigation of their entire facility in June 2008. We obtained the *ct*-product (CTP) and the Half Loss Time (HLT) for both SF and MB the using Fumiguide™. It is a computer program created by Dow AgroSciences to guide fumigators in ProFume fumigations.

Dome traps

Dome traps (Trece Inc) that are specific for trapping flour beetles were placed throughout the facilities, and the insects removed and counted each week. The traps were in the facilities 6-20 weeks before the SF fumigations. The traps were baited with a pheromone for the confused and red flour beetles (*Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst)) and a vegetable oil attractant. The vast majority of insects caught in the traps were flour beetles, and those data are reported here. The insect numbers are expressed as a percentage of the pre-treatment populations. The mean number of insects/trap/day in the pre-treatment periods was calculated and the means divided by pre-treatment mean and multiplied by 100 to give a standardized measure of efficacy.

Plant #1 started trapping (eighteen traps) in July 2007, several weeks after a MB fumigation, but well before the SF fumigation. They have made the trapping part of their pest management program and made available the data from 2008 and 2009. Plant #2 started trapping (twelve traps) well before the MB and the SF fumigation, but no flour beetles were ever found in pheromone traps, despite flour beetles being present in the plant. Plant #3 started trapping (fourteen traps) after the MB fumigation. As there is no pre-treatment trapping, no trap data for Plant #3 is reported. Plant #3 started trapping 6 weeks prior to SF fumigation and continued 20 weeks after fumigation.

Bioassays

The red flour beetle, *T. castaneum* (Steinbach strain), was used as a test insect. They were reared on white wheat flour with 5% brewer's yeast at 30°C, 60% r.h. Twenty unaged adults of unknown sex were placed in 16 g of culture medium in plastic vials, 4-8 d before the

treatment, and held at 20-30°C, 60% r.h. So at the time of the treatment there were twenty adults per vial and an unknown number of immatures, of which most would be in the egg stage. Eggs are the stage most resistant to SF. Eight vials were used as untreated controls. They were treated as the insects exposed to the treatment, but they were not held in the plant during the treatment. Twenty-five vials were placed throughout the facility a few hours before the treatment and retrieved a few hours after the treatment. About half of the vials were placed in the middle of the facility and half of them near windows or doors. Data loggers (Hobo Dataloggers, Onset Computers Inc.) were placed with each vial, and the temperature recorded every 15 minutes.

RESULTS and DISCUSSION

Fumigant

Full results are reported in Harrison (2009). Plants took 8-23 h to prepare for the fumigation, the gas was held for 22-26 h, and the plant employees were allowed back into the buildings 5-17 h after the end of the fumigation, total shutdown was 48-138 h. MB and SF fumigations had similar durations.

Less MB (26 g/m^3) was added than SF (58-62 g/m^3) (Table 1). The HLT for MB fumigations were longer than SF fumigations for Plant #1 and 2. The resultant CTP varied considerably between treatments (Table 1). For the SF fumigation in Plant #1, regrind and bin rooms were especially leaky with HLT of 2-5 h, resulting in CTP (412-492 gh/m^3, Table 2) below the target of 658 gh/m^3. In Plant #2, the CTP for SF (461 gh/m^3) was below the target of 600 gh/m^3, due to a leaky structure (HLT of 4 h). The regrind room of Plant #1 and the semolina room of Plant #2 had interior walls common to other areas of the plant that were not being fumigated. Normally, these adjacent areas would also be under fumigation, so leakage is not normally a problem. Even with that taken into consideration, the remaining areas of Plant #1 had a much lower HLT with SF (8.8 h) compared with the previous fumigation with MB (13.3 h). Higher winds, fluctuations in temperature or changes in sealing may account for these differences.

Methyl bromide CTP values were much higher (298-573 gh/m^3, Table 1) than that seen in the fumigations with flour mills (108-443 gh/m^3 average 286 gh/m^3, Harrison 2007). HLT in the pasta plants were much longer (9.9-17 h, Table 1) than in flour mills (1.2-12 h, average 5.4 h, Harrison 2007). This could be due to the pasta plants being newer structures and do not require explosion panels compared to flour mills.

Bioassays

Both MB and SF treatments were effective in killing 100% of adult *T. castaneum* in the bioassays. In the sulfuryl fluoride treatments, average egg mortality ranged from 69 to 81%. All eggs were killed in the MB fumigation (Table 1). Note, that the estimation of egg mortality is approximate, as the number of eggs varied from vial to vial. In the control vials, the number of adults that emerged from vials varied from 192-309 adults (247 $\pm$ 16, 8; mean $\pm$ SEM, n) for SF fumigation of Plant #1. Therefore, egg mortalities from individual vials, only give a rough estimate of survival at a given location, but all the vials taken together should give a good estimate of overall survival.

The lower than target CTP due to leakage was probably the cause of the egg survival in SF fumigations. There were 25 bioassays, located throughout the building. Whereas, the gas was sampled at ten locations in Plant #1 and Plant #3 and at two locations in Plant #2. This is sufficient sampling to estimate if more gas is needed in a particular area of the plant.

Table 1. Summary of efficacy of MB or SF fumigations in Canadian pasta plants.

Gas	Plant #	Start Date (month/yr)	Temp. Inside (°C)	Temp. Outside (°C)	Total Gas Added (g/m³)[1]	Gas CTP (gh/m³)	Gas Half Loss Time (h)	Bioassay Adult Mortality (%)	Bioassay Immature Mortality (%)	Trap catches Rebound Time (wks) Regularly Found[2]	100%[3]
MB	1	05/07	27.3	16.3	26	447	13.3	100	100	5	-
MB	1	05/08	-	-	26	494	12.3	-	-	1	never
MB	1	10/08	-	-	26	488	12.3	-	-	3	+ 20
MB[4]	2	06/07	29.0	20.0	-	573	17.1	100	100	-	-
MB[4]	3	06/07	30.9	17.4	22	298	9.9	100	100	12	-
SF	1	10/07	29.2	11.0	62	863	7.7	100	81	3	12
SF	2	10/07	27.6	12.1	59	461	4.0	100	69	-	-
SF	3	06/08	30.5	21.8	58	712	16.5	100	94	5	12

[1] g/m³ is very close to oz/1000 ft³
[2] 3 consecutive weeks of insects trapped some where in plant, Plant #1 MB 05/07 trapping only started 5 weeks post fumigation
[3] Weeks for populations to return to 100 % pre-treatment levels
[4] No pre-trapping before fumigation, therefore unable to calculate time to rebound to 100%

Table 2. Concentrations of SF in different areas of Plant #1 and survival of bioassay insects

Fumigant	Plant #	Start Date (month/yr)	Location	Gas CTP (gh/m^3)	Gas Half Loss Time (h)	Bioassay Adult Mortality (%)	Bioassay Immature Mortality (%)
SF	1	10/07	New Bin Room	492	1.9	100	53
SF	1	10/07	Regrind	412	5.1	100	33
SF	1	10/07	Rest of Plant	965	8.8	100	89

Howver, there can be significant differences in gas at different locations in the building. For example, a vial was located beside a door and had 61% immature mortality, but the CTP for this area is 1005 gh/m^3, which should be sufficient to kill all eggs, but the CTP at the vial is probably lower due to leakage of gas out the door.

Traps
Only trap data from the SF fumigations in Plants #1 and 3 are presented. Flour beetles were found in Plant #2, however, Plant #2 never caught any insects in the traps, despite traps being deployed well before the MB and SF fumigations.

After MB fumigation in Plant #1, there were adults present right after the fumigation. Insects caught in traps rose after the MB fumigation in Plant #1. Insects were consistently caught in traps 1-5 weeks after MB fumigation. However, insects never returned to the pre-treatment levels after the MB fumigation in Plant #1 within in the 19-20 weeks post fumigation. The Plant #3 MB fumigation placed traps 3 weeks after fumigation, so there is no estimation of populations before the MB treatment. Insects were consistently caught in traps 12 weeks after MB fumigation in Plant #3.

After the SF fumigation, insects caught in traps in Plants #1 and 3 rose to 100% of pre-treatment levels 11 and 12 weeks respectively, after the SF fumigation. Insects were consistently caught in traps 3-5 weeks after SF fumigations (Table 1, Fig. 1).

Alternatives to MB
There are several alternatives to MB fumigation to control stored-product insects, the main ones being SF, heat, IPM and phosphine combination treatment (Banks, 2002; Fields and White, 2002; Harrison, 2007; MBTOC, 2007). These alternatives have mainly been used in flour mills, with only a few studies being done in pasta manufacturing facilities (Subramanyam, 2006; Trematerra and Süss, 2006).

This study followed three SF fumigations in pasta plants. Two of the fumigations were partial fumigations, with sections of the facility sealed off from SF. This caused excessive gas loss through leaking into other sections of the facilities. In 2007 and 2008, the label for ProFume only allowed fumigation of empty structures. Dow AgroSciences has applied for food tolerances for sulfuryl fluoride in cereals. This is under review by Health Canada. Being able to fumigate the entire structure, as done with Plant #3, will simplify the fumigation, and allow for better retention of the gas and hence more effective fumigations.
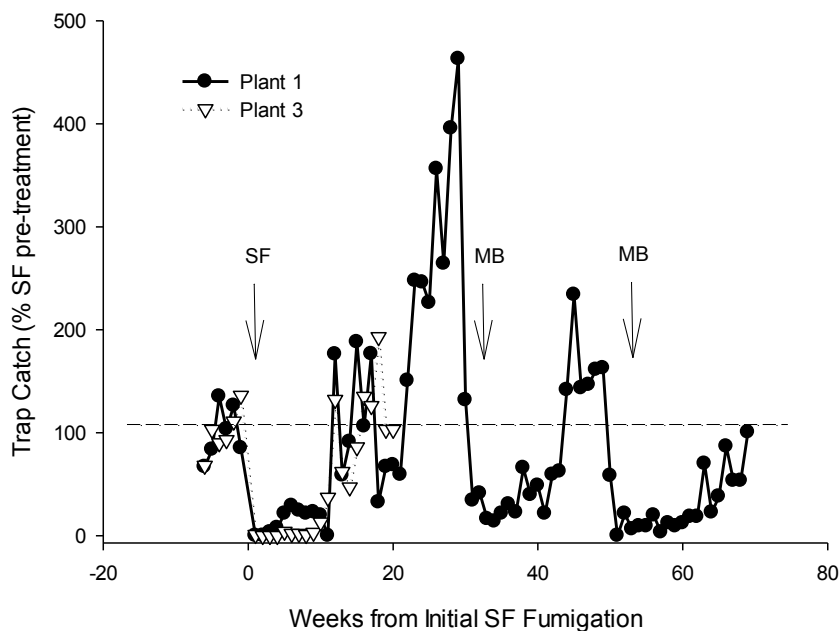
Fig. 1- The pheromone trap catch of flour beetles in Plants #1 and 3 before and after a fumigation with sulfuryl fluoride. Plant #1 continued trapping 60 weeks after SF fumigation (10/07) with a spring and fall MB fumigation in 2008. All numbers as % of SF pre-treatment.

Comparing fumigations is difficult, because pest pressures change from year to year, weather conditions change from year to year, two of the three SF fumigations were partial fumigations, pheromone trapping did not start before MB fumigations in Plant #3, Plant #2 had populations too low to measure the effect of fumigation and there are only three tests of SF. Immature mortality tended to be less with SF than with MB, although additional replication would be needed to verify this. Some of the immature survival could be due to doing partial fumigations, leaky sections of the plant and not achieving the target CTP values.

Plants #1 and 3 replaced a MB fumigation with the SF, and did not have to redo the fumigation with MB. Plant #2 did not conduct a full plant SF treatment. In Plant #3, after the SF fumigation in June 2008, nine additional foggings with dichlorvos were needed starting 2 months after the fumigation. Also, an additional cleaner was hired to increase sanitation to prevent an increase in insect populations. These measures had not been required in the past after MB fumigations.

All pasta facilities have extensive capacity for heating, so unlike flour mills, there would be no capital investment needed for boilers. Several issues would need to be addressed before heat could be used to control insects (Fields and White, 2002). However, there is one facility in the USA that has been using heat for insect control for several years (Subramanyam, 2006). The phosphine combination treatment (phosphine at 100 ppm, carbon dioxide at 5% and temperature at 30°C) has been used extensively in the USA and tested three times in Canada (Harrison, 2007). The European Community phased out MB in flour mills

and pasta plants since 2008 (MBTOC, 2007). The alternatives have been mainly, increased sanitation, increased contact insecticides, SF or heat treatments (Trematerra and Süss, 2006).

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THE DISINFESTATION OF HISTORICAL MONUMENTS: GAS SUBSTITUTES FOR METHYL BROMIDE

Daniel Floréal [1], Baslé Katia [2], Mounier Aurélie [1], Queixalos Inocence [3], Robbiola Luc [4], Ducom Patrick [5], Ciesla Yann [5], Fritsch Jérôme [5]

1 Institut de recherche sur les Archéomatériaux, UMR CNRS 5060, IRAMAT-CRPAA, Université Bordeaux 3, France. fdaniel@u-bordeaux3.fr
2 Centre interrégional de conservation et restauration du patrimoine, Marseille, France
3 Laboratoire Materia Viva, Toulouse, France
4 Laboratoire TRACES - UMR 5608 CNRS, Université Toulouse le Mirail, France
*Laboratoire National Denrées Stockées – LNDS-QUALIS, France
*Corresponding author e-mail: *fdaniel@u-bordeaux3.fr*

ABSTRACT

All areas involved by heritage protection are concerned by infestation problems. Historical buildings and their collections show more significant problems due to the difficulty in intervening in situ and the fact that, quite often, artefacts cannot be conveyed or disassembled. Environment of such areas is favourable to the development of insects and moulds. Attacks to materials, especially wood, require disinfestation by chemical treatments. Until recently, methyl bromide was the fumigant of choice because it has no adverse effects. Because of its phase out under terms of the Montreal Protocol due to its ozone - depleting properties it has been necessary to find alternatives. Candidate alternatives are sulfuryl fluoride (Vikane®), phosphine, dimethyl disulfide (DMDS) - use for soil fumigation as nematicide and fungicide - and cyanogen ('ethanedinitrile'), general disinfectant. These alternatives if used for furniture disinfestation within the framework of historical monuments may have effects on decorative elements. Also, wall paintings may include pigments and reactive gildings (gold containing decoration). It is of the highest importance to study, on selected samples, the effect of the fumigation treatments on these units of our cultural heritage. Consequently, an experiment with all these gases was carried out to define the various important physicochemical interactions with artefacts. In order to observe the effects of such gases with time on optical properties of pigments on historical artefacts, samples were artificially aged, then compared with reference samples.

Keywords: fumigation treatments, gildings, infestation, metal artefacts.

INTRODUCTION

Conservation of historical monuments and artifacts in them need pest control measures. When preventative measures fail, it is necessary to use curative methods, and, among them, fumigation. Until recently, methyl bromide was used for this purpose but it was banned under the Montreal Protocol in 2005 in developed countries. It may be replaced by sulfuryl fluoride (SF) registered in France in 2006, in the heritage field. It has already been subject of

publications related to its application to cultural heritage (Su, 1999). Other fumigants considered in this study are phosphine (PH_3), already well known, mainly for wood objects, and two other compounds, dimethyl disulphide (DMDS) and cyanogens ('ethanedinitrile', (EDN), which have valuable properties as insecticides but also as fungicides. In order to observe the effects of such gases with time, samples were artificially aged, then compared with reference samples kept in an air conditioned room.

MATERIALS AND METHODS

Fumigations

Fumigations were carried out in gastight stainless steel fumigation chambers of 1 m^3 for PH_3, DMDS and SF, and in a rigid PVC 200 L chamber for EDN. These chambers were held in an air conditioned room, maintained at a temperature of 15°C ±1°C.and r.h. 50 % ± 5 %. The mode of gas introduction was different, according to their properties.

Phosphine was generated from aluminum phosphide pellets to give 2 g m^{-3} of phosphine for an exposure time of 7 days. Ten pellets giving by hydrolysis 0.2g PH3 each were put in a dish.

DMDS was placed as a liquid in a petri dish. A fan was used to increase vaporization and to mix the gas in the chamber. The dosage was 60 g m^{-3} and exposure time 24h.

SF was injected as a gas from a small cylinder containing 1.5 kg of liquid and the quantity was measured by weight placing this cylinder on a scale. A fan was run to mix the gas in the chamber. The dosage was 150 g m^{-3} and exposure time 24h.

C_2N_2 was injected as a gas at a dosage of 200g m^{-3}, exposure time 24h. The gas was introduced with a 2 L Hamilton gastight syringe with the displaced air allowed to escape at the opposite side.

The ct-product (CTP) obtained was calculated to give a good measure of the activity of the introduced fumigant, except for phosphine were the only rule was that the concentration was maintained at over 200 ppm anytime (Ducom, 1999). Concentrations were measured at three levels in the chambers with an electrochemical cell for PH_3 (model MX2100, Oldham), and by thermoconductivity for DMDS, SF and EDN, (Fumiscope). The gas concentrations presented in table 3 show average concentrations, but the values throughout the chamber were very close.

Gilded Samples

To analyze the fumigation effects on the heritage materials, test pieces were produced according to ancient techniques of gilding (gold leaf application) on panel paintings and on stone, with both supports commonly found in the heritage area.

On panel paintings, nine types of gilding have been applied, following the data sourced from ancient texts (Perrault, 1992). The basic preparation is common to all samples. The underlayer was composed of several calcium carbonate layers to which a 10 % skin glue had been added. This is known as distemper gilding here. Various types of gildings were applied to this preparation (Table 1).

On stone (limestone), seven types of gildings have been tested. The stratigraphic structure of the gilded samples is shown in Table 2.

Table 1. Gildings on wood panels, used in the studies

B1	Distemper gilding (gold) on Armenia bowl (skin glue)
B2	Distemper gilding (gold) on Armenia bowl (skin glue) + rabbit skin glue varnish
B3	Distemper gilding (gold) on yellow pigment layer (skin glue)
B4	Powder gilding (gold) on yellow pigment layer
B5	Distemper gilding (silver) on Armenia bowl (egg)
B 6	Distemper gilding (silver) on Armenia bowl (egg) - dammar varnish
B7	Distemper gilding (brass) on Armenia bowl (egg)
B8	Mixtion gilding (brass) on yellow pigment layer
B9	Powder gilding (bronzine) on yellow ochre pigment layer

Table 2. Gildings on stone, used in the studies

P1	Mixtion gilding (tin) on red ochre layer
P2	Mixtion gilding (tin) on blue azurite layer
P3	Mixtion gilding (tin) on red cinnabar layer
P4	Distemper gilding (gold) on Armenia bowl (skin glue)
P5	Distemper gilding (gold) on Armenia bowl (egg)
P6	Mixtion gilding (tin + gold) on red ochre pigment layer
P7	Mixtion gilding (gold) on red ochre layer

Artificial ageing

Artificial ageing seeks to simulate the degradation processes of the materials. The hygrothermal ageing protocol developed previously (Aze, 2005) was used. This ageing (V1) reproduces 8-hour cycles that follow the temperature and relative humidity variations recorded in the Aix-en-Provence cathedral during a year. Samples were submitted to 90 8-hour cycles (= 1 month) each cycle consisting in a succession of 4 climate phases for a duration of 90 minutes each, with linear transitions of 30 minutes: (a) High humidity phase (r.h. = 85 %, T = 18°C); (b) Low temperature phase (r.h. = 0 %, T = -10°C); (c) Dry heat phase (r.h. = 25 %, T = 40°C); (d) Wet heat phase r.h. = 60 %, T = 30°C).

Light ageing (V2) is cumulative, unlike the hygrothermal ageing (Feller, 1994). Samples were submitted to UVB (313 nm) for 400 hours at constant temperature, close to 45°C. The equipment (QUV-Panel, Q-LAB) supplied UVB centered around 313 nm. The spectral irradiance was 0.71 W m^{-2} nm^{-1} for a standard distance to the tubes of 4.5 cm.

Methods
Sample preparation (stratigraphic sections): some samples have been embedded in a resin and observed by optical microscopy to examine the alterations due to gases, on the metallic coat and on the preparation coat (underlayer). Samples and their cross-sections are examined and photographed using a Leitz polarizing microscope connected to a digital camera.

A colorimetric survey of the metal surface of the sample has been performed to see the chromatic variations before and after treatment and ageing. The system used is a HunterLab portable equipment, type Miniscan XE Plus 4000S, operating under standard light D65 with an observation angle of 10° allowing spectra acquisition in the 400-700 nm spectral field with a resolution of 10 nm. The analyzed surface was a 6mm diameter disc. Thirty observations were made for each sample, before and after treatment. The average results have been used for the calculation of the colour differences DE* (Dupont and Steen, 2006).

Some of the materials have been analyzed using a JEOL scanning electron microscope (SEM-EDS) JSM 6460LV (low vacuum) coupled to an EDSX (Oxford INCA 300) energy dispersion X-ray detector.

RESULTS

Evolution of gas concentrations during the exposure times
Table 3 shows the evolution of gas concentrations in each chamber. As usual, sorption is high with EDN, but very small with SF. DMDS was vaporized very slowly, 6 hours to give the maximum concentration. The hydrolysis of phosphine was slow, probably because the r.h was low (50%) when the chamber was closed. The rate of evolution of gas was similar to those obtained in good commercial fumigations.

Table 3. Evolution of gas concentrations in each chamber for initial dosages of 200 g m^{-3} for EDN, 150 g m^{-3} for SF, 60 g m^{-3} for DMDS and 2 g m^{-3} (1400 ppm) for PH$_3$

EDN		SF		DMDS		PH$_3$	
Exposure Time (h)	Concentration (g m^{-3})	Exposure Time (h)	Concentration (g m^{-3})	Exposure Time (h)	Concentration (g m^{-3})	Exposure Time (h)	Concentration (ppm)
0	0	0	0	0	0	6	380
1	193.5	1	123.5	2	31.2	24	1033
3	165	5	122	3	45.5	27	1081
6	147	8	123.5	5	56	29	1105
22	94.5	24	120.5	8	48.75	48	1159
24	91.5			24	42.9	72	1108.5
						150	895.5
						168	850
CTP (g h m^{-3})	**3041**		**2873**		**1061**		**-**

Gilded panel paintings
Visually, nearly no chromatic variation has been noticed in literature (Koestler et al., 1993). The colorimetric results « refine » these impressions. Some small decreases in brightness and a yellowing have been observed. Variations in DE* lower than 5 are perfectly acceptable and

are typically reported as insignificant. Generally speaking, on the chromatic point of view, artificial ageing had only a small effect on the gold and silver foils. On the other hand, copper foils were darker.

DMDS and sulfuryl fluoride gave satisfactory results on the whole samples. Concerning B1, B5 and B7 samples processed by sulfuryl fluoride, sulphur and chlorine, concentrations higher than those of the reference samples (untreated) were found on a red gesso area revealed by a tear on the metal leaf. These same deposits have been noticed on the rabbit skin glue which covers the gold leaf of sample B2. Studies performed by Getty Conservation Institute (Baker et al., 1990: Derrick et al. 1990) already showed this fact. It seems that they are due to gas impurities. For this reason, purification of the matter before fumigation was required. So, a filter consisting of small marble stones was installed between the sulfuryl fluoride cylinder and the treatment chamber.

In the case of DMDS, higher sulphur concentrations have also been noted by SEM-EDS on the surface of the red gesso. As with sulfuryl fluoride, sulphur does not seem to induce alterations to the red gesso. Sulphur was also found on the dammar varnish on the surface of sample B6. No significant variations were found on the dammar varnish spectrum processed with DMDS versus the spectrum of the unprocessed sample, but under the influence of the UV ageing. The sample covered with dammar varnish and processed with DMDS became highly yellowed ($Db* = 12.9$).

Concerning C_2N_2, very poor results were found on samples B1, B3 and on copper alloys (B7, B8 and B9).

Hydrogen phosphide gave satisfactory results on samples B1 to B6. But the effects were disastrous on the copper alloys of samples B7, B8 and B9. Such results could be anticipated based on a previous study (Bertholon, 1993). These samples as well as samples B4 showing a too small gilded surface have been intentionally discarded from the colorimetric investigation. Some surface depositions have been featured by SEM-EDS. Concerning hydrogen phosphide, high phosphorus concentrations were found on samples B7, B8 and B9 (copper alloys). Phosphorus traces are also found on the skin glue of sample B2 as well as on the silver leaf. Because of its action on copper, C_2N_2 should be prohibited from use for historical monument treatment. The three other treatments gave satisfactory results as a whole, except hydrogen phosphide on copper alloys.

Gilding on stone
Colour evaluations were performed on the reference samples, on samples treated by the three gases and then on the aged samples (Fig. 1).

The effect of the treatments on the luminance is not statistically significant. All the evaluations are based on the colour difference DE*. For the four treatments, a slight blackening or tarnishing of the surfaces of the tin leaf gildings (P1, P2 and P3) was noted.

C_2N_2 treatment has the highest effect on the colour of the gildings. The cyanogen (C_2N_2) treated and non-aged gildings (T4V0) showed a blackening of the metal surface of gold leaves (PM5, PM6 and PM7) and a marked yellowing of P4 (as if an opaque varnish coat had been superficially applied). C_2N_2 has a small effect on tin (P1, P2 and P3), just a small «lightening» of the metal leaf.

DMDS is the treatment having the lowest effects.
An assessment of the effects of some fumigants on pigments and metals shown that sulfuryl fluoride induces visible modifications of the zinc and lead appearance (Kigawa et al., 1999). As to pigments, tests performed on painting samples made of proteinaceous lacquer coating

containing linseed oil plus a mixture of white lead and oil and at last a pigment coat (cobalt blue, Prussian blue, yellow ochre,...) showed that sulfuryl fluoride affected most of the samples in terms of change in colour and brightness (Koestler et al., 1993).
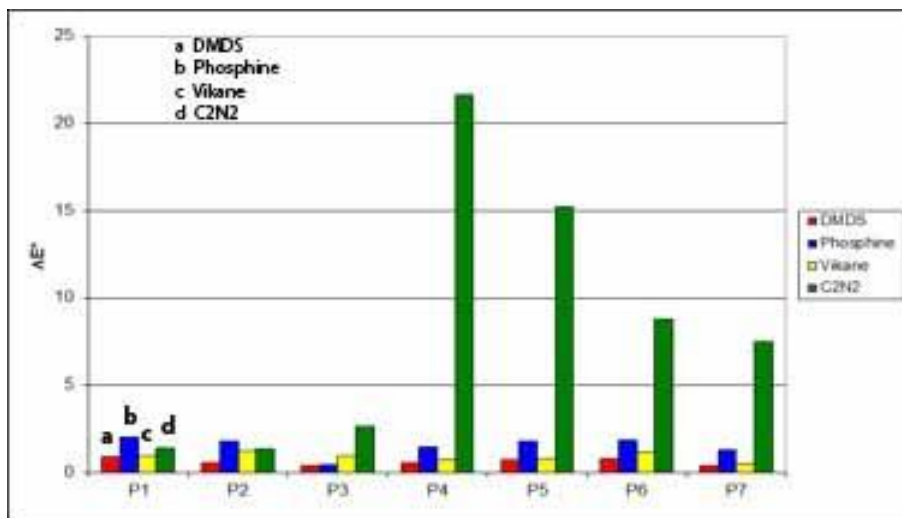

Fig. 1- Colour difference (DE*) of the gildings with regard to the references (controls).

An alteration of azurite with phosphine was visible on tin gildings on azurite (P2). When the copper based pigment was used, it became black (Fig. 1). This reaction was expected as azurite is a copper based pigment $Cu_3(CO_3)_2 (OH)_2$ and it has been shown previously that phosphine had a high corrosion effect on copper and copper alloys (Bertholon, 1993).

Effect of ageing on treated gildings
Figs. 2 and 3 show the effects of the hygrothermal (V1) and light (V2) ageing on the treated samples. Hygrothermal ageing appeared to be the most harmful to the conservation of gildings (Fig. 2), especially on samples treated following distemper gilding. The highest effect was noticed with DMDS (DE* values from 4 to 8) and C_2N_2 treated gildings (DE* values > to 15).
Concerning C_2N_2, on P2, the oil containing binder yellowed and it was noticed that the blue azurite pigment turned to green. V1 results in small modifications for tin gildings treated by C_2N_2. For zwischgold gildings, a deep yellowing of the P4 and of the P5 gold was noticed as well as a network of surface small cracks. The highest alteration pertinent to all ageings relates to gold gildings with a proteinaceous binder: high metal shrinkage and « deflaking » of the metal leaf carrying the red ochre coloured layer and a «Dry» appearance with, sometimes, a nearly complete disappearance of the gold. Concerning gold gilding, a high yellowing of the metal surface followed by dulling was noted. For both distemper gilding samples no data has been recorded, the metal leaf having suffered a lot: gold leaf « deflaking » and shrinkage, and material loss.
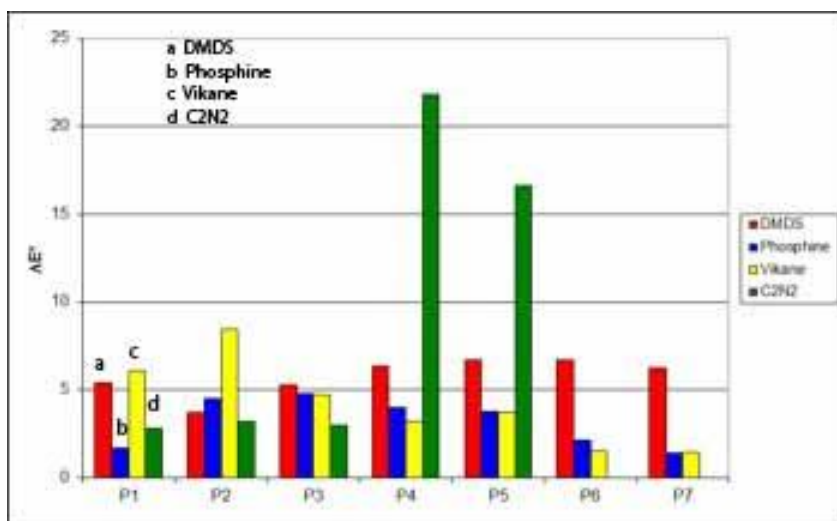
Fig. 2- Colour difference (DE*) with regard to the aged references (hygrothermal ageing V1).

Except for C_2N_2, UV ageing does not seem to have produced such effects on gilding (Fig. 3), but gold gilding intensive dulling and yellowing was observed. No significant colour deviation after UV ageing was noted.
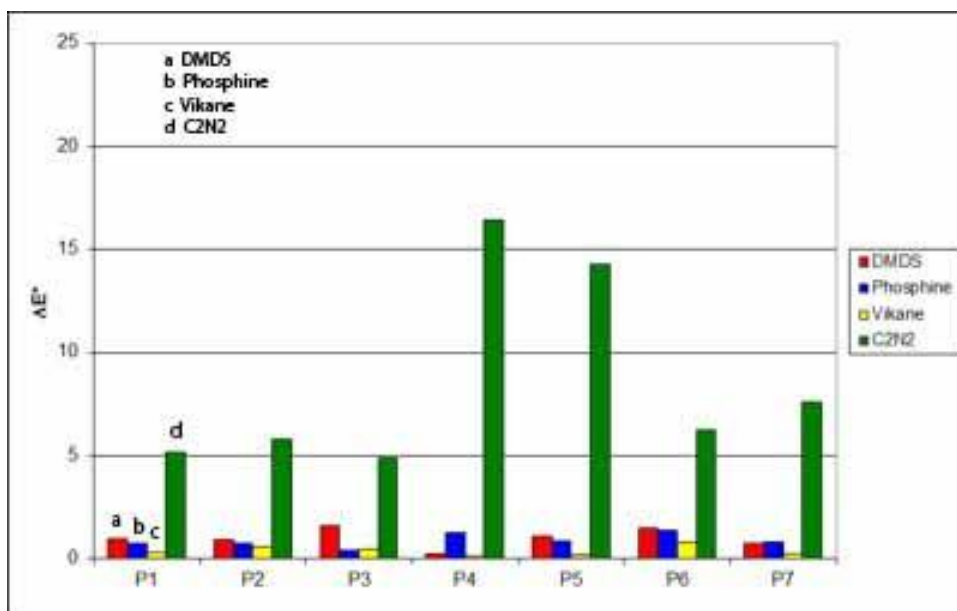
Fig. 3- Colour difference (DE*) with regard to aged references (light ageing V2).

For C_2N_2 treated samples, a dulling of the metal surfaces (loss of the bright metal aspect, for P1, P5 and P7) and sometimes a yellowing of gold which becomes more «pasty», more orange (P2, P4) has been noticed. Also there was metal delamination and the metal

formed a network of small cracks with P4 and P5. These results and observations show that C_2N_2 is not a suitable disinfestation method for this type of materials.

CONCLUSIONS

The objective of this study was to be able to select the best fumigant as a fumigant for historical monuments and artefacts as an alternative of methyl bromide. However, each gas showed advantages and limits and the best choice should be a compromise considering all the parameters, whatever the processing, cost and alterations of the materials.

Phosphine does not give satisfactory results. Indeed, it is totally discarded due to its irreversible effects on copper alloys, in spite of its easy application. On painted plasters, this gas induces the highest colour alterations after treatment, especially on gold gildings. It should not be recommended for the disinfestation of heritage premises in the presence of metal artefacts with silver, copper (or alloy containing copper), tin or lead.

Study performed on gilded wood panels shows that DMDS, which is not registered in France today, could give satisfactory results, except its very persistent garlic smell and dammar varnish yellowing under light. Should these effects be confirmed, they, undoubtedly, would result in the withdrawal of this gas for this utilization.

Results and observations show that using C_2N_2 as disinfection agent seems to be compromised and not suitable to this type of materials.

DMDS and EDN have less corrosive effects than phosphine. However, they lead to changes on all metals tested, higher for copper and lead, and result in instability of the compounds during ageing. They should not be recommended.

Sulfuryl fluoride gives satisfactory results, as the impurities in the technical grade can be eliminated thanks to the installation of a filter. Studies performed by the Getty Conservation Institute were favourable as far as the use of SF on cultural heritage artefacts is concerned. Among others, several churches have been treated in Germany and no noticeable effect has been reported. Recently, in France, the first fumigation applied to a historic monument (Hauteluce church) has been performed in 2007. Despite of its very high cost and that the required active substance has to be at least double that for methyl bromide for the same efficiency, sulfuryl fluoride (Vikane) is the most suitable substitute gas for methyl bromide.

ACKNOWLEDGEMENTS

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APPENDIX

Nomenclature of gases and samples:
Gas: No treatment (T0), DMDS (T1), hydrogen phosphide (T2), sulphuryl fluoride (T3), C_2N_2 (T4).

Ageing: No ageing (V0), hygrothermal ageing (V1), light ageing (V2).

The effects of gases with time can be analyzed from the comparison of artificially aged samples and compared with the reference samples (T0V0): no treatment, no ageing. Indeed, the effects of gases with time can be analyzed from the comparison of artificially aged

samples and compared with the reference samples submitted to the same ageing (T0V1): no treatment and hygrothermal ageing and (T0V2): no treatment and light ageing.

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CONTROL OF *BREVIPALPUS CHILENSIS* WITH PHOSPHINE ON FRESH FRUITS UNDER COLD STORAGE FUMIGATIONS

Pedro Horn*

FOSFOQUIM S.A., Chile
Corresponding author's e-mail: *phorn@fosfoquim.cl*

ABSTRACT

The fruit export industry in the world faces a significant problem with the phase out of methyl bromide, the most important fumigant for the control of quarantine pests on export fresh fruits. In 2005, Fosfoquim developed in Chile a method where pure cylindered phosphine, VAPORPH$_3$OS®, is applied through the Horn Diluphos System™, for the control of pests on fresh fruits under cold storage conditions. The Horn Diluphos System™ is a phosphine and air blending equipment developed in the year 2001 in Chile that allows the dispensing of pure cylindered phosphine mixed with air at a concentration below the point of ignition. Since then this method has been used commercially in a large scale in Chile for the control of different pests like mealy bugs, thrips, moths and scales. Normal fumigation conditions are cold storage temperature (normally 0ºC), 24 – 48 h exposure time and 1,000 to 2,000 ppm phosphine concentration. In Chile only, almost 12,000 fumigations have been made applying pure cylindered phosphine VAPORPH$_3$OS to fresh fruits.

Some countries have specific pests that limit their export to other countries. This is the case of Chile with the false Chilean Mite. Although good control is achieved with phosphine under the normal fumigation conditions at 0ºC, 24 - 48 h and 1,000-2,000 ppm, the pest cannot be controlled 100 % for all stages. After extended research, new conditions were found to effectively control the false Chilean mite using pure cylinderized phosphine blended with air. The tests for *Brevipalpus chilensis* were carried out on apples at a temperature of 0ºC, a concentration of 300 ppm and 10 days exposure time. The tests showed that adults and nymph stages could be controlled at 100 %, while the control showed a survival rate of 87.9 %. Tests were made with 100 individuals with 3 replicates.

Key words: phosphine, cylindered phosphine, Horn Diluphos System, HDS, VAPORPH$_3$OS®, fumigation, fresh fruit fumigation, vegetable fumigation, cold storage fumigation, postharvest phosphine fumigation, mites, quarantine fumigation.

INTRODUCTION

Phosphine gas, has been used for over 75 years as an insecticide, and it is presently the most accepted fumigant for stored products, like grain, nuts, dried fruits and others

Phosphine has the great ecological advantage that its application in pure form from cylinders does not leave harmful residues in the environment or in treated products.

Nevertheless, because phosphine forms explosive and self flammable mixtures with air at concentrations over 18,000-ppm, it had not been possible to apply phosphine in its pure

state by means of direct dilution with air, even though this would be the best way to apply the gas.

Phosphine has been always obtained by on site hydrolysis of a metal phosphide compound, like aluminum phosphide or magnesium phosphide.

In the year 2001, a new a method was developed to blend directly pure phosphine with air. Since then, this new tool has been used now for some years in many countries for different applications such as:

- Fumigation of grain in silo bins, flat storages and bunker storages
- Fumigation of flour mills, minimizing risk of corrosion
- Sea containers
- Fumigations chambers

With this new tool at hand, where pure phosphine could be applied without generating the byproducts ammonia and carbon dioxide, phosphine started to be used in Chile for the control of pests in fresh fruits and vegetables in the year 2005.

For several years, before this new treatment was developed, phosphine had been investigated as a fumigant for the treatment of fresh fruits and vegetables. The research has shown good results as for mortality of insects. However, acceptable results were not obtained in reference to the quality of treated fruit, which had always suffered damage. This damage has been caused by two reasons mainly: presence of ammonia and relatively high fumigation temperature, over 15°C, to which the tests have been exposed. At high temperature the metabolic activity of the fresh fruits and vegetables is still important and, as phosphine acts through the blocking of the metabolic activity, phosphine also affects the quality of the fruits at high temperature.

Aluminum phosphide or magnesium phosphide based products have the great disadvantage that, if they are used at low temperature, they produce phosphine very slowly and that they always produce ammonia as a by-product and ammonia is known to be very phytotoxic. For that reason, damage to the fruit is always expected when using metal phosphides.

But on the other hand, because of the pyrophoric characteristics of the product, until the direct blending of pure phosphine with air was developed in the year 2001, it was not possible to apply pure phosphine for fumigations.

When phosphine started to be used in Chile for fresh commodities, like fruits and vegetables, it was found that it is possible to carry out fresh fruit fumigation with pure phosphine free from ammonia, at low temperatures and with high gas concentrations, without affecting the quality of the fruit and eliminating most of the pests hosted on fresh fruits and vegetables.

The gas is normally applied in fumigation chambers, cooling chambers or controlled atmosphere chambers.

It was determined that this can be done successfully if the fumigation is carried out at a temperature between −1.5 and 6ºC with a concentration of pure phosphine free from ammonia, between 1000 and 1500 ppm in a sealed enclosure, with an exposure time between 24 and 72 h.

It was discovered that when lowering the temperature, it is possible to carry out the fumigation with a very high concentration of phosphine with no damage to the fruit, since at that temperature, the metabolic activity of fruit is slowed down. This high concentration compensates the low activity of the insects at low temperatures, controlling the pests.

Some small off-taste in fumigated fruit was observed following the fumigation, but this disappeared after 5 or 6 days of storage at low temperatures.

The best way to do the treatment with phosphine is to fumigate the fruit directly in the cooling chambers, where the fruit is stored after the selection process, and leaving the cooling system working during the whole fumigation period.

The fruit is preferably treated at the optimum cold storage temperature of each species. For example, for apples, grapes, kiwis and berries, pears, nectarines, peaches, etc. it is preferable the treatment with temperatures from –1.5 to 2°C. Other fruits like avocados, citrus and mangoes are preferably to be treated to their corresponding cold storage temperature.

So far, the treatment with high dosages of methyl bromide (30-60 g m^{-3}) was the most used and effective fumigation method for fresh fruits, which, although quick and efficient, has a series of disadvantages, such as the known ozone depletion, phytotoxicity, residues and worker exposure.

The use of pure phosphine has as main advantages, compared to methyl bromide, that it does not leave bromine residues in the fruit after treatment and that after liberated into the atmosphere, phosphine is oxidized into phosphates by the action of sunlight. It has also great advantages for workers, as the fumigation operation could be done from the outside of the facility, avoiding workers exposure to confined spaces, and it is not required to manipulate directly the fumigant as the fumigation equipment is a closed system

It has been demonstrated that it is possible to control the main pests of the fruit, such as the mealybugs, *Pseudococcus spp*; apple moth, *Cydia pomonella*; *Eulia* spp,, *Proeulia* spp.; fruit tree weevil, *Naupactus xanthographus*; Mediterranean fruit fly, *Ceratitis capitata*; fruit flies, *Bactrocera* spp*., Anastrepha* spp.; and *Thrips* spp. (Horn 2006).

Only in Chile, over 12,000 fumigations have been made applying pure cylindered phosphine VAPORPH$_3$OS® to fresh fruits

But, even though, most of the pests on fresh fruits could be controlled in an effective way, some pests had not been effectively controlled with the current fumigation protocol. The pests that had not been possible to control were two important quarantine pests for the Chilean export markets. One is the false Chilean mite, *Brevipalpus chilensis* (Baker), and the other pest is the European grapevine moth, *Lobesia botrana* (Denis and Schiffermüller).

Recently the conditions were found at which also these two pests can be controlled effectively.

MATERIALS AND METHOD

The treatments where performed in 28.3 L fumigation chambers on apples that were conditioned for 24 h between 0 and 10ºC. The apples were infested with *B. chilensis* that were grown on *Ligustrum sinensis* without having being treated with other chemicals.

Fumigation conditions were:

Phosphine concentration: 300 ppm, Control 0 ppm.
Fumigation temperature: 0ºC to 1ºC.
Exposure time: 10 days
Fruit species: Apple
Filling factor: 50% v/v

Two treatments with three replicates were made with 100-300 individuals in mobile stages for each replicate.

Pure phosphine blended with nitrogen was injected into the chamber to obtain a concentration of about 650 to 800 ppm of phosphine that would allow achieve 300 ppm phosphine at the end of the fumigation.

Phosphine concentrations were monitored on a daily basis using 0-2,000 ppm colorimetrical tubes. According to concentration readings, top ups were made to maintain the concentration according to the fumigation schedule. Four top ups were required to keep the concentrations within the schedule. Mortality was evaluated 5 days after aeration through visual inspection under stereoscopic microscope

Table 1. Phosphine concentration durinsg fumigation

| Exposure time | Phosphine concentration in ppm | | | Top ups |
	Replicate 1	Replicate 2	Replicate 3	
2 min	700	650	650	
24 h	300	250	200	
24 h after topup	800	750	800	1st
96 h	200	150	150	
96 h after topup	850	800	900	2nd
120 h	500	550	400	
144 h	50	50	50	
144h after topup	700	700	750	3rd
168 h	450	400	400	
168h after topup	600	550	600	4th
240 h	300	200	250	

Table 2. *Brevipalpus chilensis* mortality 5 days after fumigation with phosphine at 300 ppm for 240 h

| Treatment | Replicate | Mobile stages treated | Mortality 5 days after aeration | | |
			N° of live individuals	N° of dead Individuals	% of mortality
Phosphine at low concentration for 10 days at temperature of 1ºC.	R1	250	0	250	100
	R2	223	0	223	100
	R3	301	0	301	100
	Average		0	258	100
	Std. Deviation		0	35,4	0
	Std. Error		0	20,5	0
Control without fumigation at temperature of 1ºC	R1	129	119	10	7,8
	R2	442	384	58	13,1
	R3	405	343	62	15,3
	Average		282	43,3	12,1
	Std. Deviation		127,6	25,9	3,5
	Std. Error		73,7	14,9	2

RESULTS AND DISCUSSION

It could be demonstrated that pure cylindered phosphine is able to control 100% of mobile stages of *B. chilensis* in fresh fruits when exposed for 10 days to concentrations averaging 457.5 ppm with average temperatures of 1ºC.

Mortality of control was 12.2% which corresponds to the natural mortality of the tested population.

The proposed treatment is an effective tool for the control of mites on fresh fruits, especially considering those fruits that are stored for longer periods of time, and where the same cooling infrastructure can be used as fumigation facility such as table grapes, kiwifruit, apples, and others.

Similar results as with *B. chilensis* have also been obtained with other quarantine pests like the grapevine moth, *L. botrana* .

There are a series of advantages when fumigating fresh fruit with pure cylindered phosphine using the HORN DILUPHOS SYSTEM[TM]:

- No changes in taste, smell, texture, color or shelf life of the fruit after 6 days of aeration, if fumigation has been conducted at low temperature.
- It is possible to fumigate at low temperature with the cooling system running.
- It is not necessary to warm up the fruits prior to fumigation.
- There are no residues left after fumigation.
- Pure cylindered phosphine does not produce ammonia and it is, therefore, not phytotoxic.
- The fumigation can be performed in the same cooling chambers where the fruit is stored.
- Hydrogen phosphide is rapidly deactivated by the action of sunlight once released to the atmosphere.
- Phosphine does not damage to the ozone layer and it is not a gas with greenhouse effects.

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EVALUATION OF PROFUME® GAS FUMIGANT (SULFURYL FLUORIDE) IN AMBIENT AIR ADJACENT TO A BULK GRAIN STORAGE IN AUSTRALIA

Ellen Thoms[1], Robert Annetts[2*], Peter Williamson[3]; Daniel Cornally[4], Simon Bishop[5] and Robert Ryan[6]

[1] Dow AgroSciences, Gainesville, 32607 USA,
[2] Dow AgroSciences, Toowoomba, QLD 4350 Australia,
[3] SA Rural Agencies, Wingfield SA 5013 Australia,
[4] Dow AgroSciences, Millthorpe NSW 2798 Australia,
[5] A-Gas (Australia) Pty Ltd, Gordon NSW 2072; Australia,
[6] VAPORFAZE, PO Box 4, Sans Souci, NSW 2219 Australia,
*Corresponding author's e-mail: rannetts@dow.com

ABSTRACT

ProFume® gas fumigant (99.8% sulfuryl fluoride, Dow AgroSciences, Indianapolis, IN, USA) has been used for control of mixed age populations of insects infesting grain storages in Australia since 2007. The increased use and reliance on ProFume as a pest management tool in Australia is due in part to the widespread distribution of multiple insect species resistant to commonly used insecticides in grain protection. In particular, high levels of phosphine resistance have been documented in *Cryptolestes ferrugineus* in north eastern Australia, where existing phosphine label rates are no longer effective. In addition to large scale grain storages, fumigations using ProFume have been conducted in a variety of structures including houses, mills, shipping containers, buildings, ships, bag stacks, silo bags, and silos sheds. In Australia, ProFume is championed and distributed by SA Rural Agencies, now a division of A-Gas. SA Rural is also responsible for product stewardship which includes product specific training of fumigators and oversight of compliance in use of ProFume. The safe use of ProFume is of primary concern. One trial was carried out in Australia to monitor ProFume in ambient air around a large scale grain storage during fumigation and aeration. The results demonstrated that the 3 m exclusion zone, currently used for phosphine fumigations, and label directions for ProFume prevent bystander and worker exposure to SF concentrations which exceed permissible levels during fumigation and aeration.

Key words: ozone depletor, stored product fumigant, ProFume gas fumigant, sulfuryl fluoride, product stewardship.

INTRODUCTION

The listing of methyl bromide as an ozone depletor by the Montreal Protocol initiated development work by Dow AgroSciences to find an alternative stored product fumigant. ProFume® gas fumigant (99.8% sulfuryl fluoride [SF], Dow AgroSciences, Indianapolis, IN USA) has been used for control of a range of insects in a variety of structures including houses, mills, shipping containers, buildings, ships, bag stacks, silo bags, silos sheds, and grain bunkers. ProFume has been used for control of mixed age populations of insects infesting grain storages in Australia since 2007 and is registered for control of a variety of insect species in dried commodities. The most common species encountered in grain storages in Australia are: the rusty grain borer *Cryptolestes ferrugineus* (Stephens), lesser grain borer *Rhyzopertha dominica* (F.), flour beetles, *Tribolium spp.*, and rice weevil, *Sitophilus oryzae* (L.), which are all controlled by ProFume. The widespread discovery of insects resistant to phosphine, especially *C. ferrugineus* in north eastern Australia where existing phosphine label rates are no longer effective (Emery *et al.,* 2011), has led to the increased use and reliance on ProFume as a pest management tool (Nayak *et al.*, 2010).

In Australia, ProFume is championed and distributed by SA Rural Agencies, now a Division of A-Gas (Australia). SA Rural is a leading supplier of a comprehensive range of fumigation products and services to established agents and distributors throughout Australia and countries in the Asia Pacific region. In conjunction with Dow AgroSciences, SA Rural Agencies introduced Telone®, chloropicrin and ProFume as viable replacements for methyl bromide in Australia.

The use of ProFume brings many benefits to the user, including a product stewardship program and the Fumiguide® Program, a highly effective tool for calculating the dose needed based on pest species, temperature, estimated gas retention and duration of fumigation. Other benefits of ProFume include: a different mode of action for use as a fumigant alternative in phosphine resistance management programs, superior material compatibility (inert to most materials), ease of application and to "top up" a fumigation, and rapid aeration of fumigated commodities.

The safe use of ProFume is of primary concern. The currently available, battery-operated low-concentration SF detectors are too large to use as a clip-on personal monitoring device (such as those available to detect phosphine). This trial was conducted to determine if the standard 3 m exclusion zone established around grain bunkers as a label requirement for ProFume (as it is for phosphine) prevents bystander and worker exposure to SF concentrations above permissible levels.

MATERIALS AND METHODS

The trial was carried out at a grain storage facility operated by GrainFlow in Jondaryan, Queensland, Australia. One metal-sided bunker for sorghum storage was evaluated. The tarped bunker measured approximately 8 m (height) x 34 m (width) x 253 m (length), and stored about 33,500 tonnes of sorghum.

During the fumigant introduction and exposure periods, eight air monitoring stations were positioned 3 m from the basal perimeter of the bunker at the four corners and at two locations midway on each lateral side. Each monitoring location consisted of a stand made of 2.5 cm

diameter PVC pipe attached to a commercially available bollard in order to collect an air sample drawn from 1.5 m in height from the ground using a battery-powered (2 size "D" cells) aquarium air pump (Marina, Rolf C. Hagen Corp., Mansfield, MA) modified to have the fresh air intake port connected to 0.64 cm OD Tygon tubing. A Kinar[TM] 20-L air sample bag, fitted with an on-off valve using 0.64 cm OD Tygon tubing was attached to the pump exhaust port. Air flow rate from the air pump was controlled by a plastic air control valve (Elite) between the pump and quick release connector. The air flow valve was adjusted in ml/min using the small hand screw valve and a mass air flow meter (Cole-Palmer Instruments Co., Chicago, IL,) to provide sufficient sample air without over-inflating the bags according to the planned sampling interval. Air flow rates were 20-30 mL/min for day time sampling and 15 mL/min for overnight sampling. Batteries on the pumps were changed after two days of operation.

At the start and conclusion of each monitoring period, the air flow rate was measured and the time recorded. SF concentrations were measured in the air sample bags using the SF-ExplorIR (Spectros Instruments, Hopedale, MA) which uses non-dispersive infrared sensor technology to measure low SF concentrations. During the daytime, air bags were sampled at two intervals, ~4 h each, during the first four days after fumigant introduction and one interval, ~8 h, at five days after introduction through aeration. Overnight, air bags were sampled at one, ~16 h interval. Prior to initiation of aeration, seven additional ambient air monitoring stations were placed on the east side of the bunker where fumigant was to be vented. Stations were spaced at 20 and 40 m intervals in front of, in back of, and at a 90 degree angle from the exhaust vent of the aeration fan. During aeration, air bags were sampled at ~8 h during the first day of aeration and after ~15.5 h overnight.

The SF concentration was measured ca. 1 m in the grain at 16 locations; nine along the bunker peak and seven along the lower perimeter, about 3 m from the metal wall, at each corner and intermediate on the western and eastern sides. Fumigant monitoring hose (0.32 cm OD x 90 m long) was folded at the terminal end and taped with electrical tape, and at least ten 1-mm diameter holes were punched into the hose before the fold using a leather tool punch. This was done to prevent grain from lodging inside the hose during insertion and monitoring. The monitoring hose was inserted using a grain sample probe through a tarp slit, which was sealed with double-sided, 1 mm thick butyl tape. The labeled, proximal ends of the monitoring hoses extending outside the grain bunker were connected to a manifold (LeBeau Inspections, Inc., Mt. Sterling, OH). An electric pump powered by a car battery was used to rapidly draw air samples from within the grain mass to the manifold. SF concentrations in the air samples were measured using an SF-ContainIR version 1.88 (Spectros Instruments, Hopedale, MA) consisting of a portable, battery-operated monitoring device which also uses non-dispersive infrared (NDIR) sensor technology to measure SF. SF concentrations were measured at least twice daily throughout the fumigation exposure period.

The target dose, calculated using the Fumiguide Program, was 24 g m^{-3} of ProFume. A total 1021 kg of ProFume (18 cylinders of ProFume) was calculated based on the tonnage of grain stored and was applied using commercial methods. ProFume was introduced into the bunker through 0.44 cm ID introduction hose that was approximately 150 m in length. A manifold was used at the terminal end to connect two short lengths of 0.44 cm ID introduction hose which were inserted through slits cut in the tarp about 3 m down from the peak on the east and west sides of the bunker. These insertions occurred at ~15 m intervals along the peak. Introduction was on the 22 September, 2011. Each slit in the tarpaulin was sealed with butyl tape after the introduction

hose was removed. Additional gas 56.7 kg was introduced on 27 September 2011 in order to achieve the specified dose in this area of the bunker. The introduction hose configuration was the same as described previously, with two hose insertions made on the northeast corner of the bunker. This was the only section of the bunker in which monitoring indicated the accumulated dosage was less than required for control of target pests. The amount of ProFume added was calculated to obtain the required accumulated dosage based on the measured conditions of confinement and the remaining fumigation exposure period. All fumigant introductions were conducted by certified applicators of ProFumigation, Inc.

The fumigant exposure period was 11 days due to scheduling of aeration to begin on Monday, 3 October 2011 when the required grain facility personnel were present. Fumigant aeration was carried out using a custom built ventilation unit, consisting of an Aerovent fan powered by a 37.8 amp electric motor (Western Electric) mounted on a trailer installed on the southeast corner of the bunker. The tarp in the northwest corner of the bunker was unsealed to permit fresh air input. The aeration fan was started to initiate aeration at 9:56 am, 3 October 2011 and operated continuously throughout aeration which was completed at 9:40 am the next day. Readings were taken using an SF-ExplorIR until the bunker was certified cleared. Then all sampling equipment (hoses, temperature data loggers, and bioassays) in the bunker were removed.

RESULTS AND DISCUSSION

The SF accumulated dosage in the grain was sufficient for the targeted insect control. The lowest accumulated dosages of 848 and 880 g h m^{-3} were at the north end of the bunker where additional ProFume was added.

SF was detected in four air sample bags, representing three sample locations and two sample intervals; introduction and initial fumigation exposure period. For all other sample locations and intervals, including aeration, no SF was detected in the air sample bags. The highest SF concentration measured in an air bag was 3 ppm, which is the permissible exposure limit of ProFume for workers and bystanders. The 24 hour Time Weighted Averages (TWAs) of SF for introduction and initial fumigant exposure for the three locations in which fumigant was detected were 0.17 ppm for two locations and 1.15 ppm for one location. These TWAs are well below the 3 ppm TWA for SF established in Australia for protection of workers and bystanders. TWAs could not be calculated for other monitoring locations and time periods because concentrations of SF were below the limit of detection (1 ppm) of the SF-ExplorIR. Therefore, any potential exposure of workers and bystanders to SF at the 3 m exclusion zone boundary during the fumigation and aeration period periods would have been well below the TWA of 3 ppm for a 24 hour exposure.

These results demonstrate that at the 3 m exclusion zone worker and bystander exposure to ProFume was well below the permissible threshold concentration and 24 hour TWA during fumigation and aeration. During aeration, an additional exclusion zone set up 20 m from the exhaust fan vent also ensured that worker and bystander exposure to SF was well below the permissible threshold concentration and 24 hour TWA of 3 ppm. The size of the exclusion zone from the aeration fan, particularly down wind and down air stream, may vary based on concentration of fumigant in the bunker at initiation of aeration, aeration fan capacity, wind speed, and other conditions. An SF clearance detector, such as the SF-ExplorIR, can delineate

the appropriate boundaries from the aeration fan for excluding personnel during aeration. This monitoring is required by labeling for ProFume as follows; "The perimeter of the fumigation area, especially downwind, must be monitored to ensure that ProFume concentrations are kept within acceptable levels outside the fumigation area."

These data from this trial indicate that if both the prescribed exclusion zone of 3 m and label directions for ProFume are observed, bystander and worker exposure to SF concentrations will not exceed permissible levels. Dow AgroSciences is currently undertaking work in order to expand the usage of ProFume into new stored produce in order for these industries to remain viable under the threat of insect resistance and withdrawal of methyl bromide.

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CURRENT STATUS OF PROFUME ® GAS FUMIGANT FOR DISINFESTATION OF COMMODITIES

Stanislas Buckley[*1], Ellen Thoms[2]

[1]Dow AgroSciences *S.A.S., 790 Avenue Dr Donat, 06254 Mougins Cedex, France.
sbuckley@dow.com
[2] Dow AgroSciences LLC, 7257 NW 4th Blvd #20, Gainesville, FL 32607, USA.
*Corresponding author's e-mail: sbuckley@dow.com

ABSTRACT

Since its first approval for fumigation of flour mills in Switzerland in 2003, ProFume® gas fumigant (99.8% sulfuryl fluoride; Dow AgroSciences LLC) has been approved for the control of stored product insects in structures (flour mills, food processing plants) and/or commodities in over 20 countries. It has been established as the fumigant of choice to replace methyl bromide to treat dried fruits and tree nuts in the United States, and cocoa beans in the United States and Northern Europe. It is also used as an effective alternative to control phosphine-resistant insects infesting grain in the United States and Australia. Ten years of studies in cooperation with six stored product research laboratories in the United States and Europe have established reliable dosages to control all stages of main stored product insects under a wide range of conditions. An extensive program of food quality studies conducted with the Dried Fruit and Tree Nut Association of California, Ege University of Turkey, Purdue University and Kansas State University in the United States, and the National Confectioners Association in the United States, confirmed that ProFume has no adverse effect on taste or quality of fumigated commodities.

Due to its fast penetration, ProFume is also an excellent wood fumigant and an effective control method for quarantine insects and nematodes in wood logs and wood packaging materials. The inclusion of ProFume in International Standard for Phytosanitary Measures (ISPM) would offer an alternative fumigant to methyl bromide to prevent the distribution of economically-important forest pests.

Key words: Commodity, grain, dried fruits, tree nuts, sulfuryl fluoride, wood, quarantine

INTRODUCTION

Sulfuryl fluoride (SF) was first marketed under trade name Vikane® (Dow AgroSciences, Indianapolis, IN) in the United States in 1961 as a structural fumigant to control wood-destroying and structure-infesting insects. In the last nine years, the use of SF, under trade name ProFume®(Dow AgroSciences, Indianapolis, IN), as a structural and commodity fumigant in the food processing industry to control stored product insects has been considerably developed around the world. The use of SF as a quarantine fumigant against undesirable forest pests globally, and as an alternative to phosphine on grain are in development.

Kenaga(1957) documented that SF was effective on a large selection of stored product insects. The study found that postembryonic stages were more susceptible to SF than the eggs were. This property of SF has been confirmed in efficacy studies conducted since (Thoms and Scheffrahn 1994, Bell et al. 1999, Baltaci et al. 2009). Outram (1967) demonstrated SF had reduced penetration through and bound to the proteinacious egg shell and embryonic membranes.

Initial research on pests of dried fruits and tree nuts was conducted by USDA-ARS in Fresno, California (Zettler et al. 1998) and Dried Fruit and Tree Nut Association (DFA) of California (Schneider and Hartsell1998). These studies established that low dosages of SF could eliminate larvae from field insects (*Cydia pomonella* (L.) and *Amyelois transitella* (Walker)) present on the crop and higher dosages would control all stages of the stored product moth *Plodia interpunctella* (Hübner). Further work demonstrated that ProFume could control all stages of closely related pyralid moth *Ephestia kuehniella* (Zeller) (Bell et al. 1999), and *Ephestia elutella* (Hübner) (Baltaci et al. 2009) with some differences of tolerances according to the age of eggs and ambient temperature.

The research conducted at DFA and Central Sciences Lab (Bell et al. 1999) was also focused on stored product beetles (*Tribolium castaneum* (Herbst) , *T. confusum* (Jacquelin du Val)*, Trogoderma variabile* (Ballion)*, Cryptolestes turcicus* (Grouvelle), *Ptinus tectus* (Boieldieu), *Sitophilus granarius* (L.), *Gnatocerus cornutus* (F.), *Tenebrio molitor* (L.)) and psocids *Liposcelis bostrychophila* (Badonnel) . Most species were completely controlled at all stages at cumulated dosage (CTP = Concentration x Time products) of 500 g-h/m^3 at 30°C and 1000 g-h/m^3 at 25°C. *T. castaneum* and *T. variabile* were the species needing the highest dosage to achieve complete control of the egg stage.

More research on important stored product insects was conducted with independent research institutes to confirm effective dosages on all stages of stored product moths and beetles (Thoms et al. 2008). The results confirmed that SF was effective in controlling all stages of insects with temperature ranging from 20 to 40°C without exceeding the maximum approved dosage of 1500 g-h/m^3. These research studies have been submitted and evaluated by government scientists in Europe and have resulted in approval of fumigation of mills and food processing facilities in more than 20 countries in the world. Many countries have also approved ProFume on a wide range of food commodities (Table 1).

With the increasing complexity of SF dosage in food commodity, proprietary software, the ProFume Fumiguide™, was developed by Dow AgroSciences to calculate the CTP for 19 insect pest species (Table 2) for a wide range of temperatures and exposure times. The data used to produce the Fumiguide is the result of ten years of research by six stored product research laboratories in the United States and Europe (Thoms et al. 2008), and nearly 1,200 bioassays of the key cosmopolitan stored product insects evaluated during 51 commercial fumigations (unpublished, Dow AgroSciences). When monitoring data are entered into the Fumiguide, the program will calculate the actual half loss time and accumulated dosage, predict the dosage outcome for the planned exposure period, and update instructions on exposure time (on target, shorten or lengthen) and fumigant concentration ("on target" or "add more") (Dow AgroSciences, 2005).

Table 1. Approval of ProFume gas fumigant on raw Food commodities in the world

Countries [1]	Me	USA	Be	Fr	It	Ge	Ne	Tu	Gr	Au
Cocoa		x	x			x	x			
Cereals [2]	x	x								x
Maize (Corn)	x	x								
Rice	x	x								
Bean	x									
Dried fruits [3]		x		x		x		x	x	x
Walnuts		x		x	x	x				x
Hazelnuts		x		x	x	x		x	x	x
Pistachios		x		x	x	x			x	x
Pecan		x		x	x	x			x	x
Almonds		x		x	x	x			x	x

[1] Me: Mexico; Be: Belgium; Fr: France; It : Italy; Ge: Germany; Ne: Netherland; Tu: Turkey; Gr: Greece; Au: Australia
[2] Wheat, barley, oats
[3] Raisins, apricots, figs, dates, prunes – Raisins, apricots, and figs for Turkey

Table 2. Insects currently in the ProFume Fumiguide (version 2011)

Order	Family	Name
Coleoptera (beetles)	Tenebrionidae	*Tribolium castaneum*
		Tribolium confusum
	Dermestidae	*Trogoderma variabile*
		Dermestes maculatus
	Curculionidae	*Sitophilus granarius*
		Sitophilus oryzae
	Bostrychidae	*Rhyzopertha dominica*
	Lameophloeidae	*Cryoptolestes ferrugineus*
	Chrysomelidae	*Callosobruchus maculatus*
		Acanthoscelides obtectus
	Anobiidae	*Lasioderma serricorne*
		Stegobium paniceum
	Silvanidae	*Oryzaephilus surinamensis*
Lepidoptera (moths)	Pyralidae	*Ephestia kuehniella*
		Ephestia cautella
		Ephestia elutella
		Plodia interpunctella
		Amyelois transitella
	Tortricidae	*Cydia pomonella*

Many trials have been conducted to assess fumigation with SF on the quality of the commodity. Four taste tests were conducted on dried fruits and tree nuts from 1998 to 2001 to determine the potential for sulfuryl fluoride to affect the taste, in association with DFA and two groups of sensory researchers: National Food Laboratory in Dublin California and Department of Pomology, University of California, Davis. (Unpublished, Dow AgroSciences). Raisins, apricot, dates, prunes, figs, walnuts pistachios and almonds received single or multiple SF fumigations, and their taste was compared to unfumigated controls by panelists in a blind study. Results indicate that the taste, quality and commercial value of these eight commodities are not affected with treatment of SF at 2000 g-h/m^3.

Tests have also been conducted in Turkey (Fatih Şen et al. 2009) on the Sarılop fig variety. Fruit quality was evaluated after short (15 d), medium (100 d) or long-term storage (210 d). No negative impact occurred on fruit surface colour, sugaring, water content, water activity, total soluble solids, titratable acidity contents, pH and firmness following SF fumigation.

On grain, several trials were conducted at Kansas State University from 2000 to 2002 to determine the potential for SF to affect quality and nutritional characteristics of wheat grain (Unpublished, Dow AgroSciences). Wheat kernels were fumigated once or twice with SF at 2000 g-h/m^3. There was no significant difference between fumigated and nonfumigated kernels in physical/chemical characteristics (test weight, 1000 kernel weight, % ash) and nutritional quality mould infection, % fiber, % protein, % lipid, thiamin (vitamin) content). The flour made from fumigated and nonfumigated kernels did not significantly differ based on the Hagberg falling number, Alveograph and baking tests. Similarly, the quality of spaghetti (brightness, colour, cooking and tensile test) made from fumigated and nonfumigated durum wheat did not differ significantly.

In 2005, sensory evaluation of cocoa beans fumigated with SF was conducted by the National Confectioners Association (NCA) in the United States. Dried, unroasted test cocoa beans were from the Ivory Coast and Indonesia, both major sources of cocoa beans. The beans were treated with 3 SF dosages (400, 800, and 1500 g-h/m^3). Fumigated and nonfumigated cocoa beans were made into chocolate liquors and sent to nine chocolate manufacturers for sensory evaluation. The NCA members concluded that there was no significant adverse effect on the sensory properties of liquors made from SF treated beans, and subsequently adopted SF for cocoa beans fumigation.

Its successful commercial use in many countries of Europe and America prove that ProFume is a technically and economically viable alternative to methyl bromide for commodity fumigation.

CURRENT DEVELOPMENT FOR SF

Currently, high levels of phosphine resistance in the flat grain borer, Cryptolestes ferrugineus, are resulting in control failures for phosphine treatment of central grain storages in Australia. Sulfuryl fluoride and phosphine have different modes of action (Thoms and Phillips 2004), phosphine resistant insects are not cross-resistant to sulfuryl fluoride (Bell et al. 2002), and there is no known insect resistance to sulfuryl fluoride (Thoms and Phillips 2004). These characteristics make ProFume a primary candidate for rotating with phosphine to combat resistance. In cooperation with government researchers and commercial bulk grain handlers in Australia, Dow AgroSciences is evaluating the practicality and effectiveness of SF

fumigation for typical Australian grain storage bunkers. The effectiveness of low SF concentration for long exposure times (10-14 d) in bunkers is a new area of research since previous research with SF on stored product insects was conducted for shorter exposure times, typically 24-48h.

It has been demonstrated that SF is effective in controlling a wide range of insects infesting unseasoned wood, such as Asian long horn beetle *Anoplophora glabripennis* (Motschulsky) (Barak et al. 2006), Bamboo borer *Chlorophorus annularis* F.(Daojian et al. 2010), Emerald ash borer *Agrilus planipennis* (Fairmaire) **(**Barak et al. 2010**)** and various species of Cerambycidae, Scolytidae, and Platypodidae (Soma et al.1996, Mizobuti et al. 1996). Studies by leading nematologists and quarantine experts have shown effective control of pine wood nematode *Bursaphelenchus xylophilus* (Steiner & Buhrer) (Soma et al. 2001, Dwinell et al. 2005; Flack et al. 2008 unpublished; Sousa et al. 2010.

The only current approved treatments for wood packaging in ISPM N°15 (International Standard for Phytosanitary Measures) are heat and methyl bromide (MB). Heat can damage commodities and their packaging. MB has been phased out in many areas in the world, even for use in quarantine and preshipment treatments which are excluded from the Montreal protocol. Therefore, it is critical that an alternative fumigant is approved in international trade for treatment of wood packaging fumigation, and Dow AgroSciences is pursuing research with ProFume to obtain inclusion of SF in ISPM N° 15. ®Trademark of Dow AgroSciences LLC

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"VAPORMATE™" AN ALTERNATIVE FUMIGANT FOR QPS TREATMENTS

Simcha Finkelman[1]*, Shlomo Navarro[1], Hagit Navarro[1] Ervin Lendler[2]

[1]Food Technology International Consultancy Ltd., P.O. Box 3300, Beit Yehoshua, 40591, Israel
[2]Agriterra Ltd., P.O. Box 1071, Haifa Bay, 26110, Israel.
*Corresponding author's e-mail: *Finkelman@ftic.info*

ABSTRACT

For the registration of Vapormate[TM] as an alternative to methyl bromide and for quarantine and post harvest treatments, efficiency tests were conducted at the laboratory, at semi- commercial and commercial trials in Israel. Vapormate[TM] contains 16.7% ethyl formate mixed with carbon dioxide. The effect of three Vapormate[TM] dosages 210, 420 and 630 g/m^3 was tested at fixed exposure times of 24 h with control at a temperature of 30°C and 70% relative humidity at laboratory conditions. For laboratory trials, test insects were adults of *Tribolium castaneum* and *Rhyzopertha dominica* and larvae of *Trogoderma granarium* in 3-liter desiccators containing 1 kg of rice or corn. The optimal results were obtained at 24 h exposure of 420 g m^{-3} that caused 100% mortality of *T. castaneum* and *R. dominica* adults and *T. granarium* larvae. Semi-commercial tests were carried out on green coffee beans stored in a 3 m^3 plastic cube fumigated for 24 h exposure to 420 g m^{-3} Vapormate[TM]. Fumigations resulted in total mortality of *T. castaneum* adults with no adverse effects on green coffee beans. Commercial tests were carried out on 20 tonnes of rice stored in bags inside a 33.2 m^3 standard transport container. The rice was fumigated for 24 h by exposure to 420 g m^{-3} Vapormate[TM]. Adults of *R. dominica* and *Sitophilus oryzae* exposed to Vapormate[TM] resulted in total mortality. Dosage of 420 g m^{-3} and exposure time of 24 h that resulted in high toxicity to the test insects, and the rapid desorption from the commodities, offer Vapormate[TM] to be a potential fumigant to replace methyl bromide in QPS treatments and when rapid disinfestations of storage commodities is essential.

Key words: Vapormate[TM], ethyl formate, QPS, fumigation, insect control, methyl bromide, *Rhyzopertha dominica*, *Tribolium castaneum*, *Orazophilus surinamensis*, *Trogoderma granarium, Sitophilus oryzae*

INTRODUCTION

Methyl Bromide (MB) alternatives were considered for quarantine and pre-shipment (QPS) treatments throughout the world before and after its phase out in 2005 in all the developed countries. In Israel, the only alternative fumigant for treatment of post harvest of durables remained phosphine. A fumigant that most exporters or importers as well as their pest controller are reluctant to use because of the long exposure time needed for successful implementation and the resistance that many pest insect have developed to this fumigant.

The global concern from the introduction of new pests or new resistant strains of known grain storage insect pest increased the interest in additional alternatives. A fumigant that will be user friendlier to environment, effective, rapid action and at the same time should be on an acceptable cost basis. One of the fumigant of choice considered was ethyl formate (EF) known in its commercial formulation as Vapormate™.

Vapormate™ is a low human risk fumigant formulated by BOC Australia, a member of the Linde Group, and contains 16.7 wt% EF in liquid carbon dioxide (CO_2) (Ryan and Bishop, 2003). The CO_2 in Vapormate^{TM} has been added to eliminate the flammability of the EF and to enhance efficacy by its synergistic effect in reducing the time required to kill insects (Haritos et al., 2006). EF occurs naturally in many natural products as orange juice, honey, apples, pears and wine. It is used as a synthetic flavoring agent in the food industry and as fragrances; it is also a GRAS registered food additive (Ryan et al., 2006). It decomposes slowly in water releasing formic acid and ethanol. Laboratory tests as a fumigant against insect pests of food commodities and field trials on bagged cereals, spices, pulses, dry fruits and oilcakes have been carried out (Muthu et al., 1984). EF is currently registered as a fumigant in Australia as ERANOL® by Orica Chemnet for the elimination of insect pests in packed dried fruits like raisins. It is toxic to storage insects including psocids (Annis et al., 2000). It was registered for use in grain and horticultural products in Australia, and in New Zealand for use in grain and for quarantine treatment of bananas (Krishna et al., 2002). It is registered in Israel for dates disinfestation and control of nitidulid beetles (Finkelman et al., 2010). In this work we report on the efficiency tests that were conducted at the laboratory, at semi-commercial and commercial trials to register Vapormate^{TM} in Israel for QPS treatments as an optional MB alternative fumigant.

MATERIALS AND METHODS

Test insects

All test insects were reared at FTIC laboratory at 30º±1C and 70±2% r.h. Test insect species were: *Rhyzopertha dominica* (F.)*, Tribolium castaneum* (Herbst), *Trogoderma granarium* Everts, *Sitophilus oryzae* (L.) and *Oryzaephilus surinamensis* (L.)

Laboratory trials

Adults of *R. dominica* and *T. castaneum* were placed into a 22 mL glass vials together with about 3 g of ground wheat. Each glass vial together with the test insects was placed into a 3 L gas-tight desiccator used as a fumigation chamber. Each desiccator contained either 1 kg of polished rice (11.5% m.c.) or corn (11.0% m.c.). The Vapormate^{TM} was introduced into the desiccator to achieve concentration of 630, 420 and 210 g m^{-3} at 30±1ºC or 24 h. Dosage calculations were converted to the gaseous phase and the required volume of Vapormate™ was obtained by evacuating the desiccator to the desired pressure, followed by restoration of atmospheric pressure using Vapormate™ supplied from a pressurized cylinder. The desired pressure was first calculated by converting the dose into a percentage of the desiccator volume to be treated, then desiccator was evacuated to the desired absolute pressure using a laboratory vacuum pump and the pressure measured using a portable transducer manometer (SE-2000, Celesco, Chatsworth, CA, USA), and then the equivalent to the partial pressure of in air was supplied by restoration of atmospheric pressure using the Vapormate™ supplied from the pressurized cylinder. The same process of evacuatingto the desired pressure was carried out in the control desiccator but instead of the gas mixture, the pressure was restored using ambient air at atmospheric pressure (Finkelman et al., 2010). At the end of the exposure

time, the glass vials with the test insects were removed from the fumigation chamber and placed in an incubator at 30±1ºCfor 24 h before mortality counts were made. For every test 4 replicates were used.

The efficiency of Vapormate[TM] was tested on non-diapausing larvae of *T. granarium*, since this species is considered one of the most important quarantine pests. The larvae were exposed to 420 g m^{-3} at the same conditions as the other tested insects in the desiccator. The desiccator was then placed at 30ºC for the predetermined exposure times. The effectiveness of Vapormate[TM] on *T. granarium* larvae was tested at three exposure times: 12, 16 and 24 h. At the end of each exposure time, the glass vials with the test insects were removed from the fumigation chamber and placed in an incubator at 30ºC and 70±2% r.h. for 24 h before mortality counts were made.

Semi-commercial trials
Two semi-commercial trials were carried out using two types of flexible fumigation chambers to test the efficiency of Vapormate[TM]. In the first trial 1.2 tonne of polished rice stored in 50 kg bags were fumigated with 420 g m^{-3} Vapormate[TM] at 26º±2C for 24 h. The rice bags (11.85±0.85 m.c.) were placed in a 2 m[3] cube consisted of welded PE-PP laminated with aluminum foil barrier sheets. Test insects were placed at the top, middle and bottom of each chamber. In each test location at least 20 adults of *R. dominica*, *S. oryzae* and *O. surinamensis* were placed in glass vials of 22 mL together with about 3 g of flour. Each test was replicated four times. In the second trial 12 bags containing green coffee beans (60 kg each), imported from Vietnam, were arranged on a pallet and the pallet was placed in a 3 m[3] Rentokil flexible PVC fumigation chamber. Test insects were placed at the top, middle and bottom of the chamber among the bags. In each test location three glass vials of 22 mL, each containing three developmental stages of *T. castaneum*; adults, pupae or larvae were placed with the glass vials contained about 3 g of flour and 20 individual insects. The bags were fumigated using 420 g m^{-3} Vapormate[TM] at 27ºC for 24 h. Vapormate™ supplied from the pressurized cylinder was mounted on a scale while the pressure tube was hold inside the sealed liner and secured to prevent movement of the injection tube due to back pressure of the gas. During the injection the opposite top of the chamber was kept open to release excessive pressure and to prevent sudden ballooning of the fumigation chamber. A gas-sampling opening in the chamber was used to measure gas concentration. An initial dosage of 420 g m^{-3} was used and concentrations were measured immediately after the gas release using a CO_2 gas analyzer. After exposure to fumigants the glass vials were taken to the laboratory and placed in an incubator at 30ºC and 70±2% r.h. for 24 h before mortality counts were made.

Commercial trials
The trials were conducted at the sea port of Haifa as part of scheduled fumigation activities in 33.2 m[3] containers on imported commodities. Three commercial containers each loaded with 20 tonnes of polished rice in bags were prepared to accommodate the test insects which were placed in the front at the four corners of the container among the bags. In each test location two glass vials of 22 mL were placed with adults of *R. dominica* and *S. oryzae* arranged separately to contain about 3 g of flour. Vapormate™ supplied from the pressurized cylinder as in the semi-commercial trials as well as the method of measuring of gas concentration. After fumigation the glass vials were taken to the laboratory and placed in an incubator at 30ºC and 70±2% r.h. for 24 h before mortality counts were made.

RESULTS AND DISCUSSION

Laboratory trails

Exposing adults of *R. dominica* and *T. castaneum* to 630 and 420 g m^{-3} Vapormate^{TM} at 30°C for 24 h resulted in 100% mortality both in rice and in corn. *R. dominica* adults were more sensitive to the fumigant then *T. castaneum* adults and at 210 g m^{-3} only 3.2% of *T. castaneum* died in comparison to 70.5% *R. dominica* adults. Furthermore the mortality of *R. dominica* adults was higher when they were exposed in rice to the fumigant then in corn, resulting in 70.5% and 42.9%, respectively. These differences are probably due to the lower r.h. % in rice that may have caused higher desiccation rate to enhance mortality (Table 1).

Table 1. Laboratory trials on mortality of *R. dominica* and *T. castaneum* adults after exposure to 630, 420 and 210 g m^{-3} of Vapormate^{TM} at 30ºC for 24 h (average of 4 replicates)

Vapormate^{TM} dose (g m^{-3})	Type of grain	*R. dominica*		*T. castaneum*	
		Average number of adults	Average mortality (%)	Average number of adults	Average mortality (%)
630	rice	23.8	100	20.5	100
control	rice	20	5	23	0
630	corn	22	100	20.8	100
control	corn	23	26.1	21	9.5
420	rice	28.5	100	28.8	100
control	rice	20	25	23	8.7
420	corn	28	100	20.3	100
control	corn	19	4	21	3.2
210	rice	23.5	70.5	21.5	3.2
control	rice	20	0	20	0
210	corn	21	42.9	21	4.4
control	corn	21	0	26	0

Khapra beetle (*T. granarium*) is one of the most important quarantine grain pest beetles mainly because they developed a high resistance to phosphine. When the larvae of the of Khapra beetle were exposed for 12 h to 420 g m^{-3} of Vapormate^{TM} at 30ºC, the average mortality was 76.3% and after 16 h mortality was 97.5%. Only when the exposure time was extended to 24 h the target mortality of 100% in all 4 test replicates was achieved (Table 2).

Table 2. Laboratory trials on mortality of Khapra beetle larvae (*T. granarium*) after exposure to 420 g m^{-3} of Vapormate^{TM} and three exposure times of 12, 16 and 24 h, at 30ºC (average of 4 replicates)

	12 h		16 h		24 h	
	Mortality (%)	Number of larvae	Mortality (%)	Number of larvae	Mortality (%)	Number of larvae
Treatment	76.3	141	97.5	50	100	121
Control	2.2	45	NA	NA	0.1	60

Semi-commercial trials

Using portable flexible fumigation chamber made of welded PE-PP laminates or using the PVC fumigation chamber was effective. Fumigation of 1.2 tonnes of polished rice in 50 kg bags stored was carried out in the PE-PP laminated chamber using 420 g m^{-3} Vapormate^{TM} at 26±2°C for 24 h resulted in 100% mortality of *R. dominica*, *S. oryzae* and *O. surinamensis* adults (Table 3). The same successful control of 100% mortality of larvae, pupae and adults of *T. castaneum* was obtained when fumigated green coffee beans using 420 g m^{-3} Vapormate^{TM} at 27°C for 24 h in Rentokil flexible fumigation PVC chamber (Table 4).

Table 3. Semi-commercial trials on mortality of *R. dominica* and *T. castaneum* adults after exposure to 420 g m^{-3} of Vapormate^{TM} for 24 h in stored rice (average of 4 replicates)

Test insect		Vapormate^{TM} (420 g m^{-3})		Control	
		Average number of adults	Average mortality (%)	Average number of adults	Average mortality (%)
R. dominica	Top	21.3	100	21.5	16.0
	Middle	21.0	100	NA	NA
	Bottom	21.0	100	21.0	4.0
S. oryzae	Top	20.8	100	22.8	11.0
	Middle	22.5	100	22.8	27.0
	Bottom	21.0	100	20.8	9.0
O. surinamensis	Top	19.5	100	17.3	7.0
	Middle	19.5	100	NA	NA
	Bottom	20.3	100	20.8	7

Table 4. Semi-commercial trials on mortality of *T. castaneum* adults, pupa and larvae after exposure to 420 g m^{-3} of Vapormate^{TM} for 24 h in green coffee beans (average of 4 replicates)

Position in the chamber	Average mortality (%)			Control		
	Adult	Pupa	Larvae	Adult	Pupa	Larvae
Top right	100	100	100	NA	NA	NA
Top left	100	100	100	NA	NA	NA
Top middle	100	100	100	0	0	10
Middle	100	100	100	0	0	5
Bottom middle	100	100	100	0	10.5	5

Commercial trials

Commercial fumigation was carried out as part of the fumigation activities at the sea port of Haifa. A container of 33.2 m^3 was used for importing the 20 tonnes of polished rice stored in bags. The rice was fumigated with 420 g m^{-3} Vapormate^{TM} for 24 h fumigation of the three containers resulted in 100% mortality of *R. dominica* and *S. oryzae* adults (Table 5).

Table 5. Commercial trials on mortality of *R. dominica* and *S. oryzae* adults after exposure to 420 g m^{-3} of Vapormate^{TM} for 24 h in rice (average of 3 containers)

Test insect	Vapormate^{TM} (420 g m^{-3})		Control	
	Average number of adults	Average mortality (%)	Average number of adults	Average mortality (%)
R. dominica	68.3	100	85	4.7
S. oryzae	92.3	100	82	62.1

CONCLUSIONS

The results of this work indicate that exposure to Vapormate^{TM} at the concentration of 420 g m^{-3} at 30ºC for 24 h is an effective fumigation treatment. This treatment was found successful for QPS treatments of grain and other product that are sensitive to infestation by stored products insects. Although, the exposure time needed for Vapormate^{TM} is longer than the time needed for MB, but it is faster and more effective then the use of phosphine. Vapormate^{TM} can be implemented using the same facilities or techniques used by the pest controllers for MB fumigation. Vapormate^{TM} is registered in Israel for the use by the date industry as an alternative to MB since 2008 and is registered for grain treatment since 2010. It is important that it will be implemented for the use of QPS to replace the use of MB or provide an effective substitute.

ACKNOWLEDGMENTS

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EFFICACY OF METHYL IODIDE, SULFURYL FLUORIDE AND CYPERMETHRIN AGAINST THE SIX-TOOTHED BARK BEETLE: *IPS SEXDENTATUS* (BÖRNER)

Ciesla Yann*, Guéry Bernard[1], Ducom Patrick[2]

* Sitona AgroExpert – 9, avenue Marcel Pagnol - 33600 Pessac, France
[1] Ministère de l'agriculture – SRAL-DRAAF Aquitaine - 71, avenue Edouard Bourlaux - BP 81 - 33883 Villenave d'Ornon Cedex, France
[2] Captsystèmes – 20, rue de Lormont Village - 33310 Lormont, France
*Corresponding author's e-mail: *yann.ciesla@laposte.net*

ABSTRACT

Since the ban of methyl bromide for QPS uses in European Union, sulfuryl fluoride is the only fumigant registered in France for log disinfestation Treatments must be done in fumigation chambers. This study was carried out to investigate the efficacy of two fumigants: sulfuryl fluoride and methyl iodide and a contact insecticide: cypermethrin under different conditions against all stages of the six-toothed bark beetle *Ips sexdentatus*. Ten infested pieces of pine (*Pinus pinaster*) were treated for each test series. Cypermethrin was sprayed on all surfaces of the logs with an application rate of 5 g of cypermethrin per cubic meter of wood. Sulfuryl fluoride fumigation was carried out in a 17 m^3 chamber at 30 g m^{-3} and an exposure time of 24 hours. The methyl iodide fumigations were carried out in a 17 m^3 chamber at 35 g m^{-3} and a in a 20 feet container at 50 g m^{-3} and an exposure time of 24 hours. The efficacy of each insecticide was evaluated by comparing the emergence reduction of adult six-toothed bark beetles between control and treated logs. Ten weeks after treatment, the treated logs showed an emergence reduction of six-toothed bark beetle of 88.53% with cypermethrin, 99.92% with sulfuryl fluoride and a concentration time product (CTP) of 716 g h m^{-3} and 100% for methyl iodide fumigations with a CTP of 825 and 942 g h m^{-3} respectively in the fumigation chamber and container.

Key words: Six-toothed bark beetle, *Ips sexdentatus*, cypermethrin, sulfuryl fluoride, methyl iodide

INTRODUCTION

After the phase out of methyl bromide in European Union in 2010, including for QPS uses, methyl iodide was identified as a possible ozone-safe alternative to disinfest timber logs (Ohr et al., 1995). Fumigation is a crucial step to kill insects inside pine wood and to avoid introducing quarantine species into foreign countries. Methyl iodide seems to have the same action spectrum (insecticide, fungicide, nematicide…) as methyl bromide (Waggoner et al.,

2000; Ciesla et al., 2010; Muthu and Srinath, 1974). In France, the six-toothed bark beetle *Ips sexdentatus* (Börner) (Scolytinae, Curculionidae) is the main pest which infests pine wood (*Pinus sp.*). The aim of this trial is to compare the efficacy of two fumigants: methyl iodide (MI), not yet registered, sulfuryl fluoride (SF), and a contact insecticide: cypermethrin. For the moment, sulfuryl fluoride is registered in France for the fumigation of timber but with a restriction: the fumigation has to be done in a fumigation chamber, not in containers. This study was carried out, in Bordeaux, France, on infested pine logs and the efficacy of each insecticide was assessed by comparing the emergence reduction of insects between each treatment and control.

MATERIALS AND METHODS

Insecticides

Three insecticides were tested in this study:

- methyl iodide with a fumigation in a 17 m^3 chamber at 35 g m^{-3} and a fumigation in a 20 feet container at 50 g m^{-3}
- sulfuryl fluoride (Profume$^®$) at 30 g m^{-3} in a 17 m^3 fumigation chamber
- cypermethrin sprayed with an application rate of 5 g of cypermethrin per cubic metre of wood

Methyl iodide (Midas Technical) was supplied by Arysta LifeScience in 1 litre flasks. Methyl iodide is liquid at ambient temperature with a boiling point of 42.5°C. The fumigation was carried by introducing liquid methyl iodide on a stainless shallow pan next to a fan to mix the gas in the fumigated volume. In the 33 m^3 container, 1650 grams of MI were introduced and 595 grams in the 17 m^3 fumigation chamber. Methyl iodide concentrations were measured by thermoconductivity with a Fumiscope.

Sulfuryl fluoride (Profume$^®$) was supplied by Dow AgroSciences in cylinders under pressure. The cylinder was connected with the fumigation chamber and the fumigation was carried out by introducing 510 g of sulfuryl fluoride into the chamber through a 2 mm stainless tube. The concentration of sulfuryl fluoride was measured with an infra-red analyser (Spectros IR).

Cypermethrin (Forester EW) is a pyrethroid and was supplied by Agriphar. The formulation (100 g active, per litre) was applied as a1% mixture. The treatment of wood needs 1 g of cypermethrin per litre of mixture in order to spray the surfaces of the logs, equivalent to 5 L of mixture per m3 of wood. The 10 pine logs represent about 0.3 m^3, so 1.5 L of mixture was applied.

The temperature and relative humidity were recorded for the fumigation and the insecticide treatment with Captsystèmes$^®$ loggers.

Wood

The treated pine logs (*Pinus pinaster*) were 1m in length with diameters ranging from 9 to 19.5 cm. A total of 50 logs were used, with 10 pieces of wood in each test series. The selection of the pine logs was made at random. The infestation of six-toothed bark beetles was natural, so infestation varied between logs. The pine logs came from pine trees cut at the beginning of 2011. Each piece of wood was identified with a number (Table 1).

When the six-toothed bark beetle reaches the adult stage, it emerges from the bark and come out the log. Emerging insects are trapped in a cage composed of an insect-proof net They are attracted by sunlight, then move to the funnel and fall into the insect box (Fig). Every day, the 50 boxes were collected in order to count the emerged insects in test series. These boxes were collected for 10 weeks in 2011. This study began on 27 June (week 26) and was finished on 5 September (week 35).

Table 1. Identifying numbers of each pine log for each test series

Control									
T1	T2	T3	T4	T5	T6	T7	T8	T9	T10
Cypermethrin									
F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Sulfuryl fluoride									
SF1	SF2	SF3	SF4	SF5	SF6	SF7	SF8	SF9	SF10
Methyl iodide in fumigation chamber									
MICH1	MICH2	MICH3	MICH4	MICH5	MICH6	MICH7	MICH8	MICH9	MICH10
Methyl iodide in container									
MICO1	MICO2	MICO3	MICO4	MICO5	MICO6	MICO7	MICO8	MICO9	MICO10
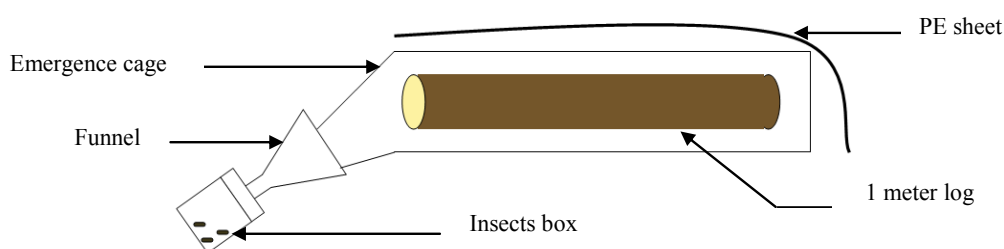
Fig. 1- Composition of an emergence cage.

Insects

The six-toothed bark beetle *Ips sexdentatus* is the largest bark beetle in France, measuring up to 8 mm in length. Generally there are two adult flights per year, one in April-May and the second in July-August, these flights depending of the climatic conditions. The pine logs were naturally infested during the 2011 spring. The efficacy of each treatment was assessed by comparing the reduction of emergence of six-toothed bark beetles with the control. In the case of cypermethrin treatment, the death of insects is not instantaneous, so it is necessary to discriminate between insects which emerge and die after few hours and insects which can survive several days after emergence. An adult insect was considered as alive if it can move and reach the insect box. An adult insect was considered as dead if it died in the emergence cage without ever reaching the lighted part of net and subsequently the insect box.

RESULTS AND DISCUSSION

The measured concentrations show that there was no leakage from the fumigation chamber and the sorption is insignificant. The concentration of sulfuryl fluoride (30 g m^{-3}) and methyl iodide (35 g m^{-3}) at the beginning of the fumigation was the same as 24 hours later (Fig. 2). The actual CT product was practically the theoretical CT product, with 716 and 825 g h m^{-3} respectively for sulfuryl fluoride fumigation (Fig. 2- Evolution of concentrations of sulfuryl fluoride (points), methyl iodide in the 17 m3 chamber (triangles) and methyl iodide in the container (rhombs) during the fumigation of 10 pine logs respectively at 30 g/m3, 35 g/m3 and 50 g/m3.Table) and the methyl iodide fumigation in chamber (Table). The container was not as well sealed, with the theoretical concentration at 50 g m^{-3} but, after 24 hours, only 35 g m^{-3} methyl iodide remained. The CT product achieved for this fumigation in the container was 942 g h m^{-3} (Table).
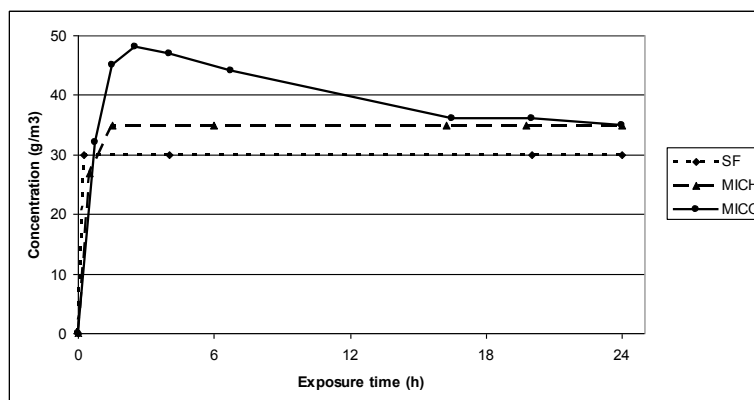
Fig. 2- Evolution of concentrations of sulfuryl fluoride (points), methyl iodide in the 17 m^3 chamber (triangles) and methyl iodide in the container (rhombs) during the fumigation of 10 pine logs respectively at 30 g/m^3, 35 g/m^3 and 50 g/m^3.

Table 2. Concentrations of sulfuryl fluoride measured and calculated concentration-time products after a fumigation of 10 pine logs at 30 g m^{-3} in a 17 m^3 chamber

Exposure time (h)	Concentration (g m^{-3})	CT product (g h m^{-3})
0	0	0
0.25	30	4
4	30	116
20	30	596
24	30	716

Table 3. Concentrations of methyl iodide measured and calculated concentration-time product after a fumigation of 10 pine logs at 35 g m^{-3} in a 17 m^3 chamber

Exposure time (h)	Concentration (g m^{-3})	CT product (g h m^{-3})
0	0	0
0.5	27	7
1.5	35	38
6	35	195
16.25	35	554
19.75	35	676
24	35	825

Table 4. Concentrations of methyl iodide measured and calculated concentration-time product after a fumigation of 10 pine logs in a container at 50 g m^{-3}

Exposure time (h)	Concentration (g m^{-3})	CT product (g h m^{-3})
0	0	0
0.75	32	12
1.5	45	41
2.5	48	87
4	47	159
6.75	44	284
16.5	36	674
20	36	800
24	35	942

For these treatments, the temperature was recorded and the data show that the spraying of cypermethrin was applied at 34.8°C in average (Table 5). In average the temperatures recorded for fumigations (MI and SF) carried out in the chamber were about 24°C. The fumigation with MI in the container was carried at 20.4°C in average.

Table 5. Temperature and relative humidity recorded for each treatment

Test series	Temperature (°C)	Relative humidity (%)
Cypermethrin	34.8	34
Sulfuryl fluoride	24.2	78
Methyl iodide in chamber	23.6	69
Methyl iodide in container	20.4	70

Table 6. Total of emerged adult six-toothed bark beetles collected in the insect boxes per week (W) and percentage of emergence reduction after ten weeks in the test series: T = control; F = cypermethrin; SF = sulfuryl fluoride; MICH = methyl iodide in chamber; MICO = methyl iodide in container

Total emerged adult six-toothed bark beetles/week/test										Total 10 weeks	Emergence reduction (%)
W26	W27	W28	W29	W30	W31	W32	W33	W34	W35		
T	T	T	T	T	T	T	T	T	T	T	T
244	59	52	21	406	216	167	80	29	34	1308	-
F	F	F	F	F	F	F	F	F	F	F	F
9	21	30	19	33	19	13	4	2	0	150	88.53
SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF	SF
0	0	0	0	0	0	0	0	1	0	1	99.92
MICH	MICH	MICH	MICH	MICH	MICH	MICH	MICH	MICH	MICH	MICH	MICH
0	0	0	0	0	0	0	0	0	0	0	100
MICO	MICO	MICO	MICO	MICO	MICO	MICO	MICO	MICO	MICO	MICO	MICO
0	0	0	0	0	0	0	0	0	0	0	100

Ten weeks after treatment, 1308 adult six-toothed bark beetles emerged from the 10 pine logs in the control, 150 from the pine logs treated with cypermethrin, just one from sulfuryl fluoride modality, in the pine log SF8, and none from the 10 pine logs fumigated with methyl iodide in chamber or methyl iodide in container (Table 6). The emergence reduction was 100 % between the control and the methyl iodide tests. The percentage of emergence reduction in the sulfuryl fluoride test was 99.92 % and 88.53 % with cypermethrin. In the emergence cages corresponding to pine logs treated with cypermethrin, a lot of insects emerged and died in these cages without being able to reach the insect boxes. The infestation varied with each pine log. The average number of emerged adult insects per pine log after 10 weeks in the control was about 131 ± 86.5, with 309 insects in the most infested pine log (T8) and 23 insects in the least infested pine log (T2).

CONCLUSION

The aim of the study was to investigate the efficacy of cypermethrin treatment, methyl iodide and sulfuryl fluoride fumigations against the six-toothed bark beetle in order to disinfest pine logs. Ten weeks after treatment, methyl iodide fumigations with a CTP of 825 g h m^{-3} at 23.6°C and a CTP of 942 g h m^{-3} at 20.4°C were effective, killing all stages of this insect. A fumigation with sulfuryl fluoride at 24.2°C and a CTP of 716 g h m^{-3} was almost sufficient to kill all stages of this insect. Actually the percentage of emergence reduction was 99.92 % and just one adult emerged from the 10 treated pine logs after 10 weeks compared with the 1308 adults insects emerged in the control. The surviving adult insect emerged nine weeks after treatment, so it is possible that it was an egg when the pine logs were fumigated. A fumigation with a higher temperature or a higher CTP could improve this efficacy and give an emergence reduction of 100 %. Cypermethrin treatment, with a spraying on all the faces of pine logs, shows an emergence reduction of 88.53 % compared with control. This result could be improved with an higher application rate, but for quarantine pest control it will be very difficult to reach an efficacy to reach the desire probit 9 level. Moreover the spraying needs to apply the insecticide on all the faces of pine wood to ensure a better efficacy, this is difficult to carry out well in the field on pine logs or timbers. Methyl iodide and sulfuryl fluoride are fumigants with the potential to replace methyl bromide to control six-toothed bark beetles.

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POTENTIAL FOR OZONE FUMIGATION AGAINST ANOBIID BEETLES INFESTING STORED PRODUCTS AS AN ALTERNATIVE TO METHYL BROMIDE

Md. Mahbub Hasan*, Thomas W. Phillips and Michael J. Aikins

Department of Entomology, Kansas State University, 123 W. Waters Hall
Manhattan, KS 66502, USA
*Department of Zoology, Rajshahi University, Rajshahi 6205, Bangladesh
*Corresponding author's e-mail: *mmhbgd@yahoo.com*

ABSTRACT

Most anobiids are wood boring beetles, but two species, the cigarette beetle, *Lasioderma serricorne* (F.) and the drugstore beetle, *Stegobium paniceum* (L.), are serious pests of stored products. They are the most common storage insect pests found in botanicals. Both species infest dry plant or animal materials, they have been recorded from a wide range of foods, but their distributions are more temperate than tropical. We investigated the efficacy of ozone as a methyl bromide alternative for controlling two anobiids viz. *L. serricorne* and *S. paniceum*. The ozone fumigation showed promise for controlling both the species. Ozone treatment at concentrations ranging from 150 to 195 ppm was highly effective against adults of both species after 36 h of exposure at room temperature (24ºC). Generally *S. paniceum* was more tolerant to ozone treatment than *L. serricorne*. Results showed ozone concentration requires 144.73 ppm to kill 99% of adult *S. paniceum* while, 107.42 ppm was needed for *L. serricorne*. Ozone treatment at the low concentration of 24 ppm showed no effective control against both species since the mortality rate was less than 50%, even at 48 h exposure. The work reported suggests that the ozone treatment could be a fumigant alternative to environmentally hazardous chemicals for controlling two key botanical insect pest species.

Key words: Botanicals, anobiids, non-chemical alternatives, ozone, fumigation, cigarette beetle, drugstore beetle, stored products.

INTRODUCTION

Stored-product pests are responsible for tremendous damage and economic losses to post-harvest products, stored grains and seeds, packaged food products, and animal and plant derived items and other durable commodities. There are over 1000 described species of Anobiidae (Coleoptera). Most anobiids have wood boring larvae, but two, the cigarette beetle, *Lasioderma serricorne* (F.) and drugstore beetle, *Stegobium paniceum* (L.) are important pests of stored products. The most common insects found in botanicals are *L. serricorne* and *S. paniceum* (Abdelghany et al. 2010). *L. serricorne* is also the most serious pest of stored tobacco, tobacco products, cereal grains and processed foods (Maroof and Phillips 2008) while *S. paniceum* frequently infests dry plant or animal materials, it has been recorded from wide range of food, but its distribution is more temperate than tropical (Abdelghany et al. 2010). Control of both species around the world is primarily dependent upon continued

applications of fumigation including phosphine and methyl bromide (MB) (White and Leesch, 1995; Abdelghany et al. 2010). Although effective, repeated use of fumigants has disrupted biological control by natural enemies and led to outbreaks of insect species, development of resistance to the chemical, undesirable effects on non-target organisms, and environmental and human health concerns (Champ and Dyte, 1976; Phillips and Throne, 2010). Therefore, it is essential to develop alternative pest control techniques for protecting stored commodities.

Ozone (O_3) has received recent attention in disinfesting stored foodstuffs, particularly durable products as an alternative to MB (Sousa et al. 2008; Işikber and Öztekin, 2009; Bonjour et al. 2011). There are only a few published data on the effectiveness of ozone as an insecticide against stored products insect pest (Erdman, 1980; Mason et al. 1999; Kells et al. 2001; Mendez et al. 2003). Therefore, this study was undertaken to investigate the effect of ozone toxicity on the adult annobids *L. serricorne* and *S. paniceum*.

<div align="center">MATERIALS AND METHODS</div>

Insects

The adult beetles *L. serricorne* and *S. paniceum* used in the ozone experiment were obtained from the Center for Grain and Animal Health Research, USDA-ARS, Manhattan, USA. Subsequent cultures were routinely maintained on white flour (95%) and brewers yeast (5% by weight) at 27°C and 60–70% relative humidity (r.h.), in a constant 16 h light: 8 h dark lighting regime in the Department of Entomology, Kansas State University, Manhattan, USA.

Experimental procedures

To determine the ozone efficacy against *S. paniceum* and *L. serricorne,* mixed sex adults of both species were collected from the stock culture. For each species of anobiids, ten insects were placed in cylindrical glass vials (4.5 cm in height by 1.2 cm in diameter), and then five glass vials for each species were placed in an Erlenmeyer glass flask (1L) for ozone treatment. The vials had lids with the inside top edge coated with liquid Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to inhibit insect climbing and with 0.42-mm openings in the caps to permit air and ozone entry yet prevent beetles from escaping. A small quantity of diet was added to each vial. Lids were screwed on tightly after insects and the diet had been placed in the vials. The ozone concentrations evaluated were 24, 59, 96, 128, 146, 153 and 195 ppm and exposures were 3, 6, 12, 18, 24, 30, 36 and 48 h. The adult mortality was assessed 24 h later. Ozone concentration–time exposure response data were subjected to probit analysis (SAS, 2004), for generating mortality curves. Ozone application was carried out in an ambient temperature of approximately 25°C and 55-60% r.h., while the control chambers were placed in atmospheric air under the same conditions.

Ozone generation and system functioning

An ozone generator of laboratory scale was provided by the Adaptive Ozone Solutions Inc., Kansas, USA (http://adaptiveozone.com/). Ozone gas was generated from ambient room air using an electric discharge system. The air flow rate was adjusted to 2L min^{-1}. The generated ozone was initially injected into and stored in a large plastic chamber (approx. 48 L) to facilitate uniform distribution of the air-ozone mixture into the insect treatment chambers (Fig 1). The amount of generated ozone was regulated by adjusting the electric tension through a voltage regulator (dosing button). The exhaust of the ozone-treated chamber was connected to a Tygon tube and then passed through an Erlenmeyer glass flask (1L) containing water, before releasing into the insect chamber, to humidify the gas mixture and minimize the desiccation of test insects. The ozone carrier flow rate was adjusted to 2 L min^{-1} while entering into the Erlenmeyer glass flasks (1L) containing insect samples. The output of the insect treatment flasks were directly connected into the ozone monitoring instrument through

a filter chamber for cleaning the generated ozone. The ozone concentration indicated by the generator was checked with a continuous UV ozone monitor (model IN-2000 LoCon Ozone Analyzer, USA) and the data were directly recorded onto a laptop computer equipped with appropriate software (Taltalk ver. M5000).
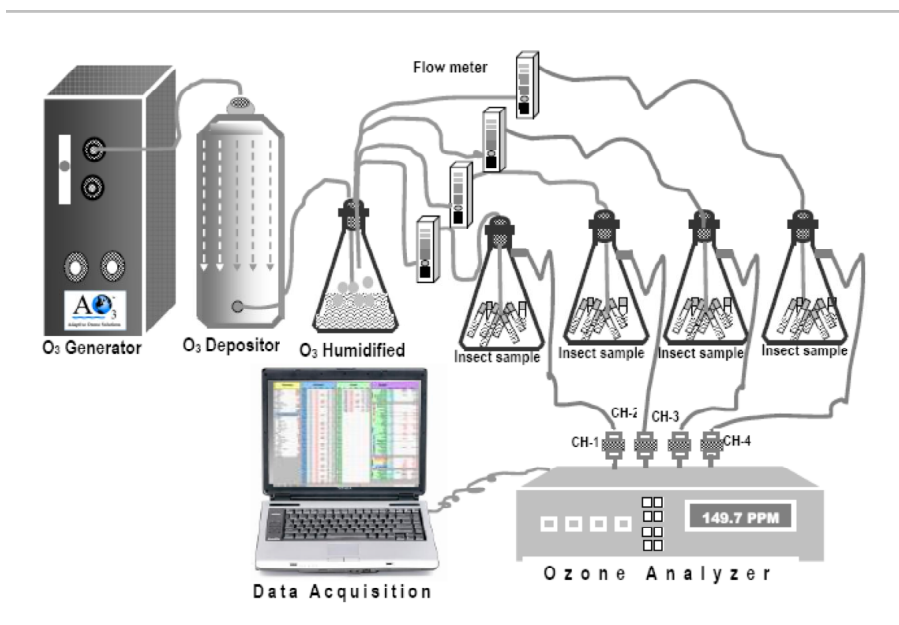
Fig. 1- Schematic of ozone generation and monitoring system.

RESULTS AND DISCUSSION

The probit estimates derived from mortality responses of adult *S. paniceum* and *L. serricorne* exposed to ozone at different exposure periods and gas concentrations are summarized in Tables 1 and 2. In general, adult *S. paniceum* were found to be more tolerant than *L. serricorne*. An ozone concentration of 195 ppm caused 100% mortality in *L. serricorne* at 12 h exposure (Fig. 2), while in *S. paniceum* the same concentration required 24 h exposure (Fig. 3). At longer exposures, i.e. 48 h, 100% kill was achieved with 24 ppm and 59 ppm ozone, for *L. serrricorne* (Fig. 2) and *S. paniceum* (Fig. 3) respectively.

Table 1. Probit analyses of mortality for *S. panicium* fumigated with ozone at different exposure periods.

Exposure h	N	LC_{50} ppmv (95% fiducial limits)	LC_{99} ppmv (95% fiducial limits)	Slope ± SE	Intercept±SE	χ^2 values (df)
12	55	87.86 (73.98 – 100.85)	344.12 (258.61 – 561.62)	13.26 ± 0.91	33.89 ± 6.14	177.53 (53) P=0.0001
18	30	72.94 (65.59 -80.82)	276.75 (220.65 – 382.54)	16.13 ± 0.83	8.65 ± 4.97	35.46 (28) P=0.15
24	50	65.76 (57.13 – 73.64)	215.69 (179.65 – 281.13)	14.09 ± 0.75	11.15±5.75	85.14 (48) P=0.008
30	25	50.64 (35.99 – 64.55)	296.15 (176.21 – 1093.00)	11.34 ± 1.25	8.75 ± 8.15	66.98 (23) P=0.001
36	40	37.14 (31.77 - 42.10)	144.73 (119.75 - 188.76)	14.13± 1.05	-13.19 ± 8.67	31.81 (38) P=0.75
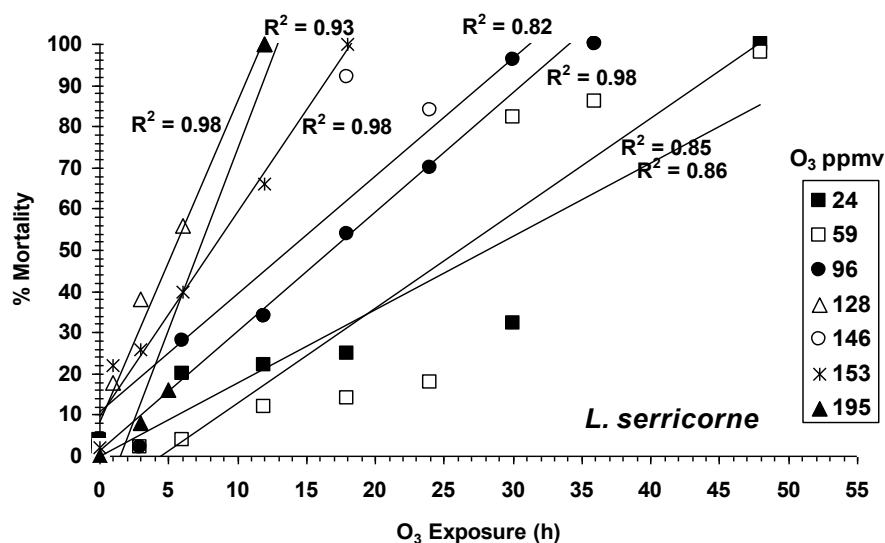
Fig. 2- Mortality of adult *L. serricorne* fumigated with ozone at different concentrations and exposures.

Table 2. Probit analyses of mortality for *L. serricorne* fumigated with ozone at different exposure periods

Exposure h	N	LC$_{50}$ ppmv (95% fiducial limits)	LC$_{99}$ ppmv (95% fiducial limits)	Slope ± SE	Intercept±SE	χ^2 values (df)
12	55	102.79 (81.29 – 127.46)	631.73 (373.05 – 2033.00)	11.62 ± 1.21	52.37 ± 7.38	264.05 (53) P=0.001
18	30	78.36 (60.43 – 102.25)	351.16 (217.18 – 1017.00)	14.85 ± 1.04	19.62 ± 6.09	120.89 (28) P=0.0001
24	50	70.09 (54.65 – 84.26)	285.98 (207.81 – 513.17)	14.09 ± 0.75	11.15±5.75	215.50 (48) P=0.0001
30	25	22.14 (9.01 – 31.88)	195.85 (118.51 – 766.05)	10.69 ± 1.65	-7.48 ± 13.15	43.36 (25) P=0.006
36	40	31.17 (26.36 – 35.56)	107.42 (88.70 – 141.77)	14.13 ± 1.05	-13.19 ± 8.67	24.57 (38) P=0.95

Mortality of adults of both the species was significantly (P > 0.001) higher at the medium (>128 ppm) and high (>153 ppm) ozone levels than at the low (> 24 ppm) levels after 24 h exposure. However, 100% mortality was never reached, even at the high ozone level at lower (< 24 h) exposure. The lethal time estimated for 50% and 99% mortality trended longer in *S. paniceum* than in *L. serricorne*, corresponding to the respective ozone concentrations (Figs. 2 and 3). These trends clearly indicate that adults *S. paniceum* were more tolerant to ozone fumigant than *L. serricorne*. The mortality data of adults of both species tested with ozone fumigation agreed with those of several authors who observed toxicity of ozone against different stored products insect pests (Erdman 1980; Mason et al. 1999; Kells et al., 2001; Sousa et al. 2008; Bonjour et al. 2011).
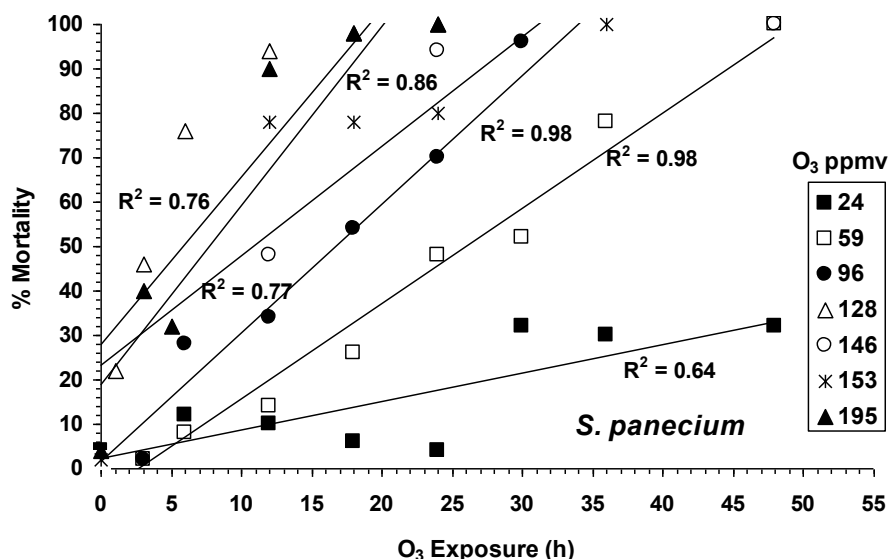
Fig. 3- Mortality of adult *S. panecium* fumigated with ozone at different concentrations and exposures.

Erdman (1980) investigated the efficacy of ozone against *T. castaneum* and *T. confusum* by mortality of larvae. Maize treated with 50 ppm ozone for 3d resulted in 92–100% mortality of adult *T. castaneum, Sitophilus zeamais* and *Plodia interpunctella* larvae and 63% reduction in the fungus *Aspergillus parasiticus* Speare on the kernel surface (Kells et al., 2001). In a follow-up study, Mendez et al. (2003) investigated the effects of ozone exposure on the food end-use properties of maize, popcorn (*Z. mays* L. var. *everta*), soybean (*Glycine max* (L.) Merr.), wheat (*Triticum aestivum* L.), and rice (*Oryza sativa* L.). Ozone has been demonstrated as an effective fumigant insecticide and fungicide to extend the storage life of dried maize; however, ozone has not been examined as a method to increase the allowable storage time of high-moisture maize. Sousa et al. (2008) assessed ozone toxicity to phosphine-resistant pests of stored products: no tested populations showed resistance to ozone. All these results reveal that ozone is a potential fumigant for stored products.

Therefore, the use of ozone against *S. paniceum* and *L. serricorne* seems a promising management alternative to MB. The species *L. serricorne* is generally much more susceptible to gaseous ozone than *S. paniceum*. Furthermore, detailed studies are required to determine mortality responses of immature life stages of anobiids and to show the products' quality that are frequently infested by the anobiids after exposing ozone fumigation.

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ERADICATION OF EUCALYPTUS WEEVILS IN APPLES BY ETHYL FORMATE

Manjree Agarwal[1,2]*, Yonglin Ren[1,2,3], James Newman[1,2] and Hui Cheng[1,2]

[1]Cooperative Research Centre For National Plant Biosecurity
[2]School of Biological Science and Biotechnology, Murdoch University, WA, 6150, Australia
[3]Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court South Perth WA 6151, Australia
*Corresponding author's e-mail: *m.agarwal@murdoch.edu.au*

ABSTRACT

Export of Pink Lady™ apples from Australia have been significantly affected by infestations of adult eucalyptus weevil (eucalyptus snout beetle or gum tree weevil). These weevils do not damage apple trees or fruit, but rest at the petiole portion of apples when selecting overwintering sites. As a result apples infested with live eucalyptus weevils leads to rejection for export. Usage of methyl bromide as post harvest treatment is restricted under the Montreal Protocol. Therefore, it has become important to develop an alternative safe fumigant as an eradication method for eucalyptus weevil on apple. Laboratory experiments were conducted to evaluate ethyl formate, which is a naturally occurring volatile chemical present in many plant commodities, as fumigant for eradication of the eucalyptus weevil on apples. Laboratory and cool storage trials show that ethyl formate is highly toxic to the eucalyptus weevil and low phytotoxic to the fruit. Complete control can be achieved at 30 g m^{-3} of ethyl formate at 25ºC for 24 hours exposure with and without apple. In comparison with untreated apples, the colour and texture have no change 1, 2 and 3 weeks after treatment. Four field trials were conducted in cool storages (the capacity ranged from 250-900 tonnes) in Western Australia. The ethyl formate was applied at dosage of 50-55 g m^{-3} and low temperature (4-8ºC) for 24 hours exposure. All eucalyptus weevils were killed and after 1 day aeration, residue of ethyl formate declined to natural levels (0.05-0.2 mg kg^{-1}). Phytotoxicity studies showed no effect on morphology or taste of apples.

Key words: apple, ethyl formate, fumigant, fumigation, eucalyptus weevil

INTRODUCTION

Australia conducts a small but important export trade in Pink Lady™ apples, but recent exports have been significantly affected by infestation of adult eucalyptus weevil. The insect was accidentally introduced to WA where it inhabits blue gum (Eucalyptus globulus) plantations, but in autumn, some adult weevils seek shelter in apple orchards during harsher weather. The weevils do not damage apple trees or fruit, but rest at the stalk when selecting overwintering sites. When subjected to quarantine inspection in Australia prior to overseas export, such fruit would be rejected, especially for the lucrative British and European markets. Until successful management programs for the weevil can be developed, the issue of bulk picking, packing and transporting is uncertain and even shipment in cartons is at risk.

Due to restrictions governing use of methyl bromide as mandated by the Montreal Protocol, use of naturally occurring plant volatiles as potential fumigants for post-harvest treatment of insect pests was considered a priority for investigation. One such compound is ethyl formate which has long history as a fumigant for stored products (Cotton and Roark, 1928) and for dried fruit in particular (Simmons and Fisher, 1945; Banks and Hilton, 1996). For the past few years, ethyl formate has been re-evaluated as an alternative fumigant for grain stored in unsealed farm bins (Annis, 2002; Ren et al., 2003; Ren and Mahon, 2006). It is registered as a fumigant for dried fruit in Australia and has a history of safe use as a food additive. Ethyl formate occurs naturally in soil, water, vegetation and a range of raw and processed foods including vegetables, fruit, grain, beer, grapes, wine and animal products like milk and cheese (Desmarchelier, 1999). Unlike other fumigants, ethyl formate kills insects rapidly and its residue breaks down to naturally occurring products, formic acid and ethanol (Desmarchelier et al., 1998). It is a colourless liquid with a low boiling point (54.1^{o}C) and has a pleasant aromatic odour. Its flammable limit is 85 g m-3. The US Food and Drug Administration (FDA, 1984) reviewed its use as a flavouring agent and characterised it as safe.

Experiments have been conducted using ethyl formate as a post-harvest fumigant for some pests of table grapes (Simpson et al., 2007) and thrips in onion (Van Epenhuijsen et al., 2007). Here we report the effectiveness of various concentrations of ethyl formate in controlling eucalyptus weevil both at laboratory and commercial scale cool storages.

MATERIALS AND METHODS

Fruit and insect samples
For both laboratory study and field trials, Cripps Pink apples (also known as Pink Lady™) were supplied by Newton Brothers' Orchard (Western Australia, WA). The fruit samples were stored at 5ºC in a cool room. Adult eucalyptus weevils collected from blue gum plantations in Manjimup (WA) were used for bioassay.

Reagents and apparatus
Ethyl formate used for laboratory study was supplied by Sigma Aldrich, reagent grade, 97% purity. For commercial scale fumigation, food grade ethyl formate, supplied by Bronson & Jacobs Pty Ltd., Australia.

One litre Erlenmeyer flasks (Bibby Sterilin, Staffordshire, Cat. No. FE 1 L/3) were used for preparation of standards; 250 mL Erlenmeyer flasks (Crown Scientific, Code FE1L3) equipped with cone/screw-thread adapter (Crown Scientific, Code ST 5313) with 7/16" blue septa (Grace Davison Discovery Sciences, catalog: 6518) were used for fumigation; 120 mL glass bottles (Plasdene Glass Pak, Perth) were used to monitor weevils after fumigation; and 4 litre glass jars (Plasdene Glass Pak, Perth) with screw tight lids were used for phytotoxicity and residue studies; 4 L glass jars were used for the fumigation of the apple samples plus insects.

A 100 µL syringe (SGE, Melbourne, Cat. no. 005250) and 5 uL syringe (SGE, Melbourne, Australia; Cat. no. 001000 5F) were used for injection of gas samples into the gas chromatographs (GC) and transfer of liquid ethyl formate to make gas standards; 50 mL air tight syringes (SGE, Melbourne; Cat. no. 008900) were used to withdraw air from empty flasks to make the standard.

Analysis of ethyl formate

Ethyl formate was determined using DPS portable GC companion 600 equipped with a flame ionisation detector (FID) after isothermal separation on a 30 m × 0.53 mm (i.d.) 3 um, metallic column, Restek 800-356-1688 phase MXTr-S, (Catalogue no. 70285, serial no. 702152) at oven temperature 90°C, detector temperature 150°C and carrier flow helium regulator 55 KPa and air regulator 100 Kpa.

All the samples and standards were injected in duplicate. The concentrations of ethyl formate were calculated on the basis of peak areas as compare to gas standards.

Laboratory bioassays

Fumigation was carried out in 250 mL Erlenmeyer flasks without apples at 5, 10, 15, 20, 25, 30, 40 and 80 g m^{-3} of ethyl formate with 25 adult weevils in each were taken. For bioassays with apples, seven 4 litre glass jars were loaded 90-95% full with apples and 100 adult weevils in each. The jars were sealed with airtight lids equipped with septa as an injection port and a cone-shaped filter paper. Three jars were treated with 40 g m^{-3}, three with 80 g m^{-3} of ethyl formate and one served as control.

The concentration of ethyl formate was measured by gas chromatography (GC-FID) at intervals over the exposure period of 24 hours. After 24 hours fumigation, flasks or jars were opened to check mortality and the insects were transferred to new 120 mL bottles containing fresh blue gum leaves at 25ºC to check for their recovery.

Laboratory phytotoxicity and residual studies

For these studies eight apples were placed in each of seven 4 litre glass jars. As mentioned above, jars were treated with 40 and 80 g m^{-3} of ethyl formate and one as control. After 24, 48 and 96 hours fumigation, one jar each of 40 and 80 g m^{-3} were opened and the apples were checked for morphological and physiological changes compared with unfumigated fruit. For morphological changes fruits were looked visually for any spots, skin damage, texture, change in colour compare to control. This was done in both whole and apple cut from the petiole.

For analysis of ethyl formate residues, one apple each from 24, 48 and 96 hours exposure with no aeration, one day, two days and four days' aeration and the untreated control were taken out and kept in a freezer prior to determination of levels of ethyl formate.

Commercial scale cool room fumigation trials
Application and bioassay

Two different methods for application of ethyl formate were tested at Newton Brothers' Orchard Western Australia. In the first electric frying pans and in second a new inhouse made unit was used for vaporization. Dosages of 50-55 g m^{-3} were applied for 24 h for all large scale trials. For bioassays plastic vials with weevils having screen lids were placed in different locations throughout the cool room, the treated and unexposed insect numbers used were 800-1200 and 200-300 adults respectively for each trial.

Gas sampling and monitoring

For analysis of ethyl formate gas samples were drawn from the storage through nylon lines using an electric pump. The gas samples were stored in Tedlar$^{®}$ sample bags (1 L) until analysis using the gas-chromatographic conditions previously described.

Laboratory bioassay of ethyl formate

All bioassay results were compared with untreated controls kept under the same conditions, with the same number of weevils. For bioassays without apples 100% adult mortality was achieved at 30 g m^{-3} and above of ethyl formate at 22-24ºC. However, mortality of 81, 72, 13 and 0% were observed at 25, 20, 15 and 5 g m^{-3} of ethyl formate (Figure 1). End point mortality readings taken at two and four days did not show any revival of weevils. In case of
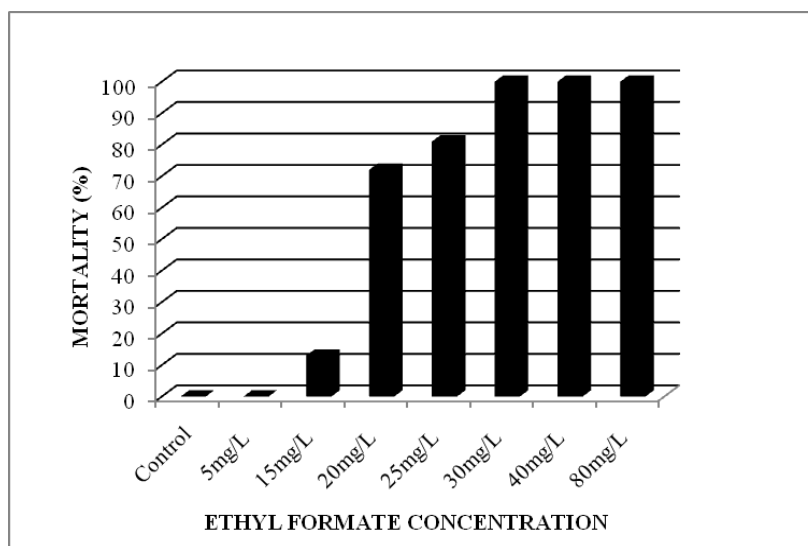
Fig. 1- Mortality of eucalyptus weevils at different levels of ethyl formate
22-24ºC for 24 hours exposure

bioassay with apples as some ethyl formate being absorbed by the fruit 100% control was achieved at 40 g m^{-3} of ethyl formate at 22-24ºC for 24 hours. The loss of fumigant in the chamber during fumigation showed that about 50% of applied ethyl formate was absorbed (Figure 2). The concentration of the formulation declined rapidly within the first 4 hours. This result is consistent with previous trials of ethyl formate on wheat, barley, oats and peas (Desmarchelier et al., 1998; Ren and Mahon, 2003, 2006).

Phytotoxicity and residue studies of ethyl formate for apples

Residue studies showed that after one day aeration ethyl formate residue in apple have declined to background levels (0.05-0.2 mg kg^{-1}) as compare to 0 day aeration. (Table1). These results are consistent with previous commercial-scale trials with ethyl formate on wheat, barley, oats and peas (Desmarchelier et al., 1998; Ren and Mahon, 2003, 2006).

In comparison with untreated apples, the colour and texture of fruit subjected to fumigation with ethyl formate showed no change and had no effect on morphology even after 1, 2 and 3 weeks of treatment.
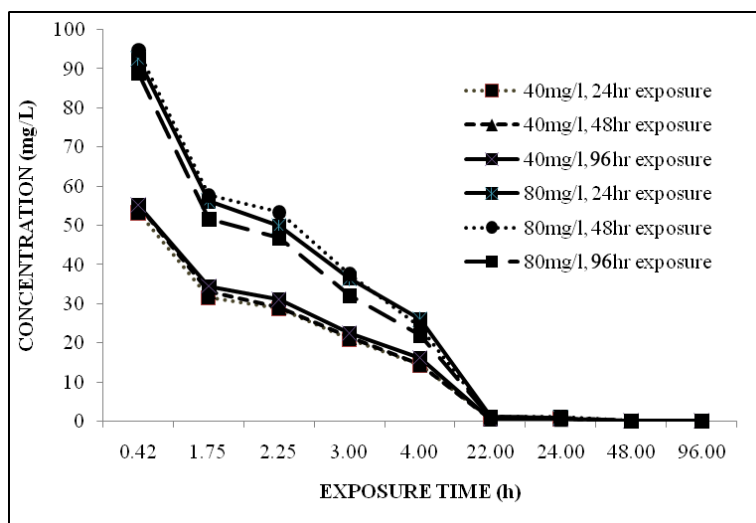
Fig. 2- Sorption of ethyl formate by apples with time for two dosage rates and three exposure time with ethyl formate after removal from fumigation chamber

Commercial scale bioassay of ethyl formate against eucalyptus weevils

Ethyl formate application methods

The new ethyl formate nitrogen purging unit, developed in house, was highly efficient in vaporising and delivering ethyl formate into the commercial-sized cool storage areas. For example, 50 litres of ethyl formate can be vaporised and delivered to a 900 m^3 cool room in less than 45 minutes. The unit works reliably and has no OH&S issue. This technology has great potential to offer application of ethyl formate for pre-shipment treatment of other insect pests of fruit and vegetables.

Table 1. Ethyl formate residues in treated apples at 40 and 80 g m^{-3} at different exposure durations as compared to untreated control samples

Dosage (g m^{-3})	Exposure time (days)	After exposure (no aeration)	One day of aeration
40	1	10.4	<0.05
	2	12.5	<0.05
	4	13.2	<0.05
80	1	18.5	<0.05
	2	16.2	<0.05
	4	16.3	<0.05
Untreated control	1	<0.05*	<0.05
	2	<0.05	<0.05
	4	<0.05	<0.05

*. <0.05 is GC detection level

Bioassays

Total mortality was achieved in all the treated plastic vials compared to no mortality in the untreated controls. 5000-6000 number of dead test insects with no survivors could be considered an acceptable result for the purpose of substantiating this use of ethyl formate as a commercial phytosanitary treatment for required quarantine inspections for export fruit.

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F.A.S.T. (FUMIGATION ABATEMENT AND DESTRUCTION) SYSTEM

P. Swords*, D. Mueller, A. Vanryckeghem

Insects Limited, Inc. and Fumigation Service & Supply
16950 Westfield Park Road
Westfield, IN 46074 USA; O: (317) 896-9300 | C: (317) 306-0160 | F: (317) 867--5757
*Corresponding author's e-mail: *p.swords@insectslimited.com*

ABSTRACT

The F.A.S.T. System scrubber accompanies a fumigation chamber/area by functioning to capture and destroy fumigant gases such as methyl bromide, sulfuryl fluoride, methyl iodide and any other alkyl halides. The F.A.S.T. system contains a depository holding scrubbing material that causes a substantially complete chemical breakdown of the fumigant introduced. The solution is non-carbon based and is mostly aqueous containing chemical degradation properties. Any alkyl halide such as methyl bromide or sulfuryl fluoride agitated through the depository can be broken down through the scrubbing solution by a SN2 chemical reaction. By-products of the reaction are retained in the scrubber depository leaving only ambient air to be released into the atmosphere.

INTRODUCTION

Many native and non-native species of moths, beetles, borers, parasites, nematodes, other microorganisms, and rodents have the ability to damage a wide range of agricultural and non-agricultural commodities and stored products. Such items can include grain, seed, flour, processed foods, cut wood, logs and a wide variety of other perishable and non-perishable goods and products. Fumigation using specialized gases with lethal properties to a broad spectrum of pests, especially insects, rodents, and pest microorganisms, are a fast and effective eradication method to prevent damage to stored commodities (Mueller, 2010). Fumigations can take place in any gas/air tight chamber, building or structure to eliminate infestations. Sealed containers, trailers, boxcars, import and export shipments, grain bins, homes and other free standing structures, where fumigant gas can be introduced and held for a long enough periods of time to, make for desirable fumigation areas and permit eradication. Other more unique situations where fumigations may take place include libraries, restaurants, ship holds, museums, and rare and/or high value artifacts.

For many years, methyl bromide (MeBr) has been widely used as a pesticide fumigant. However, because bromine released from methyl bromide has been found to contribute to depletion of the ozone layer in the troposphere, its use is being eliminated under both U.S. laws and international treaties. Nevertheless, until such time as those fully take effect, methyl bromide is still being used as a fumigant, particularly in the U.S. for quarantine and pre-shipment purposes (Mueller, 2010). From an environmental standpoint, there is therefore a strong incentive to develop systems that will ensure that fumigations performed with methyl bromide will not result in its escape into ambient atmosphere.

Because of the ozone-depleting drawbacks associated with the use of methyl bromide as a fumigant, efforts have been made to find and/or develop non-ozone depleting substitute pesticide fumigants. One such substitute is sulfuryl fluoride (SF). Although not an ozone depletor, SF is a colorless and odorless gas which is toxic if inhaled (MeBr gas is also toxic if inhaled). SF is therefore a hazardous gas, and it is necessary to take stringent safety precautions and perform fumigations using only properly trained personnel using proper safety techniques.

Most fumigant systems in present use offer little, if any, control of the spent fumigant gas, even those involving the use of toxic and/or ozone depleting substances like MeBr and SF. Frequently, all that is done is to maintain a safety perimeter (about 15 m) around the location so any persons in the area will be at a safe inhalation distance when the spent fumigant is simply exhausted to the atmosphere (Swords et al., 2011). Other systems offer some recovery of the spent fumigant; however, these systems use carbon absorption techniques that require activated carbon based filters and/or beds to capture the fumigant (Swords et al., 2011). The activated carbon does not destroy the fumigant but instead only holds the fumigant for a short time, eventually allowing it to be let back into the atmosphere. Moreover, carbon based systems are only able to be used with MeBr and are ineffective when the fumigant is SF. Thus, there is a need for improvement in this field.

MATERIALS AND METHODS

The scrubber functions to capture and destroy any harmful fumigant gas by a nucleophilic-substitution reaction. The F.A.S.T. system removes fumigant from a variety of fumigation situations including agricultural and non-agricultural commodities and/or stored products, structures and articles of value. The system includes a fumigation chamber enclosing in a substantially gastight manner an area containing an object to be fumigated therein. A fumigant gas having environmentally hazardous and/or toxic-to-humans properties is provided for introduction into the chamber. A fumigant gas scrubber containing fumigant destruction properties is also provided. A delivery system is employed for delivering the fumigant gas into the fumigation chamber and for delivering spent fumigant gas under pressure to the fumigant gas scrubber after the object has been fumigated. The scrubber functions to capture and destroy any harmful fumigant gas, such as for example methyl bromide or sulfuryl fluoride, which might otherwise be released to the atmosphere after fumigations (Swords et al., 2011). The system can be used with pallets, shipping containers, fumigation chambers, trailers and is able to fumigate small buildings and bins from a truck-based mobile system. Methyl bromide (MeBr) or sulfuryl fluoride (SF) is drawn out of the area fumigated by a regenerative air blower that forces contact with the scrubbing liquid through a multi-prong filter head. The spent fumigant gas is agitated through the solution causing a chemical breakdown of the fumigant gas into liquid and other non-hazardous by-products (Swords et al., 2011).

SN2 Substitution Reaction
The destruction process proceeds by the SN2 substitution reaction. This can be explained as a reaction of an electron pair donor, which would be a nucleophile (Nu), with an electron pair acceptor, which is the electrophile. The key to this reaction is that the electrophile must have a leaving group in order for the reaction to take place. In this case it is the halide (X) shown in Fig. 1. The halides in fumigants are bromide (Br) and fluoride (F) in methyl bromide and sulfuryl fluoride. The nucleophile that causes the breakdown is within the scrubbing solution.

The electron pair from the nucleophilic scrubbing solution attacks the electrophile which can be methyl bromide, sulfuryl fluoride or any other alkyl halide. This nucleophilic attack takes place at the carbon or sulfur molecule at the center forming a new bond, while the leaving group, Br or F, departs with an electron pair.
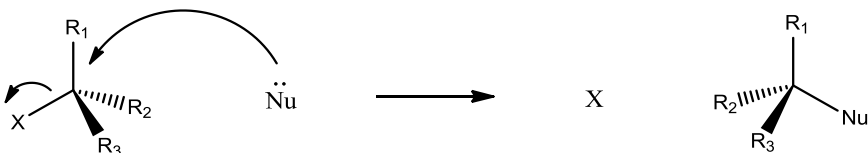
Fig. 1- Reaction of an electron pair donor, a nucleophile (Nu), and an electron pair acceptor, which is the electrophile and a leaving group halide (X).

System Workings

Following fumigation, the system is switched on from a 110 or 220 volt power source providing the necessary energy to run the system. The system employs a regenerative air blower that operates to draw out fumigant through a network of 4 and 2 inch piping to be delivered under pressure scrubbing solution depository. Once the air blower is running, the spent fumigant gas is drawn into the scrubber system inlet and travels through flexible tubing. At the other end of the flexible tubing, a reducer reduces the diameter of the inlet opening. At this point, the fumigant gas flows through piping and into the blower inlet. At the air blower inlet, the fumigant gas then moves through the regenerative air blower building pressure and then is forced through the blower outlet.

Moving from the blower outlet, the spent fumigant gas then travels through piping into the scrubber inlet in the depository and through an agitator having a filter head positioned inside and towards the bottom of the depository. The depository, containing the special scrubbing liquid, may be a wide variety of volumes (currently the largest is 950 L [250 gal]). The filter head, which is multi-pronged, is designed so that on each prong contains small apertures that allow the spent fumigant to bubble through (Swords et al., 2011). Size of apertures may vary to provide the optimum range of bubbling size and/or agitation action so that the fumigant gas can be broken down chemically much easier as it travels through the scrubbing solution from the multi-prong filter head. Spent fumigant gas bubbles through the scrubbing solution creating agitation and complete chemical breakdown of the gas introduced. Ambient air is allowed to travel up and through the exhaust and non-toxic and environmentally non hazardous or ozone depleting by products are contained within the depository (Swords et al., 2011). Gas concentrations can be monitored throughout the entire scrubbing process. The monitoring equipment provides readings of how much fumigant is left in the fumigation chamber, when the reading from the equipment within the chamber reads 0-0.0353 g m^{-3} (0-1 g/ft^3), the process has been completed and sealing may be removed. Two other locations are monitored for fumigant concentration including the exhaust from the scrubbing system and another monitoring line measuring ambient air surrounding the chamber and system. Overall, the system within 10-15 minutes can completely remove fumigant from 28.3 m^3 (1000ft^3) but is subject to change with up scaling and further testing. Our most common 950 L (250 gal) system allows more than 4 air exchanges per hour on land/sea containers or trailers and runs at a minimum of 6 m^3/min (215 ft^3/min).

RESULTS FROM SCRUBBING PROCESS

Table 1. F.A.S.T. System removing MeBr from 90 m^3 trailer

Time (min)	Conc. Trailer (g m^{-3})	Conc. Exhaust (g m^{-3})	Conc. Air (g m^{-3})	Corrected Conc. (g m^{-3})
0	128	0	0	0
5	67	2	2	0
10	28	3	2	1
15	27	1	2	0
20	24	2	1	1
25	20	1	1	0
30	14	1	1	0
35	7	1	1	0
40	5	1	1	0
45	2	1	1	0
50	1	1	1	0

Example 1: F.A.S.T. System removing MeBr from 90 m^3 (3200 ft^3) trailer.
Date: 6/14/2011   Start Time: 4:00 pm
MeBr: 6.8 kg (15 lb)   Trailer: 90 m^3 (3200 ft^3)
Note: Concentration measured in units of g m^3 (oz/1000 ft^3)
Filter: Multi-head Filter

Table 2. F.A.S.T. System removing MeBr from 28 m^3 fumigation chamber

Time (min)	Conc. Chamber (g m^{-3})	Conc. Exhaust (g m^{-3})	Conc. Air (g m^{-3})	Corrected Conc. (g m^{-3})
0	111	0	0	0
5	30	0	0	0
10	7	0	0	0
15	0	0	0	0

Example 2: F.A.S.T. System removing MeBr from 28 m^3 (1000 ft^3) fumigation chamber.
Date: 8/17/2011   Start Time: 3:45 pm
MeBr: 0.7 kg (1.5 lb)   Chamber: 28 m^3 (1000 ft^3)
Note: Concentration measured in units of g m^{-3} (oz/1000 ft^3)
Filter: Multi-head Filter

Table 3. F.A.S.T. System removing MeBr from 90 m³ fumigation trailer

Time (min)	Conc. Trailer (g m⁻³)	Conc. Exhaust (g m⁻³)	Conc. Air (g m⁻³)	Corrected Conc. (g m⁻³)
0	30	0	0	0
5	18	1	2	0
10	12	2	1	1
15	11	2	1	1
20	9	1	0	1
25	8	1	1	0
30	7	0	0	0
35	3	2	1	1
40	3	1	0	1
45	1	1	1	0

Example 3: F.A.S.T. System removing SF from a 90 m³ (3200 ft³)trailer.
Date: 6/14/2011   Start Time: 5:25 pm
SF: 6.4 kg (14 lb)   Trailer: 90 m³ (3200 ft³)
Note: Concentration measured in units of g m⁻³ (oz/1000 ft³)
Filter: Multi-head Filter

Table 4. F.A.S.T. System removing MeBr from 28 m³ fumigation trailer

Time (min.)	Conc. Chamber (g m⁻³)	Conc. Exhaust (g m⁻³)	Conc. Air (g m⁻³)	Corrected Conc. (g m⁻³)
0	70	0	0	0
5	20	2	2	0
10	5	2	2	0
15	3	0	0	0
20	1	0	0	0

Example 4: F.A.S.T. System removing SF from 28 m³ (1000 ft³)trailer.
Date: 7/1/2011   Start Time: 2:00 pm
SF: 0.7 kg (1.5 lb)   Chamber: 28 m³ (1000 ft³)
Note: Concentration measured in units of g m⁻³ (oz/1000 ft³)
Filter: Multi-head Filter

Example 5: Gas chromatograph analysis of F.A.S.T. System exhaust (small scale).

Fig. 3 represents the concentration of methyl bromide from the exhaust as a function of time. This study was done on small scale within the lab hood using gas chromatograph analysis to determine the concentration of methyl bromide by peak area after gas has been scrubbed and allowed to flow through the exhaust.

As can be seen in the above graph 100% methyl bromide has a concentration / peak area of over 1,000,000 units. After scrubbing, the gas/air flow from exhaust is analyzed showing miniscule amounts of methyl bromide present.

Fig. 2- Recent F.A.S.T. System Scrubber installed at Chicago O'Hare International Airport.

Example 6: Gas chromatograph (GC) analysis of scrubber exhaust from 794 L system (210 gal).

Fig. 4 shows the results of GC analysis for the scrubber exhaust of the large scale 794 L system (210 gal) F.A.S.T. system. The grey trace represents concentrated methyl bromide with overlays of the scrubber exhaust (the dotted line) and ambient air surrounding the system (solid black line). The results show no indications of methyl bromide present from the F.A.S.T. System exhaust as well as the surrounding ambient air.
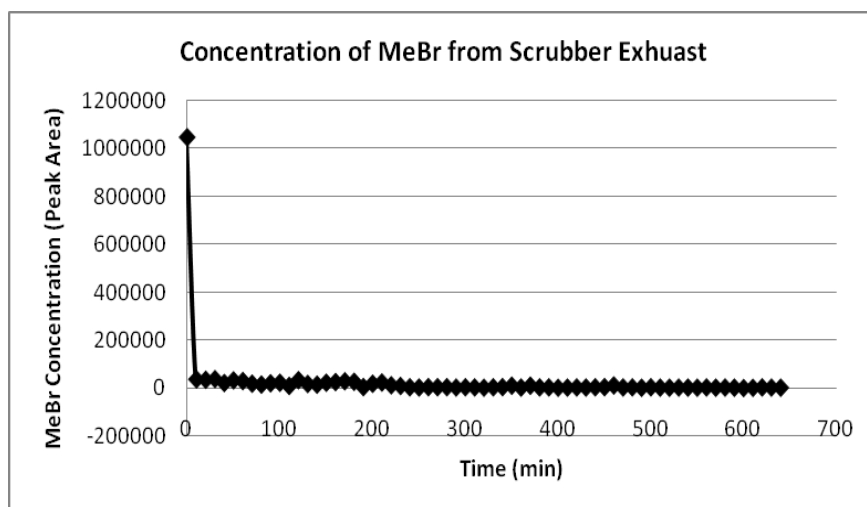
Fig. 3- Gas chromatograph analysis of F.A.S.T. system exhausts showing concentration of methyl bromide as a function of time.

Scrubbing Solution Quenching:
After repeated use the scrubbing solution depletes and needs to be replaced. This is the saturation point of the solution which is directly dependent on the molar ratio of fumigant to scrubbing material. Our 946 L system (250 gal) system will destroy approximately 363 – 454 kg (800 - 1000 lb) of methyl bromide or sulfuryl fluoride; however this is subject to change depending on the size of the system which can be customized to be more or less. Upon reaching the saturation point, an over the counter – food grade neutralizing product is used as an additive to quench the scrubbing solution before disposal. This quenching process lowers the pH of the solution making the scrubbing solution neutral. The spent solution then has a low neutral pH and has a flash point of greater than 82.2°C (180°F) making it extremely non-flammable and non hazardous. Saturated material can be disposed of at a local waste management facility for a low, economical cost of less than $40 per 208 L (55 gal) drum.
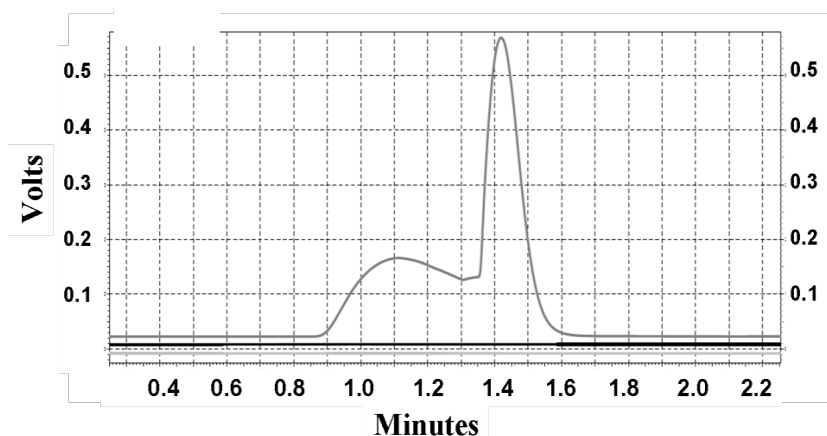
Fig. 4- Gas chromatograph analysis and comparison of scrubber exhaust, ambient air and concentrated methyl bromide. The darker grey trace represents the peaks created from concentrated methyl bromide with overlays of the scrubber exhaust (solid black line) and ambient air surrounding the system (solid light grey line).

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CONTROLLED ATMOSPHERE AND TEMPERATURE TREATMENT SYSTEM TO DISINFEST PEACH FRUIT MOTH, *CARPOSINA SASAKII* MATSUMURA (LEPIDOPTERA: CARPOSINIDAE) ON APPLES

Yerim Son[1], Ikjo Chon[2], Lisa Neven[3] , Youngmi Moon[4], Donghun Cho[5], and Yonggyun Kım[1]*

[1]Department of Bioresource Sciences, Andong National University, Andong 760-749, Korea
[2]Department of Horticulture and Breeding, Andong National University, Andong 760-749, Korea
[3]Yakima Agricultural Research Laboratory, USDA-ARS, Wapato, WA 98951, USA
[4]Plant Quarantine Technology Center, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang 480-757, Korea
[5]Plant Pest Control Division, Animal, Plant and Fisheries Quarantine and Inspection Agency, Anyang 480-757, Korea
*Corresponding author's e-mail: *hosanna@andong.ac.kr*

ABSTRACT

Carposina sasakii Matsumura (Lepidoptera: Carposinidae) is a serious insect pest of apples and peaches in Korea and Japan. Its internal feeding behavior makes difficult to control this insect pest with conventional spray pesticide applications. Due to its limited distribution, *C. sasakii* has been identified as a quarantine pest in several countries. Although fumigation with methyl bromide is used to meet phytosanitary requirements, it can cause significant damage to the ozone layer. In order to replace methyl bromide fumigation as a postharvest treatment, a Controlled Atmosphere/Temperature Treatment System (CATTS) was tested as an alternative treatment against *C. sasakii* in apples. The last instar was the most tolerant immaturestage to a heat treatment of 44°C for 20 min. CATTS conditions consisted of a linear heating rate of 16°C/h to a final chamber temperature of 46°Cand an apple internal temperature of 44°C under a 15% carbon dioxide and 1% oxygen environment. When the apples infested with different stages of *C. sasakii* were treated under CATTS conditions, young larvae (first – fourth instars) did not survive 40 min exposure, but the fifth instars required an exposure of at least 60 min to achieve complete mortality. A partial heat shock protein 90 (hsp90) was cloned and showed inducible expression in response to heat shock at 44°C. CATTS suppressed transcription in hsp90 gene expression. Apples did not show any appreciable loss of quality in relation to fruit firmness, sweetness, and decay after a 60 min CATTS treatment. These results suggest that CATTS can be applicable to control *C. sasakii* in apples.

POTENTIAL FOR PHOSPHINE AND SULFURYL FLUORIDE AS REPLACEMENTS FOR METHYL BROMIDE TO CONTROL PESTS OF DRIED MEAT

Thomas W. Phillips[1], Md. Mahbub Hasan[1],[2] and Michael J. Aikins[1]

[1]Department of Entomology, Kansas State University, Waters Hall, Manhattan, KS 66505, USA
[2]Dept. of Zoology, Rajshahi University, Rajshahi, Bangladesh
*Corresponding author's e-mail: *twp1@ksu.edu*

ABSTRACT

Fumigation with methyl bromide has been a long established and effective method for controlling the key major pests infesting dry-cured ham. Methyl bromide is the only fumigant used for dry-cured ham facilities in the USA. However, MB has been identified as an ozone-depleting chemical and its use is being restricted in accordance with an international agreement, and a more rapid alternative is desirable. This study compares the efficacy of methyl bromide versus phosphine and sulfuryl fluoride against two major key arthropod pests of dry-cured ham under laboratory conditions. Laboratory bioassay data showed that phosphine was more effective for controlling both *Necrobia rufipes* and *Tyrophagus putrescentiae* than methyl bromide. Eggs of both species were found to be highly tolerant to phosphine and methyl bromide for 48h exposure at 23°C while mobile stages were susceptible. *T. putrescentiae* was found differ from *N. rufipes* in the response either to phosphine and methyl bromide. A complete control was achieved for the both species with a dose level 0.85 and 4.0 g/m^3 of phosphine and methyl bromide respectively. Sulfuryl fluoride (SF) has been registered in many countries for stored product applications as an alternative to methyl bromide (MB). All life stages of ham beetles were easily killed by SF within low label rates, but the ham mite showed high tolerance to SF and survived concentration-time products in excess of three times the standard label limit 1500 $g.h/m^3$. Data so far suggest that phosphine will be a suitable fumigant replacement for MB in the control of dried ham pests.

EFFECTIVENESS OF HEAT TREATMENTS AGAINST *TRIBOLIUM CASTANEUM* LIFE STAGES IN TWO COMMERCIAL FOOD-PROCESSING FACILITIES

Bhadriraju Subramanyam[1], Rajshekhar Hulasare[2], Monika Brijwani[1], and Mark Leitman[3]

[1]Department of Grain Science & Industry, Kansas State University, Manhattan, Kansas, USA
[2]Temp-Air, Inc., Burnsville, Minnesota, USA
[3]Propane Education and Research Council, Washington, D.C., USA
*Corresponding author's email address: *sbhadrir@k-state.edu*

ABSTRACT

Two facilities, A and B, were subjected to heat treatments using forced-air gas heaters that were fueled by propane. At facility A, two separate rooms were heated for ~28 h. Temperature sensors and insect bioassay vials with 20 young larvae (first instars) or 20 adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) were placed in 20 or 28 locations. Temperatures reached 50°C from the ambient temperature of 23 to 27°C in 5 to 6 h. Temperatures above 50°C were held for 22 h, and the maximum temperatures did not exceed 58°C. Mortality of young larvae and adults was 90 to 96% about 12 h into the heat treatment and reached 100% at 20 h. Little or no progeny was produced 42 d later in vials exposed to the heat treatment for 12 to 28 h. In facility B heated for 24 h, time to reach 50°C from the ambient temperature of 34°C took 1.5 h, and temperatures above 50°C were held for 23 h, and the maximum temperature observed was about 60°C. Mortality of *T. castaneum* eggs was 100% in vials exposed to heat for 3 h whereas that of adults was 57% with 100% mortality occurring at 24 h. A thermal death kinetic model predicted time to 99% mortality (LT_{99}) of *T. castaneum* young larvae, which is the most heat tolerant stage, as a function of time-dependent temperature data at each location. The LT_{99} values were positively related to time to 50°C, but inversely related to time above 50°C and the maximum temperature. Observed temperatures, insect responses in bioassays, and thermal death kinetic model predictions confirmed that successful commercial heat treatments can be conducted in 24 to 28 h. No adverse effects to the electrical or structural components of the facilities occurred during the two heat treatments.

Keywords: Food processing facilities, heat treatment, demonstration trials, *Tribolium castaneum*, life stages, efficacy assessment.

INTRODUCTION

Heat treatment involves raising the ambient air temperature of the whole or a portion of food-processing facility between 50 and 60°C and holding these lethal temperatures for 24 to 36 h to manage stored product insects (Dosland et al., 2006). Brijwani et al. (2012) reported that a successful heat treatment of a pilot food processing facility (flour mill) at Kansas State University, based on lethal temperatures attained and mortality of eggs, young larvae, pupae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), can be

conducted in 24 h. In this paper, the effectiveness of heat treatment was demonstrated in two commercial facilities based on lethal temperatures attained and evaluating mortality of exposed *T. castaneum* eggs, young, larvae, and adults.

MATERIALS AND METHODS

Heat treatment effectiveness was demonstrated at two commercial facilities, A and B. Facility A manufactures roasted sunflower seeds. Two separate rooms of this facility were subjected to heat treatment lasting 27.7 h during September 25 to 26, 2009. One of the rooms is used for roasting the seeds (DRR; 52.7 m x 70.4 m x 9.2 m) while the other is used for storing sunflower seeds (BBU; 45.4 m x 37.8 m x 5.2 m). Facility B manufactures rice cakes, and in this facility a processing room (30.5 m x 12.2m x 12.2 m) was heat treated for 24 h during September 25 to 26, 2010. Heat treatments at both locations were performed by Temp-Air (Burnsville, Minnesota, USA) using forced air gas heaters, and propane was used as the fuel.

The DRR and BBU were subjected to heat treatment. A total of three heaters were used. Two heaters with a maximum heat energy output of 410.3 kW/h (1.4 million BTU/h) and 161.2 kW/h (0.55 million BTU/h) were used for heating the DRR, and one heater with a capacity of 1318.8 kW/h (4.5 million BTU/h) was used for heating the BBU. The hot air was transferred from the gas heaters into the processing rooms with fabric ductworks. A 91.4 cm diameter ductwork was placed in BBU room, and ducts of 61.0 and 50.8 cm diameter were placed in DRR. Uniform distribution of the heat was ensured with the help of 10 fans, each with a 91.4 cm fan diameter, that were placed in each room to circulate hot air.

The temperature sensor (HOBO® data loggers, Onset Computer Corporation, Bourne, MA) loggers were launched to record temperature every 2 min. A total of 28 loggers were placed at the floor level in DRR and 20 in BBU room. The effectiveness of the heat treatment was evaluated with insect bioassays placed adjacent to the temperature sensors in order to correlate temperature and insect mortality rates. Insect bioassays were prepared and mortality assessment was done in the Stored-Products Insects Research and Education Laboratory, Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, following procedures described by Mahroof et al. (2003). Young larvae and adults of *T. castaneum* were reared on wheat flour plus 5% (by wt) brewer's yeast diet at 28°C and 65% r.h. Plastic vials (2.6 cm inner diameter and 4.9 cm height) were filled with 5 g of bleached wheat flour sifted using a 250-µm opening sieve. Into each vial 20 young larvae or adults were introduced. These vials were closed with plastic lids covered with meshes to allow air flow but prevent insect escape. Four vials infested with young larvae and four vials infested with adults were placed in each of 28 locations throughout DRR. Similarly, eight vials per location were placed in each of 20 different locations in the BBU room. Two vials each infested with adults or young larvae were placed outside the DRR and BBU rooms that were unheated to determine natural insect mortality of these stages. Another set of vials (one for each life stage) remained in the laboratory growth chamber set at 28°C and 65% r.h.

During the heat treatment one vial with larvae and one with adults was sampled at 4.8, 12.2, 20.2, and 27.7 h into the heat treatment from 28 locations in DRR and from 20 locations in BBU room. After the heat treatment, all vials were brought back to the laboratory on September 27, and mortality of adults determined 24 h after incubation at 28°C and 65% r.h. Adult mortality was based on number of dead adults out of the total exposed (20). Young larvae in vials were placed in a growth chamber at 28°C and 65% r.h. for 45 d. Mortality was determined based on number of adults that failed to emerge from each vial out of the total larvae exposed. Larval

mortality was corrected for mortality of larvae in vials not exposed to the heat treatment (control vials) (Abbott, 1925).

In facility B, one heater with a maximum heat energy output of 1318.8 kW/h (4.5 million BTU/h) was used. The hot air was transferred from the gas heater into the room with 91.4 cm diameter fabric ductwork. Uniform distribution of the heat was ensured with the help of 12 fans.

In each plastic vial 5 g of bleached wheat flour sifted using a 250-µm opening sieve was added. In each vial, 20 eggs or 20 adults of *T. castaneum* were introduced. These vials were closed with plastic lids covered with mesh. Four vials infested with eggs and four vials infested with adults were placed in 24 locations throughout the room. Two vials each infested with eggs or adults were placed outside the heated room to determine natural insect mortality, and another set of vials (four for each life stage) remained in the laboratory growth chamber set at 28°C and 65% r.h. Temperature profiles in each of the 24 locations were measured using SmartButton sensors (ACR Systems, Inc., Surrey, Canada) every 2 min. These sensors were placed in 5 g of flour in an additional vial without insects. This additional vial was placed at all 24 locations along with the set of vials holding eggs and adults. During the heat treatment one set of vials (eggs and adults) were sampled at 1.5, 3, 5 and 24 h into the heat treatment from all 24 locations in the tempering room. After heat treatment, all vials were brought back to the laboratory on September 27, 2010 and mortality of adults determined 24 h after incubation at 28°C and 65% r.h. Adult mortality was based on number of dead adults out of the total exposed (20). Eggs in vials were placed in a growth chamber at 28°C and 65% r.h. for 45 d. Egg-to-adult mortality was determined based on number of adults that failed to emerge from each vial out of the total eggs exposed. Eggs mortality was corrected for mortality of eggs in vials not exposed to the heat treatment (control vials).

The mean temperature profiles across all 28 and 20 data loggers within DRR and BBU rooms, respectively, and in the heated room of facility B were plotted as a function of time using SigmaPlot 11. The mean starting temperature (°C), time to 50°C (h), time above 50°C (h), and the maximum temperature (°C) attained within each heated room was determined from the mean time-dependent temperature data. At each of the four sampling periods, the mean ± SE of temperature and mortality of adults and young larvae in DRR and BBU rooms and mortality of eggs and adults in facility B were determined.

A novel thermal death kinetic model was developed and validated to predict survival of old larvae of the confused flour beetle, *Tribolium confusum* (Jacquelin du Val), based on time-dependent temperature measured during facility heat treatments (Boina et al., 2008). The same modelling approach was used to validate survival of young larvae of *T. castaneum* (Subramanyam Bh, Mahroof R, unpublished data; Subramanyam et al., 2011) during facility heat treatment. Young larvae of *T. castaneum* are the most heat resistant life stage (Mahroof et al., 2003) among important stored-product insects and stages we tested (Boina and Subramanyam, 2004; Mahroof and Subramanyam, 2006; Yu et al., 2011; Subramanyam et al., 2011). Therefore, this stage was used in model predictions, because controlling this stage would control other stages of *T. castaneum*. Temperature data at each location in the DRR and BBU room, and in the heated room in facility B, were used to predict time in hours for 99% mortality (LT$_{99}$) of *T. castaneum* young larvae. The temperature data from each data logger were also used to determine the time to 50°C (h), time above 50°C (h), and maximum temperature (°C) obtained at each of the locations sampled in each heated room. The relationship between LT$_{99}$ and time to 50°C (h), time above 50°C (h), or maximum temperature (°C) was described using regression models (SAS Institute, 2002).

RESULTS AND DISCUSSION

The mean temperature in the BBU room was higher than the DRR room up to 6.7 h, after which the mean temperature was higher in the DRR than the BBU room (Fig. 1). Mean temperature reached 50°C in 6.3 h in the DRR and in 5.1 h in the BBU room. During the heat treatment across all 48 locations in both rooms, 37.5% of the locations were above 50°C in 4.8 h, 87.5% of locations were above 50°C in 12.2 h and 93.7% locations were above 50°C in 20.2 h. There was a sudden drop in the percent locations above 50°C (60.4%) at 27.7 h (Table 1) because the amount of heat input was reduced a few hours before the end of heat treatment.
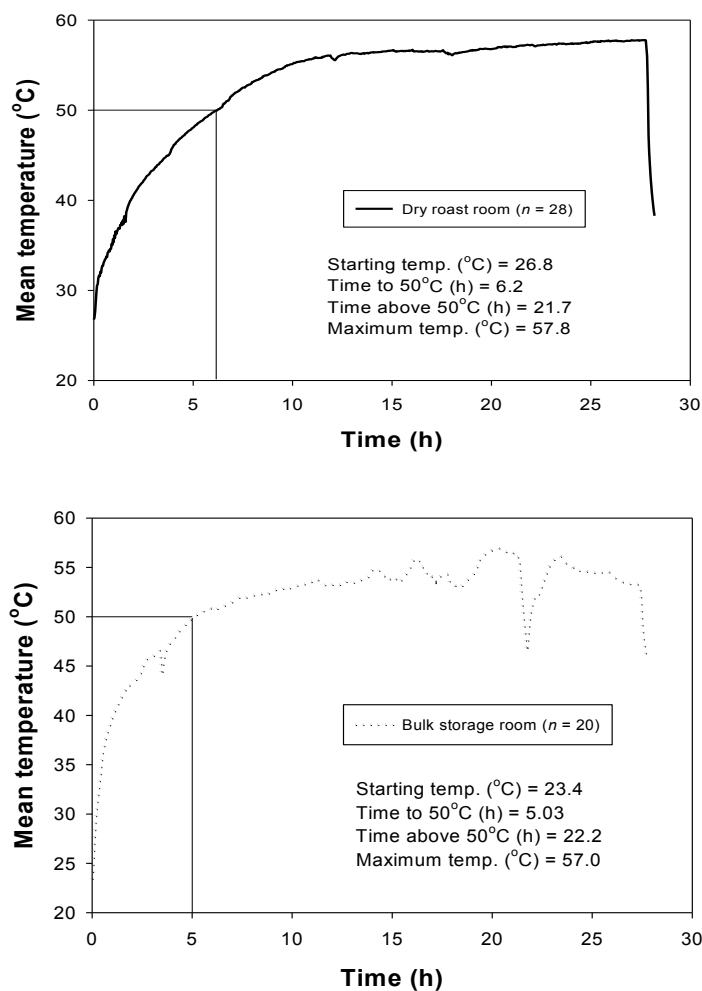
Fig. 1- Mean temperatures observed in the dry roast room (DRR) and bulk storage room (BBU) in facility A. The solid black line shows time taken to reach 50°C.

Table 1. Number of locations in dry roast room (DRR) and bulk storage room (BBU) with temperatures below and above 50°C at each of the four vial sampling periods in facility A

Time (h)	Dry roast room (DRR)		Bulk storage room (BBU)	
	No. locations	Mean ± SE Temp (°C)	No. locations	Mean ± SE Temp (°C)
4.8	22	45.9 ± 0.6	8	42.0 ± 2.2
	6	54.4 ± 1.6	12	54.5 ± 1.7
12.2	2	48.2 ± 0.8	5	44.6 ± 3.2
	26	56.2 ± 0.6	15	56.1 ± 1.4
20.2	1	43.4 ± 0.0	2	48.3 ± 0.5
	27	57.3 ± 0.6	18	58.0 ± 1.1
27.7	1	45.4 ± 0.0	18	45.3 ± 0.5
	27	58.3 ± 0.6	2	51.0 ± 0.1

The mean mortality of adults and young larvae in DRR and BBU rooms is shown in Tables 2 and 3, respectively. Mortality of adults and young larvae in vials in the DRR sampled at 27.7 h, close to the end of the heat treatment, was 100% and the mean temperature at this time was 57.8°C. In the BBU room, at 27.7 h, 19 out of 20 locations with mean temperature of 46°C achieved 100% mortality while 1 out of 20 locations with mean temperature 44°C achieved 75% mortality for adults and 92% mortality for young larvae. In general, commercial kill of adults and young larvae of red flour beetles were observed during this heat treatment at 20.2 and 27.7 h. In both DRR (Fig. 2) and BBU rooms (Fig. 3), the time required to 50°C was positively related to LT_{99}, whereas the time above 50°C and the maximum temperature were inversely related to LT_{99}.

The mean time to reach 50°C was 1.5 h after the start of the heat treatment in the room being heated in facility B (Fig. 4). During the heat treatment, across all 24 locations, 50.0% of the locations were above 50°C in 1.5 h while 91.7% of locations were above 50°C at 3 and 6 h. There was a sudden drop in the percent locations above 50°C (79.2%) at 24 h (Table 4), because the heat was turned down a few hours before terminating the heat treatment. All eggs in vials were killed 3 h into the heat treatment. Adult mortality increased with time and was 100% at 24 h (Table 5).

Table 2. Mean temperature and mean mortality of young larvae and adults of *T. castaneum* in dry roast room (DRR) in facility A

Time (h)	Mean ± SE		
	Temperature (°C)	Mortality (%) of:	
		Young larvae[a]	Adults[b]
4.8	47.7 ± 0.9	35.6 ± 10.7	15.9 ± 8.2
12.2	55.6 ± 0.7	96.2 ± 4.3	90.4 ± 6.6
20.2	56.8 ± 0.8	99.5 ± 1.6	100.0 ± 0.0
27.7	57.8 ± 0.7	100.0 ± 0.0	100.0 ± 0.0

Each mean is based on *n* = 28 samples or vials.
[a]Mean control mortality at 4.8. 12.2, 20.2, and 27.7 h was 23.4, 35.0, 33.4, and 36.4%, respectively. Mortality of young larvae was corrected for control mortality.
[b]Mean control mortality of adults was 0%.
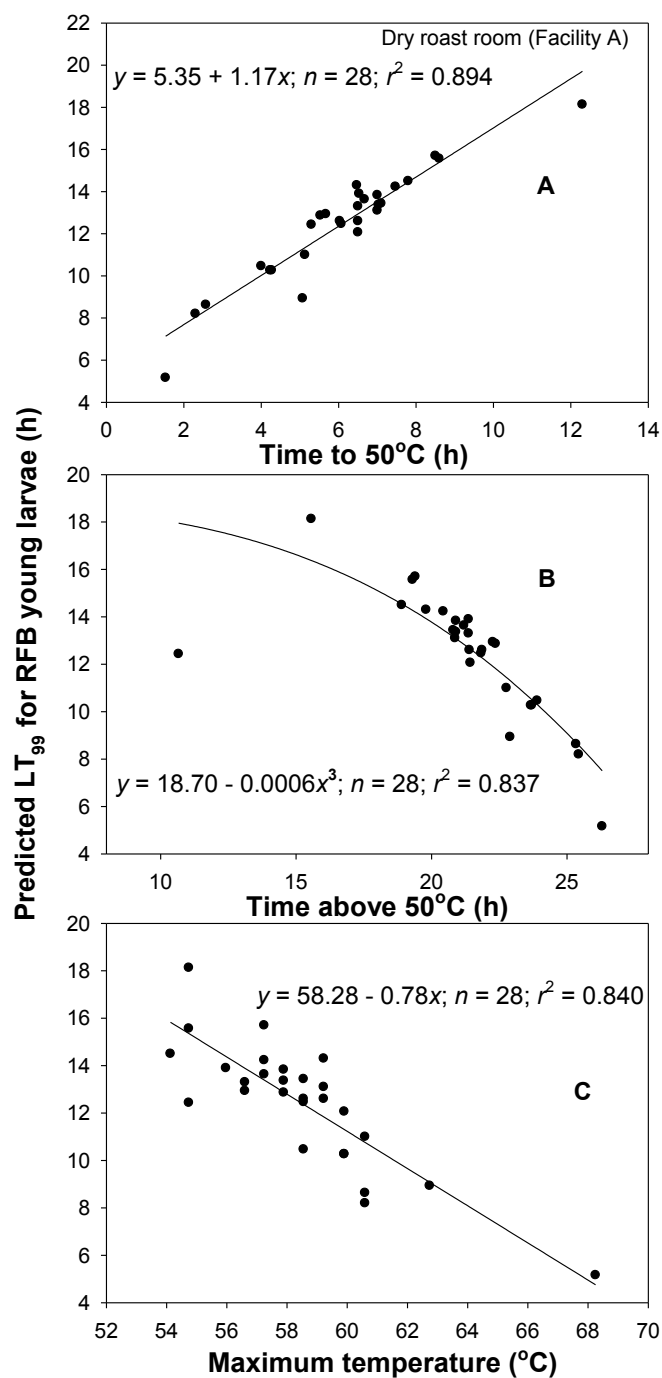
Fig. 2- Relationship between time to 50°C, time above 50°C, and the maximum temperature and time for predicted 99% mortality (LT$_{99}$) of *T. castaneum* young larvae in the dry roast room (DRR) of facility A.
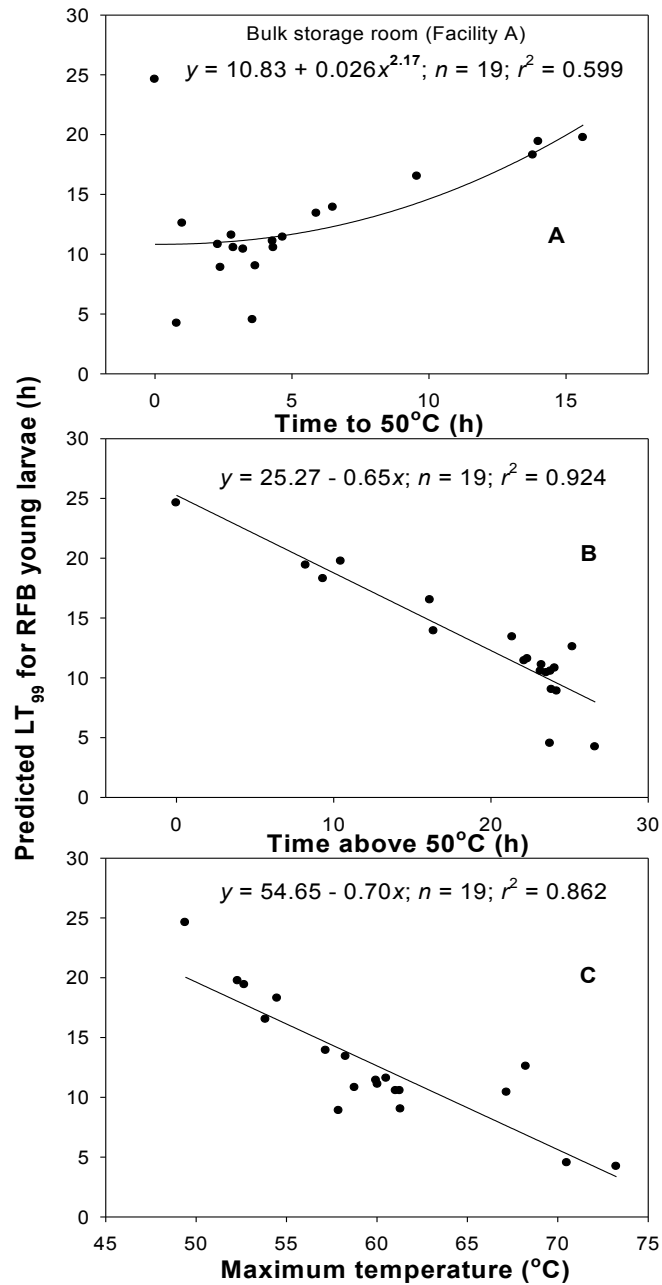
Fig. 3- Relationship between time to 50°C, time above 50°C, and the maximum temperature and time for predicted 99% mortality (LT$_{99}$) of *T. castaneum* young larvae in the bulk storage room (BBU) of facility A.

Table 3. Mean temperature and mean mortality of young larvae and adults of *T. castaneum* in bulk storage room (BBU) in facility A

Time (h)	Mean ± SE		
	Temperature (°C)	Mortality (%) of:	
		Young larvae[a]	Adults
4.8	49.5 ± 1.9	68.9 ± 10.3	58.8 ± 11.0
12.2	53.2 ± 1.7	84.6 ± 8.1	85.0 ± 8.0
20.2	57.0 ± 1.2	96.3 ± 4.2	95.3 ± 4.8
27.7[b]	45.8 ± 0.6	99.6 ± 1.4	98.8 ± 2.5

Each mean is based on *n* = 20 samples or vials.
[a]Mortality of young larvae was corrected for control mortality (see footnote to Table 2).
[b]In one location, where the temperature was 44°C, the mortality of adults in a vial was 75% whereas that of young larvae was 92%.
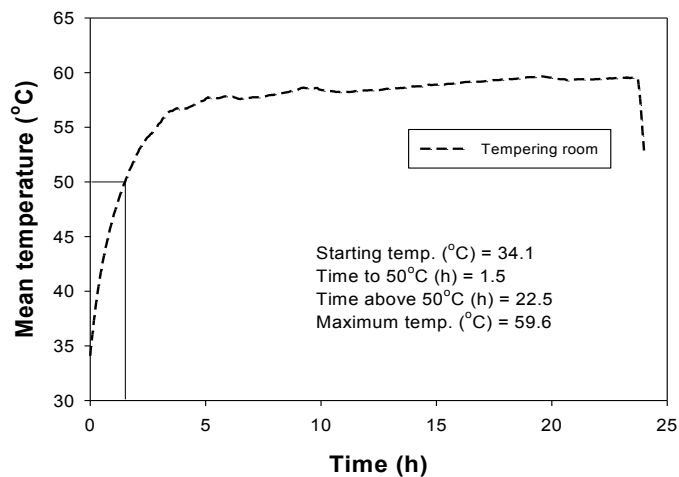
Fig. 4- Mean temperature observed in the heated room of facility B. The solid black line shows time taken to reach 50°C.

Table 4. Number of locations in the heated room with temperatures below and above 50°C at each of the four vial sampling periods in facility B

Time (h)	No. locations	Mean ± SE Temp (°C)
1.5	12	46.5 ± 0.7
	12	53.5 ± 0.7
3.0	2	47.8 ± 0.8
	22	56.0 ± 0.8
6.0	2	47.8 ± 0.8
	22	56.0 ± 0.9
27.7	5	47.7 ± 0.7
	19	54.2 ± 0.6

Our results show that an effective heat treatment to control eggs, young larvae, and adults of *T. castaneum* can be performed in 24 to 28 h. Brijwani et al. (2012) also showed that a successful heat treatment to control eggs, young larvae, old larvae, pupae, and adults of *T. castaneum* can be accomplished in 24 h. Despite careful management of temperature, in two locations in the DRR and four locations in BBU room, the maximum temperature exceeded 60°C. Except for these six locations, temperatures in all other locations in facilities A and B were maintained between 50 and 60°C. Both the facilities were heated at different heating rates and a faster heating rate (facility B) did not cause any adverse effects on the electrical or structural components. The thermal death kinetic model predictions showed that the mortality of *T. castaneum* young larvae is related to how quickly temperatures reach 50°C, and how long temperatures are held above 50°C, and the maximum temperature. In conclusion, heat treatment is an effective and environmentally benign pest management tactic to kill life stages of *T. castaneum* within food-processing facilities.

Table 5. Mean temperature and mean mortality of eggs and adults of *T. castaneum* in the heated room of facility B.

Time (h)	Mean ± SE		
	Temperature (°C)	Mortality (%) of:	
		Eggs[a]	Adults[b]
1.5	50.0 ± 0.9	74.5 ± 10.0	33.3 ± 9.7
3.0	55.4 ± 0.9	100.0 ± 0.0	56.7 ± 9.9
6.0	55.4 ± 0.9	100.0 ± 0.0	78.3 ± 8.0
24.0	52.9 ± 0.7	100.0 ± 0.0	100.0 ± 0.0

Each mean is based on $n = 24$ samples or vials.
[a]Mean control mortality at 1.5, 3.0, 6.0, and 24.0 h was 32.5, 34.2, 30.0, and 33.3%, respectively. Mortality of eggs was corrected for control mortality.
[b]Mean control mortality of adults was 0%.

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EFFICACY OF SULFURYL FLUORIDE AGAINST EGGS AND ADULTS OF *TRIBOLIUM CASTANEUM* IN COMMERCIAL FLOUR MILLS

John Lawrence[1], Bhadriraju Subramanyam[*,1], Dirk E. Maier[1], and Watcharapol Chayaprasert[2]

[1]Department of Grain Science & Industry, Kansas State University, Manhattan, Kansas 66506, USA
[2]Department of Agricultural Engineering, Kasetsart University, Nakhon Pathom 73140, Thailand
*Corresponding author's e-mail: *sbhadrir@k-state.edu*

ABSTRACT

The effectiveness of sulfuryl fluoride against the eggs and adults of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), was studied during five fumigation trials in four commercial flour mills. Mill volumes ranged from 8,495 to 28,317 m^3. Outdoor weather parameters such as wind speed and direction, ambient temperature, humidity, and barometric pressure were monitored using a weather station installed on the roofs of mills. The ambient air temperatures within mills were also monitored. Adults and eggs of *T. castaneum* in plastic vials with 5 g of flour were placed in 15 locations on each mill floor to assess insect mortality. On each mill floor, fumigant concentrations were recorded every hour during the 24 h exposure period. Temperatures inside mills during fumigation ranged from 21.4 to 36.9°C. The achieved concentrations over time (Ct products) varied among the mills and ranged from 630.3 to 1,357.6 g-h/m^3. Ct product variation among mill floors across the ranged from 28.4 to 692.4 g-h/m^3. In all the fumigation trials, there was 100% adult mortality irrespective of varying mill temperatures. Temperatures in the mill during fumigation played an important role only in *T. castaneum* egg mortality. When mill temperatures were below 25°C, egg mortality was about 80% and at temperature below 23°C, egg mortality was about 10%.The eggs that survived fumigation successfully completed development to adulthood.

Key words: flour mills, fumigation, methyl bromide alternative, surfuryl fluoride, red flour beetle, *Tribolium castaneum*, egg mortality, adult mortality, efficacy assessment

SESSION 3

POSTERS

TOXICITY OF *ROSMARINUS OFFICINALIS* ESSENTIAL OIL AGAINST IRRADIATED *TRIBOLIUM CASTANEUM*

Mehrdad Ahmadi[1*], Saeid Moharramipour[2]

[1]Agricultural, Medical and Industrial research school, Nuclear Science and Technology Research Institute, P.O.Box 31485-498, Karaj, Iran
[2]Department of Entomology, College of Agriculture, Tarbiat Modares University, Iran
*Corresponding author's e-mail: *mahmadi@nrcam.org*

ABSTRACT

Management of stored-product insect using gamma radiation could be enhanced by other feasible control methods such as essential oils as potent alternatives to chemical insecticides. In this study, the efficiency of sub-lethal doses of essential oils from *Rosmarinus officinalis* L. combined with gamma radiation was verified to assess their enhanced toxicity against the adults of *Tribolium castaneum* (Herbst). Irradiated adult insects by 720, 890 and 1200 Gy doses (LD_1, LD_5 and LD_{25}) were subjected to three levels of essential oil (LC_1, LC_5 and LC_{25}). It was shown that using of essential oil from *R. officinalis* could be statistically increased 2-4 times the intensity of gamma radiation and high mortality levels has been observed in this combination. Also the results showed a synergistic effect between rosemary essential oil and gamma radiation combinations. The results indicated that insecticidal activity of essential oils would be altered to varying degree if the insects already were exposed to radiation. The potential use of integrating essential oils and gamma radiation treatment are discussed for management of the stored-product pests.

Keywords: Sub-lethal dose, essential oil*, gamma radiation*, *Rosmarinus officinalis*, synergistic effect, *Tribolium castaneum*

INTRODUCTION

In order to control of *Tribolium castaneum* (Herbst), fumigants such as methyl bromide and phosphine were widely used. Wide use of these fumigants has raised residue levels and has lead to the development of resistance in certain species (Rossi et al., 2010). Due to environmentally unfavorable effects of the fumigants, their use has recently been banned in many countries (Carpenter et al., 2000). Irradiation and essential oils have become approved and feasible alternatives to conventional method for the direct control of stored product pests; because of residue free advantages over chemical fumigation. *Tribolium castaneum* was observed to be sensitive to irradiation (Misra and Paravathy, 1998; Tuncbilek et al., 2003). In addition, there are several reports on insecticidal effects of rosemary essential oil on stored-product pests. Clemente et al. (2003) observed that extracts of *Rosmarinus officinalis* L. have an insecticidal effect on *T. castaneum*. Ahmadi et al. (2008b) also reported that *R. officinalis* essential oil could be utilized to control *T. castaneum* due to its fumigant toxicity.

On the other hand the use of gamma radiation and essential oils alone at high doses is very expensive and time-consuming; therefore to reduce costs, it is important to look for a strategy to use low doses without reducing the efficacy. One of the alternative possibilities is using a combination of the two methods looking for synergistic effect. Combination of gamma radiation with other treatments like, microwave, infra-red radiation and insecticides has also been previously reported (Cogburn and Spiers, 1972; Tilton et al., 1972; Ramesh et al., 2002; Mehta et al., 2004). Ahmadi et al. (2008a) discovered that combination of gamma radiation with essential oils of *Perovskia atriplicifolia* (Benth) may result in synergistic interactions that would enhance the potential for control of *T. castaneum*.

In this study the toxicity of doses of gamma radiation combined with *R. officinalis* essential oil against *T. castaneum* adults was tested.

MATERIALS AND METHODS

Extraction of essential oils
Arial parts of *R. officinalis* were collected at full flowering stage from Tehran in April 2011. Essential oils were extracted from dried plant samples using a Clevenger-type apparatus. Conditions of extraction consisted of 40 g of air-dried sample (1:10 plant material: water volume ratio) and 4 h distillation.

Irradiation
Irradiation of the tested insects was administered by using ^{60} cobalt gamma sources at the Nuclear Science and Technology Research Institute at a dose rate of 0.4 Gy sec^{-1}.

Fumigant toxicity
In this experiment, filter paper was impregnated with oil and then the filter paper was attached under the surface of the screw cap of a glass vial volume 280 ml (Negahban et al., 2007). The cap was screwed tightly on the vial containing 50 adults (1-3 d old). Five replicates were setup for treated and controlled adults. A series of dilutions was prepared to evaluate mortality of the insects. Insects were exposed to the oil for 24 h and then the number of dead and live insects in each bottle was counted 48 h after fumigation. When no leg or antennal movements were observed, insects were considered dead. Probit analysis (Finney, 1971) was used to estimate LC_1, LC_5 and LC_{25} values.

Interaction of gamma radiation and essential oil
In the first part of the experiment, where irradiation was used 24 and 48 h after fumigation by essential oil, adults were exposed to *R. officinalis* (close to LC_{25}) then treated with different doses of gamma radiation within 24 and 48 h after fumigation. In the second part of the experiment, insects were treated with gamma radiation (close to LD_{25}) and then survivors after 24 and 48 h were subsequently exposed to essential oil. In the third part in which insects were subjected to irradiation, and immediately exposed to the *R. officinalis* oil. Each experiment was conducted with five replications consisting of 50 insects for each replication. Percentage insect mortality was calculated 72 h after the initial treatment, using the Abbott (1925) correction formula for natural mortality in the untreated control.

Calculation of synergistic effects

Synergistic effect in combination of gamma radiation and essential oil was calculated by the following formula (Berenbaum, 1989): $S = \dfrac{d_a}{D_a} + \dfrac{d_b}{D_b}$

S= synergistic effect (S>1: antagonism; S=1: additivity; S<1: synergism)

Where d_a and d_b are the concentration of each treatments (gamma radiation, essential oil) used in combination mode. D_a and D_b are their single concentrations which yielding the same effect level, when administered alone as the mixture.

RESULTS

Effects of different levels of gamma radiation on *T. castaneum* showed that 1-3 d old adults were susceptible to irradiation. Doses of 720, 890 and 1200 Gy caused 1.66%, 6.2% and 24.8% mortality 3 d post-irradiation, respectively. It was shown that mortality was increased with the increasing doses.

Results of fumigation by *R. officinalis* oil showed that LC_1, LC_5 and LC_{25} values were 4.2, 4.84 and 5.93 µl l^{-1} air respectively. In this experiment, mortality was noticeably increased as doses of essential oil increased. Comparative analyses for these 3 experiments testing the combination of gamma radiation with *R. officinalis* oil are shown in Fig 1. The results showed a significant synergistic effect between oil concentration and gamma treatment (Tukey, *P*<0.05) especially when fumigation was used 48 h after irradiation. Doses of 4.2, 4.84 and 5.93 µl l^{-1} air of *R. officinalis* alone resulted in average adult mortality of 1.33, 5.25 and 27.6% respectively. whereas average mortality rate increased to 18.3, 38.15 and 43.15% for treatment of 720 Gy of irradiation 24 h after fumigation and to 27.33, 34.13 and 45.2% for treatment of 720 Gy irradiation 48 h after fumigation. When the insects were exposed to 720 Gy of gamma radiation before fumigation of adults at the dose of 4.2, 4.84 and 5.93 µl l^{-1} air of *R. officinalis*, the mortality rate reached to 42.5, 51.33 and 62.5% (irradiation applied 24 h before fumigation) and 65.33, 75.5 and 75.3% respectively (irradiation applied 48 h before fumigation). During the third part of the experiment when irradiation by 720 Gy and essential oil (LC_1, LC_5 and LC_{25}) were exposed to the adults at the same time, mortality percentages increased to 27.33, 34.13 and 45.2% respectively. The results obtained from the application of gamma radiation in 890 and 1200 Gy doses with *R. officinalis* essential oil are shown in Fig 1.

Although it was shown that the combination of gamma radiation and rosemary essential oils could be provided significant increase in mortality rate of *T. castaneum*, but according to the synergism formula, synergistic effect was observed only when fumigation by 4.2 and 4.84 µl l^{-1} air applied 48 h after irradiation by 720 Gy dose and also irradiated adults by 890 Gy dose exposed to 4.2 µl l^{-1} air of *R. officinalis* after 48 h.

DISCUSSION

Currently, the combination of several independent techniques for the control of pest as integrated pest management (IPM) is one of the main strategies. Irradiation and fumigation by essential oil are two main methods that could be used as combined treatment in IPM. There are several reports on interaction of irradiation with other methods like fumigants (Mehta et al., 2004); thiodicarb (Ramesh et al., 2002); azadirachtin (Sharma and Seth, 2005) and *Perovskia atriplicifolia* Benth essential oil (Ahmadi et al., 2008a) to achieve adequate control of pests. Data on the toxicity of *R. officinalis* oil to adults after or before exposure to gamma

radiation indicated that a delay of 1-2 d between irradiation and fumigation could affect their susceptibility to the essential oil. Synergistic effect between gamma radiation and *R. officinalis* oil was observed when irradiated adults exposed to fumigation after 48 h.

Results obtained in our study are in agreement with the findings of El-Sayeed et al. (1988). They observed that *Callosobruchus maculatus* (Fabricius) adults are more susceptible to fenvalerate after irradiation. Similarly, Moustafa and Abdel Salam (1991) reported the synergistic effects of gamma radiation and chlorpyrifos on larvae of *Spodoptera littoralis* (Fabricius). Our findings further suggest that the fumigation of *T. castaneum* adults after irradiation is much more effective than the reverse method. The reason for this phenomenon is not clearly understood, but it seems that irradiation might have altered the cell resistance against non-favorable conditions and so had increased their susceptibility towards fumigation.
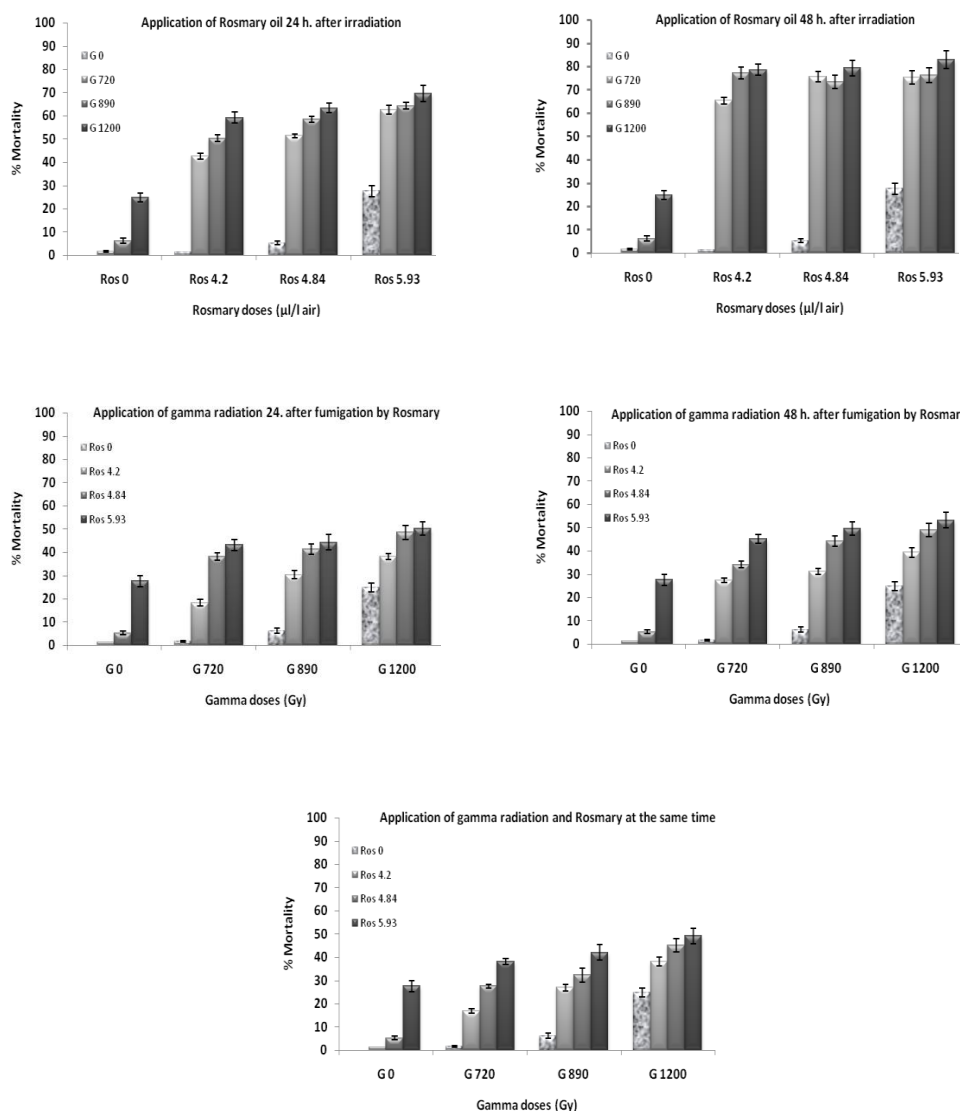
Fig. 1- Combined effect of gamma radiation and *Rosmarinus officinalis* essential oil against mortality of *Tribolium castaneum* adults.

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ECO₂FUME AS A QUARANTINE FUMIGANT FOR IMPORT OF NURSERY TREES

Young-Mi Moon [1], Min-Gu Park[1], Justin Tumambing[2]
Bong-Su Kim[3], Byung-Ho Lee[3*]

[1]Animal, Plant and Fisheries Quarantine and Inspection Agency(QIA), South Korea
[2]CYTEC Australia Holding Pty Ltd. Australia
[3]Dongbu ARI, Dongbu Hannong Co. Ltd, South Korea
Corresponding author's e-mail: *byungholee@dongbu.com*

ABSTRACT

ECO₂FUME, a cylinderized gas formulation of 2% phosphine with carbon dioxide, is a potential alternative to methyl bromide (MB). In Korea, MB has been used to treat the imported nursery tree for quarantine purpose. Currently limited use of MB fumigation is due to both low efficacy at low temperatures and phytotoxic damage to imported nursery trees: mainly dracaena and palm trees in Korea. Quarantine fumigation in nursery trees is important in terms of importer's requirements not to be damaged. Phosphine gas is outstanding alternatives of MB to reduce phytotoxic symptoms on post fumigations as well higher efficacy at low temperature due to better penetration properties than other alternatives. This study was conducted to obtain the efficacy data of ECO₂FUME to target pests and quality assessment after fumigation in import nursery trees: dracaena and palm trees.

Key words: Phosphine gas, ECO₂FUME, Quarantine, Methyl bromide chemical alternatives, Import, Dracaena tree, Palm tree, Citrus mealybug, Postharvest quality

INTRODUCTION

Thirteen species of mealybugs were intercepted in quarantine at Korean ports of entry on the imported nursery trees over the past 9 years (2000-2009). They were fumigated with 24~ 56 $g·m^{-3}$ of methyl bromide (MB) for 2 h depending on the temperature. MB can damage some nursery trees as well as it has been identified as an ozone-depleting chemical with restricted use according to the Montreal protocol. Currently, there are no restrictions on use of MB as a commodity treatment for pre-shipment and quarantine purpose, but in the future restrictions may be imposed, with the deadline for phase-out now advanced to 2015.

During the 1980s, a cylinder gas formulation of phosphine, Phosfume[®] (2% phosphine with carbon dioxide) was developed (Winks and Russell, 1994), eliminating problems of powder residues associated with solid formulations and enabling fumigation times to be reduced. Phosfume[®], recently renamed ECO₂FUME[®], has been used successfully in experiments to control several important pests of Australian wildflowers for export using phosphine concentrations of up to 1 $g·m^{-3}$ and exposure periods of up to 16 h (Muhunthan *et al*., 1997; Williams and Muhunthan, 1998).

This study was conducted to obtain the efficacy data of ECO₂FUME to target pests and assess the quality after fumigation in import nursery trees: dracaena and palm trees.

MATERIALS AND METHODS

Material

Egg and adult stages of *Planococcus citri* (citrus mealybug) were used in this test. The colony was maintained at $26\pm1℃$, 90% RH, and a photoperiod of 16:8(L:D) on potatoes for several years. Nursery trees, dracaena (*Dracaena fragrans*) and palm trees (*Chamaedorea elegans*), were purchased from nursery importer. ECO₂FUME (2% PH_3 + 98% CO_2) for fumigation studies was supplied by Cytec Industries via its local distributor, Dongbu Hannong Co., Ltd in Korea.

Measurement of Phosphine and Calculation of CT (concentration X time) Product

Concentrations of phosphine were measured at 0.5, 2, 6, 24 h after the first injection in fumigation chamber (12 L glass desiccators) and refrigerated container (28.6 m³). The gas samples were stored in gas-tight Tedlar® sampling bags before analysis. The *ct*-products (concentration x time) were calculated from the arithmetic average of phosphine concentration readings during the 24 h exposure period. Fumigant concentrations were determined with a gas chromatograph (Agilent 7890A, FPD, USA), fitted with a DB-WAX, FFD at 250℃, injection temperature of 200℃ (He as carrier gas) and oven temperature of 200℃.

Fumigation Methods for Dose Response (12 L desiccators)

The calculated amount of phosphine was injected in gas-tight 12 L glass desiccators which were known their volume for 24 h at $15\pm1℃$. The desiccators were sealed with glass stoppers containing a septum through which the mixture of gas was dosed. Several doses and gas samples were taken for analysis by gas chromatography. The dosage and the required volume for the fumigant concentration were calculated from Ren et al (2011). After 24 h of fumigation, the desiccators were opened in the fume hood for aeration. Mortality of adult stage of *P. citri* was assessed under a microscope after incubation for 1 and 3 days. The egg hatch rate of *P. citri* was examined after 7 days of incubation.

Commercial Scale Fumigation Method (Refrigerated container)

Nursery trees imported to China and Southeast Asia were stored and transported at 15℃ for 1 day and normally displayed at room temperature for sale purpose. This experiment was carried out at commercial scale temperature conditions. Fumigations using 1.5 g.m⁻³ of phosphine for 24 and 48 h were carried out with 100 dracaenas and palms trees in a 28 m³ refrigerated container. For comparative studies of MB phytotoxicity were carried out in a 0.5 m³ stainless fumigation chamber at the same temperature condition. Gas monitoring was carried out on samples taken from two points (front and rear) inside the refrigerated container to assess the distribution of the gas. Concentration of phosphine gas was determined by taking gas samples using a pump and then analyzing with GC-FPD at initial time and at the end of the fumigations. The *ct*-products were calculated as indicated by Ren et al (2011).

After fumigation, the chambers were aerated using a fan for 2 h, and then samples taken from the commodities were incubated at $15\pm1℃$ for 1 day. This was followed by

storage at 21±1℃ for 3 weeks for assessment of quality and phytotoxicity. Assessment of quality and phytotoxicity such as diameter, chlorophyll contents, color and damage of leaves was conducted for 1, 7, 14 and 21days after fumigation. Individual assessment was more than 10 replicates of each nursery trees.

Diameter was measured at the widest side with Veniera-califers tester (Coolant proof IP 67, Japan). Chlorophyll was measured using chlorophyll-meter (SPAD-502 Plus, Japan). The color was measured using colorimeter (SpectroDens A711073, Germany). Injury of leaves was subjectively scored as zero (none), one (slight, <5% affected), two (moderate, <25% affected), or three (severe, >25% affected). Mortality of adult and nymph of the insects was assessed under a microscope after incubation for 1 and 3 days. Hatching rate of eggs was investigated after incubation for 5~7 days.

<center>RESULTS</center>

1. Dose Response of Phosphine to *P. citri*

The *ct*-products (g h m^{-3}) of phosphine that achieved more than 99% kill of eggs and adults of *P. citri* was 25.23 and 1.08 g h m^{-3}, respectively. Table 1 shows the *ct*-products of phosphine on egg stage of *P. citri*.

Table 1. Calculated *ct*-products (Concentration × time) of phosphine at 50% (L*ct*$_{50}$) and at 99% (L*ct*$_{99}$) mortality of eggs of *P. citri* at 15±1℃

Temperature	No. of insects	Lct (95% C.l.)		Slope	Degrees of Freedom	X^{2b}
		L*ct*$_{50}$^{a}	L*ct*$_{99}$^{a}			
15℃	14,812	0.04 (0.001 ~ 0.267)	25.23 (13.99 ~ 50.73)	0.84	53	215.95

^{a}. Unit of **L*ct*$_{50}$**, **L*ct*$_{99}$**= g h m^{-3} for 24 h
^{b}. χ^2 is based on pooling of data with low expectation

2. Results of Refrigerated Container Fumigation

2-1. Efficacy of Phosphine to *P. citri*

The *ct*-products of phosphine for three individual trials were to 34.8 g h m^{-3} for 24 h fumigation (1st trial), 57.6 g·h·m^{-3} for 24 h fumigation (2nd trial) and 58.4 g h m^{-3} for 48 h fumigation (3rd trial). For MB fumigations, *ct*-products was g h m^{-3} for 2.0 h fumigation (Table 2).

Table 2. Efficacy of phosphine fumigation of nursery trees on egg and adult stages of *P. citri* at 15±1ºC in refrigerated container

ct-products of fumigant (g·h·m^{-3})	Fumigation Time (h)	*Planococcus citri*			
		Egg		Adult	
		No. of tested insects[1]	Corrected mortality (%)[2]	No. of tested insects[1]	Corrected mortality (%)[2]
Untreated	-	1,500	-	100	-
Phosphine (34.8)	24.0	3,587	100	60	100.0
Phosphine (57.6)	24.0	2,735	100	420	100.0
Phosphine (58.4)	48.0	997	100	60	100.0
MB (72.0)	2.0	3,014	100	60	100.0

[1] Sum of three replicates
[2] (Mortality for the treated-mortality for the control) / (100-mortality for the control)*100

2-2. Post Fumigation Damage of Dracaena and Palm Trees

Dracaena fragrans was not damaged by *ct*-product of phosphine at 34.8 g·h·m^{-3} carried out at 15℃, and after fumigation stored for 3 weeks at 20℃. Phosphine at 57.6 g·h·m^{-3} and MB at 72.0 g·h·m^{-3} caused phytotoxicity damage. However, none of *Chamaedoreae elegans* was damaged in this test (Table 3).

Table 3. Phytotoxic effect of phosphine and MB on nursery stocks at 15±1 ºC.

-	ct-products of fumigant (g·h·m^{-3})	Fumigation time (h)	Chlorophyll			Leaf browning[1] (%)	Damage[2]
			Mean ± SD	t	p		
Dracaena tree	Untreated	24.0	50.2±8.8	-	-	17.7	0
	Phosphine 34.8	24.0	48.0±8.9	1.7	0.09	14.1	0
	Untreated	24.0	50.0±9.5			10.1	0
	Phosphine 57.6	24.0	42.9±13.1	1.7	0.16	17.7	1
	MB 72.0	2.0	30.8±16.6	4.4	0.01	54.7	3
Palm tree	Untreated	24.0	46.9±6.4	-	-	12.1	0
	Phosphine 34.8	24.0	41.9±7.0	5.5	0.00	39.5	2
	Untreated	24.0	48.5±4.0	-	-	23.3	0
	Phosphine 58.4	48.0	38.7±2.9	4.3	0.01	35.0	2
	Phosphine 57.6	24.0	37.3±4.3	3.4	0.03	56.7	2
	MB 72.0	2.0	38.7±3.9	3.5	0.03	60.0	2

[1] Leaf browning = (Leaf browning/Total Leaf)*100
[2] Damage score: zero (none), one (slight), two (moderate), three (severe)

DISCUSSION

Phosphine fumigation for imported nursery plants in Korea was practically demonstrated in this study. The *ct*-products of phosphine at >30 $g \cdot h \cdot m^{-3}$ which is equivalent to 2 $g \cdot m^{-3}$ application for 24 and 48 h in well-sealed fumigation model at 15℃ could be enough for controlling egg stages of *P. citri* and without phytotoxic effect to dracaena and palm trees compared to current MB applications. These results show the similarity of previous work by Horn et al (2005) where high mortality of *Pseudococcus kraunhiae* was reported at 2.1 g m^{-3} of phosphine for 48 h fumigation.

Phosphine from ECO$_2$FUME could be a positive alternative fumigant to MB for various perishable commodities such as fruits and vegetables not only in terms of 100% efficacy at low temperature but without or minimal phytotoxic injury.

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ECO$_2$FUME AS A QUARANTINE FUMIGANT FOR EXPORT PAPRICA, CHERRY TOMATO AND STRAWBERRY

Byung-Ho Lee [1*], Bong-Su Kim[1], Justin Tumambing[2], Yong-Mi Moon[3]

[1]Dongbu ARI, Dongbu Hannong Co. Ltd, South Korea
[2] CYTEC Australia Holding Pty Ltd. Australia
[3]Animal, Plant and Fisheries Quarantine and Inspection Agency(QIA), South Korea
*Corresponding author's e-mail: *byungholee@dongbu.com*

ABSTRACT

ECO$_2$FUME, a cylinderized gas formulation of 2% phosphine and 98% carbon dioxide by weight, is an alternative fumigant to methyl bromide (MB) in grain fumigation and its applications are being extended for quarantine purposes in fruit and vegetables globally. With the increasing global trades and protection of agro-ecosystem in importing and exporting counties against quarantine pest, QPS (Quarantine & Pre-shipment) fumigation in perishable commodities is much more important in terms of quality maintenance of fumigated commodities. Currently, there is limited use of MB fumigation in perishable commodities due to its reduced effectiveness at low temperatures and its phytotoxicity at ambient temperature. Phosphine gas has demonstrated higher efficacy at low temperature and less phytotoxicity to a wide range of commodities due to good penetration properties. In this paper, more systematic data are presented on application of ECO$_2$FUME to different Korean exported perishable commodities such as paprika, cherry tomato and strawberry, in terms of correlation between *ct*-product (concentration X time) and biological efficacy to several target pests.

Key words: Phosphine gas, ECO2fume, quarantine, methyl bromide chemical alternatives, export, paprika, cherry tomatoes, strawberry, *ct*-product

INTRODUCTION

Methyl bromide (MB) is a widely used quarantine fumigant to control different quarantine insect pests. However, quarantine use of MB is also subject to phasing out in many countries globally and the European Union (EU) has issued banning announcement of MB in 2010.

For higher standards of quarantine, insect pests that remained in fruits and vegetables must be completely controlled. However, in terms of international trade, quality maintenance of fumigated commodities is no less important than quarantine control on exportation. But methyl bromide, which is often used for fumigation of export commodities, can cause phytotoxicity on several crops. For this reason, there is increasing demand on alternatives of MB. In Korea, strawberry, cherry tomato and paprika are among the most important exported

crops. According to Rural Development Administration of Korea, the most important insect pests of strawberry are *Myzus persicae* (green peach aphid), *Aphis gossypii* (cotton aphid) and *Frankliniella occidentalis* (western flower thrips), and the most important insect pest of cherry tomato is *Bemisia tabaci* (sweetpotato whitefly), and the most important insect pest of paprika is *Spodoptera litura* (tobacco cutworm).

This study was conducted to obtain the efficacy data of ECO_2FUME on treatment of these pests in export strawberry, cherry tomato and paprika as these insects can easily remain in these export commodities in big numbers.

MATERIALS AND METHODS

Materials

Three kind of important export crops (strawberry, cherry tomato, paprika) in Korea were used for this trial. Nymph and adult stages of *Myzus persicae* (green peach aphid), *Aphis gossypii* (cotton aphid) and *Frankliniella occidentalis* (western flower thrips) were gathered at field conditions and inoculated on export strawberry for the efficacy test of phosphine. In the same way, larva and adult stages of *Bemisia tabaci* (sweetpotato whitefly) were inoculated on export cherry tomato, and larval stages of *Spodoptera litura* (tobacco cutworm) were inoculated on export paprika. ECO_2FUME (2% PH_3 + 98% CO_2) for fumigation studies was supplied by Cytec Industries via its local distributor, Dongbu Hannong Co., Ltd in Korea.

Measurement of Phosphine and Calculation of *ct*-product (concentration X time)

Concentrations of phosphine were measured at 0.5, 2, 6, 24 h after the first injection into the fumigation chamber (8.3 L glass desiccators). The gas samples were stored in gas-tight Tedlar® sampling bags before analysis. The *ct*-products (concentration x time) of the fumigant were calculated from the arithmetic average of phosphine concentration readings during the 24 h exposure period. Fumigant concentrations were determined with a gas chromatograph (Agilent 7890A, FPD, USA), fitted with a DB-WAX, FFD at 320°C, injection temperature of 200°C (helium as carrier gas) and oven temperature of 200°C.

Fumigation Methods for Dose Response (8.3 L desiccators)

The calculated amount of phosphine was injected in gas-tight 8.3 L glass desiccators with known volume for 24 h at 2 and 10℃ for strawberry and at 13℃ for cherry, tomato and paprika. The desiccators were sealed with glass stoppers containing a septum through which the mixture gas was injected at several doses and gas samples were taken for analysis by gas chromatography. The dosage and required volume for the fumigant concentration were calculated according to Ren et al (2011). After 24 hours of fumigation, the desiccators were opened in the fume hood for aeration. Mortality of each insect was assessed under a microscope after incubation for 1 and 3 days.

1. Dose Response of Phosphine to *M. persicae*, *A. gossypii*, and *F. occidentalis* on strawberry

The *ct-* products (g h m^{-3}) of phosphine that achieved more than 99% mortality of nymphs and adults of *M. persicae*, *A. gossypii*, and *F. occidentalis* was 23.06 and 37.38 mg h L, 23.06 and 23.06 g h m^{-3}, 1.74 and 4.64 g h m^{-3}at 2℃ (Fig. 1), respectively; and 11.15 and 19.78 g h m^{-3}, 11.15 and 19.78 g h m^{-3}, 10.66 and 6.97 g h m^{-3}at 10℃(Fig. 2), respectively.
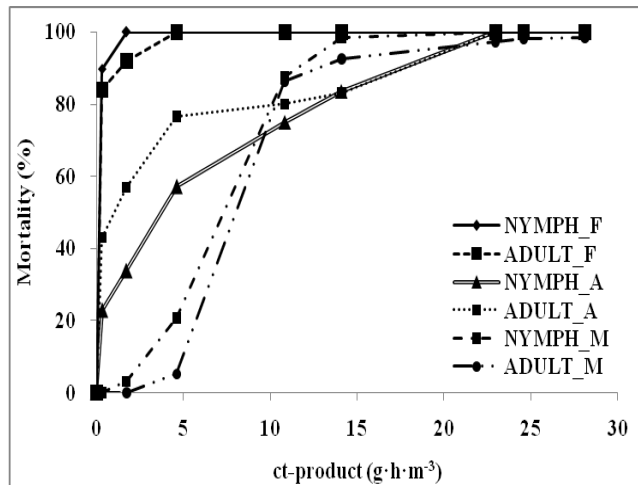
Fig. 1- Mortality of nymph and adult stages of *M. persicae* (_M), *A. gossypii* (_A) and *F. occidentalis* (_F) by phosphine fumigation on export strawberry at 2℃.
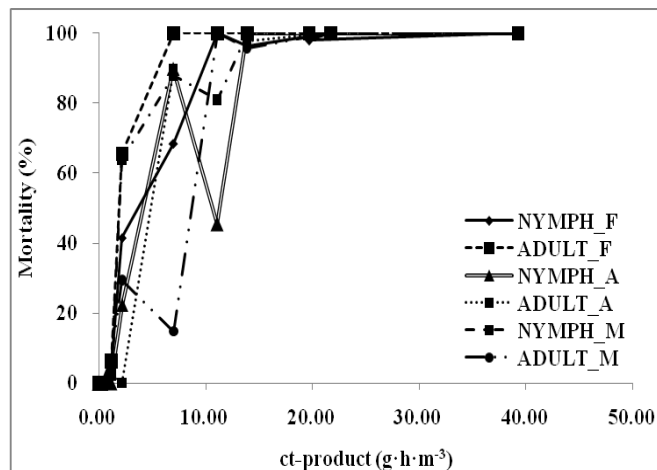
Fig. 2- Mortality of nymph and adult stages of *M. persicae* (_M), *A. gossypii* (_A) and *F. occidentalis* (_F) by phosphine fumigation on export strawberry at 10℃.

2. Dose Response of Phosphine to *B. tabaci* on cherry tomato

The *ct*-products (g h m^{-3}) of phosphine that achieved more than 99% mortality of larvae and adults of *B. tabaci* was 0.74 g h m^{-3} at 13℃. CT product of phosphine to *B. tabaci* was shown in Fig. 3.
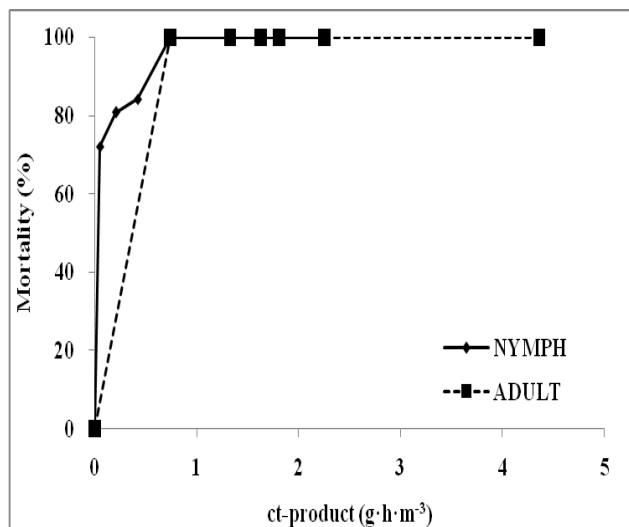
Fig. 3- Mortality of larva and adult stages of *B. tabaci* by phosphine fumigation on export cherry tomato at 13℃.

3. Dose Response of Phosphine to *S. litura* on paprika

The *ct*-products (g h m^{-3}) of phosphine that achieved more than 99% mortality of 3rd and 4th instar larvae of *S. litura* were 0.76 and 0.96 g h m^{-3} at 13°C, respectively. The *ct*-products of phosphine to *S. litura* was shown in Fig. 4.
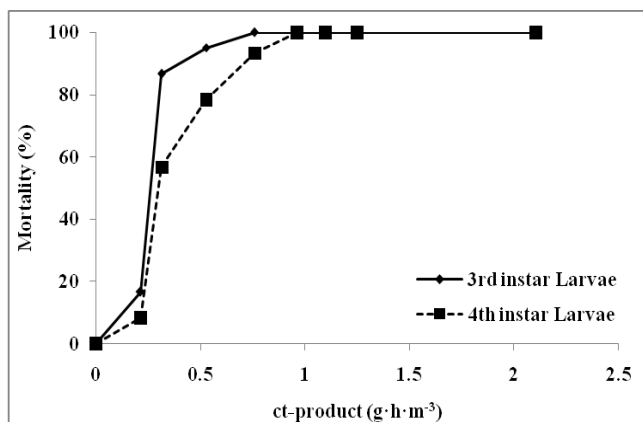
Fig. 4- Mortality of larva and adult stages of *S. litura* by phosphine fumigation on export paprika at 13℃.

DISCUSSION

Preliminary test of phosphine fumigation for export commodities in Korea was conducted in this study. The *ct*-products of phosphine at >37.38 g h m^{-3} in well-sealed fumigation chamber at 2°C and >19.78 g h m^{-3} at 10°C was shown to be suitable doses for controlling important insect pest of export strawberry compared to current MB applications. The *ct*-products of phosphine at >0.74 g h m^{-3} on cherry tomato and >0.96 g h m^{-3} on paprika were also suitable doses to control target pest.

Recently, there have been several studies on phosphine for postharvest treatment of several important agricultural pests on perishables. Liu (2011) reported that the mixture of phosphine and oxygen enhances the control efficacy of postharvest pests including *Frankliniella occidentalis* (western flower thrips), *Liriomyza langei* (leafminer), *Pseudococcus maritimus* (grape mealybug) and *Plodia interpunctella*(indian meal moth). Horn et al (2005) reported that high mortality of *Pseudococcus kraunhiae* was achieved at 2.1 g m^{-3}of phosphine after 48 h fumigation.

Phosphine from ECO$_2$FUME appears be a suitable alternative fumigant to currently used of MB for various exports of fresh commodities for quarantine purpose not only in terms of 100% efficacy but also without or minimal phytotoxic injury.

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COMPARATIVE TOXICITY OF ETHYL FORMATE FOR CONTROL OF PESTS OF SWEET PERSIMMONS FOR EXPORT

Jin-Hoon Cho[1], Chung Gyoo Park[1], Max Yoo[2], Bong-Su Kim[3], Byung-Ho Lee[3*]

[1.]Division of Applied Life Science, Gyeongsang Nat'l. Univ., South Korea
[2.]The Linde group, South Korea
[3.]Dongbu ARI, Dongbu Hannong Co. Ltd, South Korea
Corresponding author's e-mail: *byungholee@dongbu.com*

ABSTRACT

Vapormate™, a new gas formulation of ethyl formate (EF) with carbon dioxide, is an alternative fumigant to methyl bromide (MB). For applying export persimmons in Korea, the efficacy of EF was evaluated on an overwintering and summer type of *Tetranychus urticae* adults and egg stage of *Asiacornococcus kaki* which are pests in sweet (non-astringent) persimmon in Korea. Fumigated for 6 h at 5°C, the concentration × time (Ct) products of EF to overwintering and summer type of *T. urticae* was LC_{99}=147.9 and 18.8 g h m^{-3} and that was LT_{99} > 39.3 and 52.5 g h m^{-3} to *A. kaki*, respectively. We found sensitivity on EF is completely different from seasonal types of the mite as well as insect species.

Key words: Ethyl formate, Vapormate, methyl bromide chemical alternatives, export persimmon, quarantine, *Tetranychus urticae*, *Asiacornococcus kaki*, overwintering type, summer type.

INTRODUCTION

The current treatment for postharvest sweet persimmon disinfestation of pests such as overwintering two spotted spider mite [*Tetranychus urticae* Koch], grape-myrtle scale [*Asiacornococcus kaki* (Kuwana)] and persimmon fruit moth [*Stathmopoda masinissa* Meyrick] is methyl bromide fumigation (Jang, 2010). An alternative treatment is required as a result of its phase out in 2005 for all uses except quarantine treatments (UNEP, 2005) In addition, effects of human poisoning by methyl bromide are attributed as the fumigant causing great number of fatalities and injuries (EPA, 1986). Plant volatiles such as ethyl formate (EF) have been shown to have insecticidal properties (Rohitha *et al*. 1993). One important advantage of using volatiles such as EF for fumigation is that only trace residues were found on treated products (Desmarchelier and Ren, 2009). The Food and Drug Administration (FDA, 2004) has reviewed the use of EF as a flavoring agent and has characterized this compound as generally recognized as safe.

Ethyl formate has been used for disinfestation of pests in stored dried fruit since 1927 (Simmons and Gertler, 1945). More recently, EF has been tested for use in some fresh commodities. For example, packaged head lettuce infested with green peach aphid was exposed to 0.5-1.5% EF at 15°C under vacuum for up to 2 h (Stewart and Mon, 1984). EF at 35g/m^3 with CO_2 has been showed the complete effectiveness on egg, lymph and adult stages of citrus mealybugs, *Planococcus citri,* for applying banana and orange without any

phytotoxic damages (Sung et al, 2008, 2009).

For the treatment of persimmons to be exported from Korea, the efficacy of EF was evaluated on an overwintering and summer type of adult stage of *Tetranychus urticae* and egg stage of *Asiacornococcus kaki* which are the main pest in sweet persimmons in Korea.

MATERIALS AND METHODS

Test insects

Adults of overwintering and summer type of *Tetranychus urticae* were tested against a range of EF concentrations to ascertain the effect of fumigation on mortality. The overwintering type was collected from a farm in the Geochang, Gyeongsangnamdo Korea in 2011, while the summer type obtained from Gyeongsangnamdo Agricultural Research and Extension Services (Jinju, Korea) in 2006. We maintained the colony at $24\pm2℃$, 60~70% RH, and a photoperiod of 16:8 (L:D) h on kidney beans, *Phaseolus vulgaris* L. For exposure to EF, a section of kidney bean with 30 adult mites was placed in Petri dish (6 cm i.d.). After treatment, adults of overwintering and summer type of *T. urticae* were held for 24 h at $25℃$ and 50~60% RH, and then evaluated for mortality.

Grape-myrtle scale, were field collected from persimmon trees in Gyeongsang National University (Jinju, Korea). For exposure to EF, a sweet persimmon infested with 8~10 protonymph, deutonymph, or adult grape-myrtle scales were placed in desiccators (3-4 sweet persimmons per desiccator). For the egg stage, eggs were gently removed from adult female grape-myrtle scale with a small paint brush and placed in Petri dish. After treatment, the egg stage was held for 3 d at $25℃$ and 50~60% RH, before evaluating for mortality. Petri dishes containing eggs were held for 8 d at $25℃$ and $≥80\%$ RH to ensure eggs survival and evaluation for mortality.

Measurement of ethyl formate

Concentrations of EF were monitored at 0.5, 2, 6 h after the injection of EF. The gas samples were stored in Tedlar® gas sampling bags using a gas-tight, 25 mL syringe, The *Ct* products were calculated from the arithmetic average of EF concentration readings during the 6-h exposure period.

Fumigant concentrations were determined using a gas chromatograph (GC-17A, Shimadzu co., Japan) fitted with a DB-WAX, flame ionization detector at 250º, injection port was at 100º (He served as carrier gas) and oven temperature was 100º.

Fumigant and fumigation

The fumigant used was an analytical grade (99.7%) liquid formulation of EF supplied by Aldrich Chemical Company Inc.,

Each target pest was exposed to at least nine concentrations (three replications per concentration) of EF between 0.3 and 66.8 mg/L, resulting in a range of mortality between 0 and 100%. All target pests were exposed to EF for 6 h at $5℃$.

The fumigation chambers were 6.7-L desiccators, each equipped with a ground glass stopper fitted with a rubber septum (Schott Duran, German). A filter paper (55 mm i.d.) was inserted into the glass stopper to provide a liquid evaporation surface for the injected EF. Each experimental run consisted of four fumigated desiccators of different EF concentrations and one non-fumigated control desiccator.

For exposure to EF, a replicate consisted of Petri dishes or sweet persimmons containing target pests placed inside desiccators sealed with glass stopper. A partial vacuum

was pulled with a syringe, and reagent grade (99.7% purity) liquid EF was injected through a rubber septum covering an inlet port in the glass stopper onto filter paper affixed to the underside of the stopper. At the completion of the 6 h fumigation, the desiccators were opened and aired for 1 h in a fume cupboard.

Data analysis

Lethal concentration estimates were performed using Polo Plus software program (LeOra Software, 2003). For grape-myrtle scale eggs, the LC_{99} was determined from estimated mortality, which was based on subtracting the number of hatched insects in each treatment from hatched insects in the control.

<div align="center">RESULTS</div>

The observed and fitted data relating mortality to Ct products are shown Fig. 1, 2 and the results of probit analysis of the data are summarized in Table 1. The overwintering type *T. urticae* was more resistant to EF than the summer type. The Ct products for LC_{50} of EF were 66.25 and 11.46 mg h L^{-1}, and those for LC_{99} were 147.98 and 18.82 mg h L^{-1} for overwintering and summer type *T. urticae*, respectively, at 5℃, 6 h exposure.
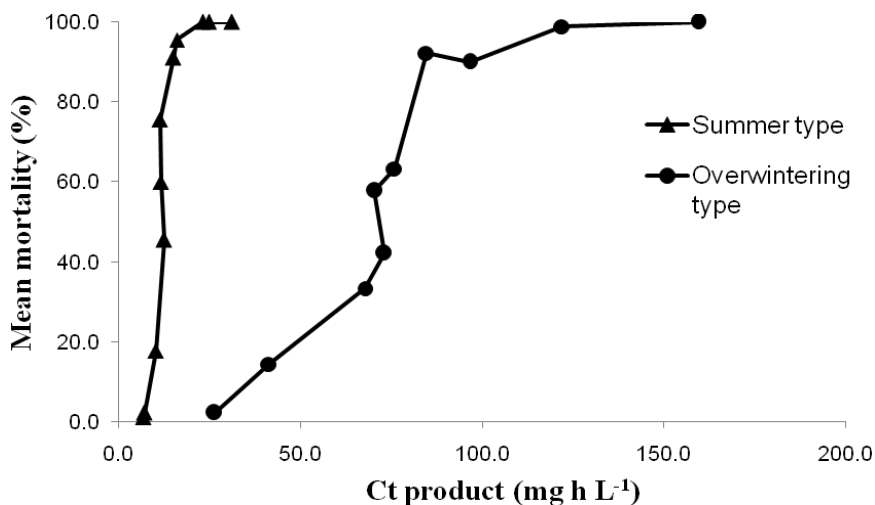
Fig. 1- Mortality of summer and overwintering type of *T. urticae* exposed to a range of Ct product for 6 h at 5ºC.

The fumigant toxicity of EF to live stages of *A. kaki* was determined using a serial range of EF concentrations. The Ct products for the LC_{99} for egg, nymph and adult was 25.18, 41.1, 15.17 mg h L^{-1}, respectively. On the basis of LC_{99} values, tolerance of the live stages of *A. kaki* was in descending order: egg < nymph < adult.
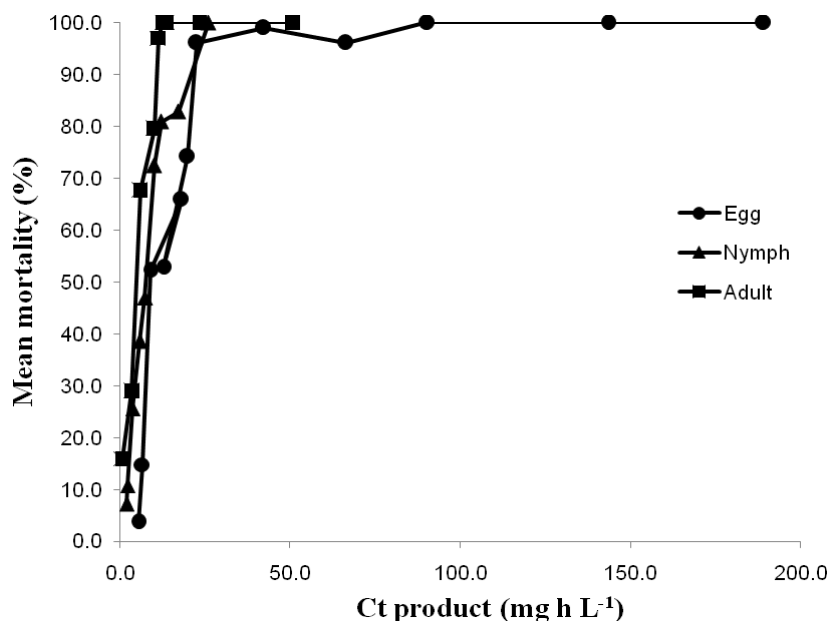
Fig. 2- Mortality of various life stages of *A. kaki* exposed to a range of Ct product for 6 h at 5℃.

Table 1. Dosage (measured as Ct product) estimates and parameters of regression of Probit mortality for exposure of the *T. urticae* and *A. kaki* to ethyl formate for the 6 h fumigation at 5±1℃.

Target pest	Type	Life stage	n^a	Slope ± SE	LC_{50} (mg h L^{-1}± 95% CL)	LC_{99} (mg h L^{-1}± 95% CL)
Tetranychus urticae	Overwintering	Adult	1080	6.67 ± 0.78	66.25 (55.64~74.96)	147.98 (115.82~275.49)
	Summer	Adult	990	10.79 ±1.31	11.46 (10.53~12.32)	18.82 (16.31~25.68)
Asiacornococcus kaki	Summer	Egg	1519	3.685 ±0.370	12.20 (9.54~15.02)	52.18 (35.35~112.43)
		Nymph	624	2.944 ±0.221	6.66 (5.89~7.52)	41.12 (30.61~62.38)
		Adult	740	5.234 ±1.001	5.45 (3.90~6.59)	15.17 (12.24~22.73)

[a] Total number treated over three replications

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DETERMINING EFFECT OF SULFURYL FLUORIDE FUMIGATION ON DRIED APRICOTS, RAISINS, AND HAZELNUTS QUALITY

Fatih Sen[1*], Uygun Aksoy[1], Gurler Tan[2]

[1]Ege University, Faculty of Agriculture, Department of Horticulture, 35100 Izmir, Turkey
[2]Dow AgroSciences, 34742 Kozyatagi, Istanbul, Turkey
*Corresponding author'S e-mail: *fatih.sen@ege.edu.tr*

ABSTRACT

Dried fruits and nuts have a long shelf life but many factors including storage pests or quality losses may limit their marketability. Storage pests pose significant threat for the dried fruits and nuts industry especially after the phase out of methyl bromide (MB). Research on MB alternatives is carried out intensively in Turkey as the major producer and trader of dried apricots, figs, raisins, and hazelnuts in the world market. Sulfuryl fluoride (SF) (ProFume, Dow AgroScience, USA) was tested on dried apricots, raisins, and hazelnuts as a MB alternative. The present study aimed at assessing the effect of SF treatments on dried apricots (cv. Hacıhaliloğlu), raisins (cv. Sultana) and hazelnuts (cv. Tombul) quality. Dried fruits and nuts samples were treated with SF at commercial scale at the storage units of TARİŞ Dried Fig Processing Plant in containers at a dosage of 100 g.m^{-3}. The temperature and relative humidity levels were monitored during fumigation and later in storage. After assessing SF as an effective fumigant, major quality parameters were analyzed right after the treatments, and after 7 months of storage under ambient conditions. Post-harvest SF treatment did not exert any negative effects on surface colour, water content, total soluble solids and titratable acidity contents of dried apricot and raisin fruits. Similarly, no negative effects were determined on colour and fatty acid composition of hazelnut kernels after 7 months of storage. Analysis also showed no SO_2 residues in treated dried apricot, raisins fruit and hazelnut kernels.

Key words: MB alternatives, ProFume, dried fruit, nut, quality, storage, residue

INTRODUCTION

Dried fruits and nuts are traditional export goods in Turkey. A major problem faced in the trade of dried fruits and nuts is the damage and losses caused by storage pests infesting fruit especially during ripening, drying and/or storage. Methyl bromide was used as the unique fumigant for disinfestations however it was banned in developed countries since 2005 and since January 1, 2008 in Turkey. It is scheduled for worldwide withdrawal from routine use as a fumigant in 2015 under the directive of the Montreal Protocol on Substances that Deplete Ozone Layer (Schneider et al., 2003) except for quarantine and pre-shipment purposes.

Integrated pest management, chemicals (phosphine, carbonyl sulfide, SF, ozone, cyfluthrin, iodomethane etc.) and physical (modified atmospheres, high pressure, heat/cold treatments, sanitation, radio frequency, long-wave energy, irradiation) methods were tested on various commodities and against different pests (Fields and White, 2002; Johnson et al., 2000; Schneider et al., 2003; Aksoy et al., 2004; Çetinkaya et al., 2006). Although there are a large

number of potential alternatives to MB, each has limitations in terms of efficiency, cost, penetration or residues that prevent it from being a direct replacement for MB in all its current uses. In many of the research works, the main target is to provide control of storage pests, however quality is generally underestimated due to more stable nature of dried produce. Dow AgroSciences released ProFume gas fumigant (SF) as an alternative to MB for the control of stored product insect pests in food storage, processing, milling and warehousing (Fields and White, 2002).

Food residue studies have already been conducted on key dried fruits and tree nuts. After testing efficacy of SF on major storage pests in Turkey, this study was designed to determine the effect of SF on the quality of dried apricots, raisins and hazelnuts.

MATERIALS AND METHODS

Sun-dried apricots (cv. Hacıhaliloğlu), raisins (cv. Sultana) and in-shell hazelnuts (cv. Tombul) packed in 50 and 25 kg (cross stitch bags for dried fruits and net for in-shell hazelnuts) bags were fumigated using SF (ProFume, Dow AgroScience, USA) at commercial scale in containers at TARİŞ Dried Fig Processing Plant in İzmir Turkey. During fumigation, SF concentration was 100 g.m^{-3} for exposure of 24 h at a temperature ranging between 11°C and 23°C. Pooled sub-samples were prepared from the treated lots of 4 kg of hazelnut kernels and 7 kg of apricots and raisins for quality assessment. Quality of SF treated samples was compared with samples from the same lots treated with MB applying 60 g.m^{-3} for 24 h. Quality tests were carried out as five replicates right after the fumigation and after 7 months of storage at ambient conditions. The temperature and relative humidity were monitored in the container during exposure and later in storage by data loggers (Hobo U12-013, Onset, USA).

Moisture content was measured by drying samples in a vacuum oven to a constant weight (AOAC, 1990) and calculated based on the percentage of weight loss. A water activity meter (TH 500, Novasina, Switzerland) was used to measure water activity values at 25°C. The surface color of dried fruits and kernels were measured on the two opposite sides of 20 fruits/kernels with a colorimeter (CR-300, Minolta Co., Japan), and average scores were recorded in terms of CIE L* a* b* values. Chroma (C*) value and hue angle (h^{o}) were calculated from a* and b* values. Total soluble solids content (TSS) was determined with a refractometer (ATC-1, Atago, Japan). Titratable acidity (TA) was determined by titration with 0.1 N NaOH up to pH 8.1 and expressed as g citric acid/100 g. Five trained panelists conducted the sensory analysis in discriminative evaluation of dried apricots and raisins. Visual appearance, flavor and texture of dried apricots and raisins were evaluated on a five-point scale (1: extremely poor; 9: excellent). The fatty acid composition of hazelnuts was determined on the lipid extracts after methylation to form fatty acid methyl esters (FAME) (AOAC, 1997) were analyzed by using a Hewlett Packard 6890N gas chromatograph equipped with a Spelco SPB-5 capillary column (Supelco, Bellefonte, PA) and a flame ionization detector (FID). Sulphur dioxide residue level was determined in duplicate by modified Monier Williams distillation method (AOAC, 1995).

The experiments were conducted as completely randomized design with five replicates. Significant differences among groups were determined using Duncan's multiple range tests at $P \leq 0.05$. Standard deviation of the mean (SD) was also calculated from the replicates. All computation and statistical analyses were done using SPSS package version 19.0 (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Dried apricots

Sulphuryl fluoride treatment did not have any adverse effect on surface colour of dried apricot (cv. Hacıhaliloğlu) fruits and the analyzed colour parameters (L*, a*, b*, C*, h^o) were similar to the control fruits after storage (Table 1). Both SF and MB treated fruits were darkened during the storage period as revealed by lowered a*, b* and C* values.

Table 1. Changes in fruit colour (L*, a*, b*, C*, h^o) of sulphuryl fluoride (SF) or methyl bromide (MB) treated dried apricots after treatment and after 7 months of ambient storage[a]

Time	Treatment	L*	a*	b*	C*	h^o
After	MB	40.44±0.22[NS]	5.98±0.45 [NS]	29.70±0.46 [NS]	30.30±0.37 [NS]	78.62±0.99 [NS]
Treatment	SF	40.41±0.21	6.55±0.42	28.30±0.55	29.05±0.51	76.97±0.91
After 7	MB	35.58±0.61 [NS]	4.64±0.59 [NS]	20.17±0.47 [NS]	20.70±0.52 [NS]	77.07±1.52 [NS]
months	SF					
storage		36.40±0.60	5.28±0.66	21.34±0.94	21.99±1.22	76.13±1.22

[NS] Nonsignificant.
[a] Results are the means of five replicate samples ±SD.

Sulphuryl fluoride treatment did not influence moisture, TSS or TA contents and sensory quality of dried apricot fruits. At the end of the storage period, moisture content decreased significantly by about 37% due to the dry conditions prevailing in the ambient. Such moisture decrease triggered the increase in TSS and TA. The sensory quality scores of SF and MB treated samples were similar and both decreased at the end of the storage period due to darkening of colour and hardening of texture (Table 2).

Table 2. Changes in water content, TSS and TA contents of sulphuryl fluoride (SF) or methyl bromide (MB) treated dried apricots after treatment and after 7 months of ambient storage[a]

Time	Treatment	Water content (%)	TSS (%)	TA (%)	Sensory score
After	MB	27.05±0.14 [NS]	60.3±0.47 [NS]	1.51±0.05 [NS]	4.60±0.55
Treatment	SF	25.02±0.55	61.3±0.00	1.57±0.02	4.80±0.45
After 7	MB	15.79±0.16 [NS]	68.44±0.38 [NS]	1.98±0.08 [NS]	3.20±0.45
months	SF				3.40±0.55
storage		17.03±0.13	68.44±0.38	1.77±0.01	

[NS] Nonsignificant.
[a] Results are the means of five replicate samples ±SD.

Raisins

Raisins (cv. Sultana) colour values (L*, a*, b*, C*, h^o) were similar for SF and MB treated samples. Colour a*, b* and C* values increased during storage whereas h^o decreased (Table 3).

Table 3. Changes in fruit colour (L*, a*, b*, C*, h^o) of sulphuryl fluoride (SF) or methyl bromide (MB) treated raisins after treatment and after 7 months of ambient storage[a]

Time	Treatment	L*	a*	b*	C*	h^o
After Treatment	MB	25.89±0.49 [NS]	6.35±0.50 [NS]	14.45±2.46 [NS]	15.78±2.09 [NS]	66.28±2.95 [NS]
	SF	25.97±0.85	5.88±0.10	13.46±1.25	14.69±1.17	66.40±1.80
After 7 months storage	MB	25.94±2.63 [NS]	11.58±0.67 [NS]	17.06±1.10 [NS]	20.63±0.94 [NS]	55.79±2.41 [NS]
	SF	26.72±1.20	12.66±0.79	18.45±0.94	22.37±1.22	55.54±1.31

[NS] Nonsignificant.

[a] Results are the means of five replicate samples ±SD.

Sulphuryl fluoride and MB treatments did not exert significant effects on moisture, TSS, TA content and sensory attributes. Water content of raisins decreased from ca 16 % to 13 % after 7 months of storage which led to the increase of TSS and TA. Sensory quality scores were lower after storage mainly due to appearance (Table 4).

Table 4. Changes in water content, TSS and TA contents of sulphuryl fluoride (SF) or methyl bromide (MB) treated raisins after treatment and after 7 months of ambient storage[a]

Time	Treatment	Water content (%)	TSS (%)	TA (%)	Sensory score
After Treatment	MB	16.33±0.64 [NS]	71.5±0.77 [NS]	1.50±0.04 [NS]	4.80±0.45 [NS]
	SF	16.06±0.52	70.2±1.54	1.46±0.05	4.80±0.45
After 7 months storage	MB	13.03±0.08 [NS]	72.44±1.54 [NS]	2.00±0.07 [NS]	4.40±0.55 [NS]
	SF	12.78±0.07	72.89±0.77	1.90±0.03	4.20±0.45

[NS] Nonsignificant.

[a] Results are the means of five replicate samples ±SD.

Hazelnut kernels

Post-harvest SF treatment had no negative effect on colour value of hazelnut (cv. Tombul) kernels. The changes that occurred during storage were similar in SF and MB treated hazelnut kernels (Table 5). The fatty acid composition of SF treated kernels was statistically similar to MB treated samples after storage (Table 6).

Table 5. Changes in fruit colour (L*, a*, b*, C*, h^o) of sulphuryl fluoride (SF) or methyl bromide (MB) treated hazelnut kernels after treatment and after 7 months of ambient storage[a]

Time	Treatment	L*	a*	b*	C*	h^o
After Treatment	MB	28.04±1.77 [NS]	11.09±0.30 [NS]	18.21±0.81 [NS]	21.32±0.87 [NS]	58.66±0.54 [NS]
	SF	28.07±1.15	10.44±0.11	19.75±0.32	22.34±0.30	62.14±0.38
After 7 months storage	MB	22.48±0.64 [NS]	5.77±0.26 [NS]	11.13±0.69 [NS]	12.55±0.52 [NS]	62.54±2.34 [NS]
	SF	24.15±1.48	5.91±0.31	11.03±0.71	12.21±0.57	61.80±1.60

[NS] Nonsignificant.

[a] Results are the means of five replicate samples ±SD.

Table 6. Changes in fatty acid composition of sulphuryl fluoride (SF) or methyl bromide (MB) treated hazelnut kernels after treatment and after 7 months of ambient storage[a]

Time	Treatment	Palmitic	Stearic	Oleic	Linoleic	Linolenic	Myristic
After 7	MB	4.93 [NS]	2.31 [NS]	82.26 [NS]	10.21 [NS]	0.11 [NS]	0.18 [NS]
months	SF						0.17
storage		5.13	2.59	82.85	9.16	0.11	

[NS] Nonsignificant.

[a] Results are the means of five replicate samples ±SD.

The quality analyses showed that SF fumigation has no negative impact on dried apricots and raisins as well as hazelnut kernels. Among quality attributes, color is considered as one of the major parameters for dried apricots, raisins and hazelnut kernels. SF exposure did not create any significant impact on colour of the tested commodities even after 7 months of storage under ambient conditions. Fruit colour changed during storage irrespective of the fumigant used due to a number of chemical and biochemical reactions. Storage period had marked effect on quality. Enzymatic and non-enzymatic browning may occur during drying and storage (Roos and Himberg, 1994; Perera and Baldwin, 2001). The darkening rate in storage is related to the substrate and the storage conditions e.g. water content, a_w, temperature and oxygen (Perera, 2005). The uncontrolled storage conditions promoted water loss in dried apricots and raisins which further concentrated TSS and TA contents. Dried fruits have direct interaction with the storage atmosphere and high temperature and low relative humidity levels (data not shown), which especially towards the end of the storage period have fastened water loss resulting in lower sensory scores (Mc Bean et al., 1971; Fennema, 1976).

Sulphur dioxide is accepted as an additive and there is a tendency for the reduction of its permissible maximum limits and labeling in case of treatment. The analysis showed no residual sulphur dioxide in SF treated raisins fruit analyzed one week after SF treatment.

Based upon the quality results SF can be recommended as a fumigant alternative to MB to control storage pests in dried fruits and nuts industry. Controlled storage conditions will further contribute to the preservation of quality even during extended storage conditions.

ACKNOWLEDGMENTS

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EFFICIENCY OF ETHYL FORMATE AND VAPORMATE[®] ON DATES FUMIGATION: EFFECTS ON FRUIT QUALITY

Cyrine Ferchichi*[1], Haithem Bessi[1], Kaouthar Grissa[2], Veronique Bikoba[3], Elizabeth J. Mitcham[3], Sihem Bellagha[1]

[1] Department of Food Technology, National Institute of Agronomy of Tunisia, University of Carthage, INAT, 43, Avenue Charles Nicolle, 1082, Tunis Mahrajène, Tunisia
Laboratory of Economy, Sciences and Food technology, LR03AGR01
[2] Department of Entomology, National Institute of Agronomy of Tunisia
[3] Department of Plant Sciences, University of California, Davis, CA 95616, USA
*Corresponding author's e-mail: *cyrineferchichi@gmail.com*

ABSTRACT

Dates are subject to infestation by several insect species during and after harvest. In Tunisia, the world first producer of Deglet Nour variety, infestation rate may reach 20%, which leads to serious damages to the fruit rendering it unfit for human consumption and unacceptable for marketing in international trade. The main pest of date is carob moth (*Ectomyelois ceratoniae*) which develops inside the fruit and continues its growth upon arrival at the packaging plant and during storage.

In this study, ethyl formate (EF) was chosen, as an alternative to methyl bromide, to fumigate dates at the laboratory scale. For this purpose, three EF concentrations (114.4 g m^{-3}; 128.7 g m^{-3}; 143 g m^{-3}) and two exposure durations (2 and 3 hours) were tested. Results showed that the most efficient combination is 143 g m^{-3} EF during 2 hours which ends up with 98.12% mortality. On a commercial scale, EF is available as a mixture of CO_2 and EF: 16.7 wt% EF in liquid carbon dioxide (Vapormate ®, LINDE GROUP). Hence, experiments at semi-industrial scale were run in order to validate previous results. Thanks to the CO_2 synergic effect, the mortality rate was confirmed.

In addition, effects of EF on fruit quality were investigated through color, sugar content and microbiological analysis.

Key words: Fumigation, Carob moth, Ethyl formate, Vapormate, Methyl bromide, Mortality rate, Fruit quality.

INTRODUCTION

Carob moth, *Ectomyelois ceratoniae* (Zeller) (Lepidotera: Pyralidae), is the pest which causes the most damages to harvested date fruits. The rate of infestation may increase from 18% at harvest to 70% during storage. Studies focusing on life cycle development of Carob moth demonstrate that the most resistant phase to EF is the larvae in L5 stage (Bessi et al., 2011).

Till now, methyl bromide has been the most used fumigant in date postharvest stack disinfestations. However, with the pending phasing-out of methyl bromide, alternatives such as EF need investigation. Research seeking alternative fumigants to methyl bromide has

involved studies with EF as a space fumigant. The practicalities and effects of space fumigation with EF are a highly attractive option for bulk disinfestations of unprocessed dates. The low molecular weight of EF is considered as an advantage over conventionally used chemicals which can persist as residues in food products (Simpson et al., 2004)

EF has been registered for application to dried fruit where it has been used as a post processing disinfestations treatment since 1927 (Simmons and Gertler, 1945) and has proven to be effective on the major insects present in the Australian processing industries. Hence, dried sultana raisins were fumigated in shipping containers using EF and an EF/CO_2 mixture (Tarr et al., 2004).

This study evaluated the use of EF alone or in combination with carbon dioxide (16.7 wt% EF in liquid carbon dioxide called Vapormate® (BOC Gases LINDE Group)) as a postharvest fumigant in order to control Carob moth (*Ectomyelois ceratoniae*), the most common pest on dates in Tunisia. In Vapormate composition, CO_2 is a carrier gas and fire retardant. However, CO_2 presence may enhance EF disinfestations.

The study reported here, summarizes the laboratory work undertaken to determine the concentration of EF and the exposure time required to obtain maximum mortality of Carob moth. The second part of the study relates field trials undertaken to apply the laboratory findings to a semi-industrial scale using a mixture of 16.7% EF in CO_2 (Vapormate®, LINDE Group). The effects of EF and Vapormate treatments on fumigated fruit quality were also investigated through color, sugar content, aromatic composition and microbiological analysis.

MATERIALS AND METHODS

1. Raw materials and target pest

Dates "Deglet Nour" variety in branch were obtained from a local distributor and stored at 4°C. An in vitro infestation of dates by the most EF resistant larvae stage (L5) of Carob moth (*Ectomyelois ceratoniae*) was applied (Bessi et al., 2011).

2. EF and Vapormate trials

Dates previously infested by the larvae L5 were exposed to EF in 1 liter glass jars (4 replications; 410g of dates fruits per treatment) sealed with rubber stoppers (Bessi et al., 2011). Three EF concentrations were tested: 114.4 g m^{-3}; 128.7 g m^{-3}; 143 g m^{-3} with two exposure times: 2 and 3 hours. The EF was injected by a microsyringe.

A fumigation pilot plant has been designed by the LINDE Group technical team. It consisted of a stainless steel enclosure (capacity 100 liters) with a sealed cover connected to the bottle of Vapormate by a copper pipe (diameter 1 cm) fitted with a barometer. A precision balance (+/- 0.005kg) was used to determinate the quantity of Vapormate injected in the enclosure. Three trays containing the artificially infested dates were placed respectively on the middle and the two opposite corner of the enclosure for each of three trials.

3. Fruit quality analysis

Post-treatment fruit quality evaluations included internal and external dates color using Minolta Chromameter (model CR-300) and sugar content by the colorimetric method. Aerobic mesophilic bacteria (NT 16-14 (2006)) and mold and yeast (NT 16-14 (2006)) were analyzed after EF fumigation and over three weeks storage at 4°C.

RESULTS AND DISCUSSIONS

1. EF effect on carob moth mortality

All EF treatments resulted in significant Carob moth mortality (Table 1). However, the highest mortality, i.e. 98.12%, was achieved with 143 g m^{-3} EF during 2 hours. Statistical analysis (P> 0.05) has shown that EF exposure time has no significant effect on Carob moth mortality. Table 1 shows no significant difference between the 128.7g.m^{-3} and 143 g m^{-3} for 2 hours treatments. However, the highest mean is obtained when 143g.m^{-3} EF concentration is used. Hence, in order to validate Vapormate assays, 143 g m^{-3} EF concentration was adopted.

Table 1. Effect of fumigants on Carob moth mortality in dates.

Trials conditions			Mortality (%)
Fumigant	Time (h)	Concentration* (g m^{-3})	
EF	2	114.4	91.6±1.32$_a$
	2	128.7	95±1.63$_b$
	2	143	98.12±2.25$_{bc}$
	3	128.7	95.5±1$_d$
	3	143	96.25±2.5$_c$
Vapormate	2	856	100±0

a,b,c,d Means in a column followed by the same letter are not significantly different at the 5% level.
*mean of four repetitions.

2. effect of Vapormate on carob moth mortality

Vapormate semi-industrial trials were run with 856 g m^{-3}, corresponding to 143 g m^{-3} of pure EF. Vapormate trials show an increase on insect mortality from 98.12% with pure EF to 100% but statistically there is no significant difference between them. Even though, reports in the literature indicate that elevated CO_2 atmospheres have a positive impact on insect mortality when combined with various fumigants (Bond and Buckland, 1978; Simpson et al., 2004).

3. Quality analysis of EF treated dates

Statistical analysis shows that there is no significant (p>0.05) difference between untreated and EF fumigated dates for water content, water activity, glucose content, and color parameters (Table 2).

4. Microbiological analysis

Total mesophilic bacteria and mold and yeast count were evaluated over 3 weeks. EF fumigation of dates gave 95% mold and yeast reduction, while over 35% destruction of mesophilic bacteria was noticed. However, after 3 weeks, the rate of microbiological reduction decreases to 77% for the mold and yeast count while it still maintained to 35% for the mesophilic bacteria count.

Table 2. Effects of Ethyl Formate on dates quality.

Samples	EF concentration (g m^{-3})	Water content (%)	Water activity (%)	Glucose Content (g/100g dates)	Color		
					L	a	b
Untreated dates	0	33,70[a]	71,63[a]	13,47[a]	53,95[a]	7,29[a]	13,68[a]
Fumigated dates	143	33,13[a]	70,05[a]	13,60[a]	51,30[a]	7,69[a]	13,69[a]

a Means in a column followed by the same letter are not significantly different at the 5% level.

These findings could be related to the fact that EF residues disappear after 7 to 10 days (Bessi et al., 2011). Nursten, (1970) has shown that EF has insecticidal and fungicidal properties when it is applied on strawberries.

ACKNOWLEDGEMENTS

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FUMIGANT TOXICITY OF *EUCALYPTUS TRANSCONTINENTALIS* ESSENTIAL OIL AGAINST EGGS AND ADULTS OF THE CAROB MOTH *ECTOMYELOIS CERATONIAE*

Samah Khemira[1], Jouda Mediouni-Ben Jemâa[1][*] and Mohamed Larbi Khouja[2]

[1]Laboratoire de Biotechnologie Appliquée à l'Agriculture, Institut National de la Recherche Agronomique de Tunisie, Rue Hedi Karray 2049 Ariana Tunis, Université de Carthage.
[2]Laboratoire d'Ecologie et Amélioration Sylvo-Pastorale, Institut National de la Recherche en Génie Rural, Eaux et Forêts, Rue Hedi Karray 2049 Ariana Tunis, Université de Carthage.
*Corresponding author's e-mail:

ABSTRACT

The Carob moth, *Ectomyelois ceratoniae* is a polyphagous species which infests a large number of crop products. It is known as a pest of dates, figs, carob, almonds and citrus in Mediterranean countries. In Tunisia, it is the most important and destructive insect pest of dates causing important damages both in field and in storage. Pest control in storage is largely based on synthetic fumigants as methyl bromide and Phosphine. Nevertheless, due to their toxicity to human and their harmful effects on the environment, methyl bromide can no longer be used. Therefore, there is an urgent need to develop safer and efficient alternatives. Recently, research showed that essential oils and their constituents may have potential as alternative compounds to currently used fumigants. The present study aims to determine the fumigant toxicity of essential oil extracted from *Eucalyptus transcontinentalis* against eggs and adults of *E. ceratoniae*.

Results showed that at the concentration 142 μL L^{-1} air, 100% of adults mortality was obtained after 2 h of exposure. The LC$_{50}$ and LC$_{95}$ values were respectively 12.94 and 27.4 μL L^{-1} air. Hatching rate was 0% at the concentration 142.86 μL L^{-1} air against 98.33% for the control. These results indicated that both adults and eggs of *E. ceratoniae* were susceptible to vapours of *E. transcontinentalis* essential oil.

Results suggested that *E. transcontinentalis* essential oil could be used as an alternative to synthetic fumigant in postharvest treatment program against the carob moth.

Key words: Insecticidal activity, Carob moth, Eucalyptus, Essential oil, Fumigation, Eggs.

INTRODUCTION

The Carob moth, *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae), is a polyphagous species which infests a large number of crop products (Dhouibi, 1989). In Tunisia, it is the most important and destructive insect pest of dates causing important damage both in field and in storage (Jarraya, 2003).

Methyl bromide and phosphine are the most commonly products used for postharvest treatment of dates in Tunisia and worldwide (Zare et al., 2002). No doubt that these synthetic insecticides play an important role in reducing losses in dates due to the carob moth. However, these fumigants have serious drawbacks such as development of genetic resistance

in the treated pests, toxic residue problems and toxicity to consumers. Moreover, due to its harmful effects on the environment, methyl bromide can no longer be used. Methyl bromide is highly reactive to ozone and is classified as a potent stratospheric ozone depletor (Cox, 2004).

Recently, research showed that essential oils and their constituents may have potential as alternative compounds to currently used fumigants (Batish et al., 2008). Essential oils are volatile and can act like fumigants, offering the prospect for use in stored product protection (Papachristos and Stamooulos, 2002).

The objective of this study was to determine the fumigant toxicity of *Eucalyptus transcontinentalis* Maiden essential oil against eggs and adults of *E. ceratoniae*.

MATERIALS AND METHODS

Insect

E. ceratoniae adults and eggs were obtained from a rearing colony established at the Laboratory of Biotechnology Applied to Agriculture, National Agricultural Research Institute of Tunisia (INRAT). The moth was reared on an artificial diet based on wheat bran (Mediouni and Dhouibi, 2007).

Essential oil extraction

Leaves *of E. transcontinentalis* were collected in May 2010 from Sidi Ismail arboretum (northern Tunisia). Essential oil was extracted by water steam distillation using a Clevenger apparatus. The distilled oils were stored in the refrigerator at 4ºC.

2.3. Chemical analysis

GC/MS analyses were performed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 μm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 220 and 290°C, respectively. The column temperature was programmed from 80 to 220°C at a rate of 4°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively.

2.4. Fumigant bioassays

The fumigant toxicity bioassays against eggs and adults of *E. ceratoniae* were conducted as described by Papachristos and Stamooulos (2002) with some modifications.

For adult assays, plastic jars of 350 mL volume were used as exposure chambers. A small piece of woven dental cotton was attached to the undersurface of the cap to serve as an oil diffuser on which different doses of pure essential oil were applied. The tested doses were 14.29, 20, 28.57, 57.14 and 142.86 μL L^{-1} air. Twenty (20) new emerged unsexed adults were put in each plastic jar. Exposure times were 6, 12, 24, 36, 48, 96 and 120 h and each treatment was replicated three times. Corrected mortality was calculated using Abbott's formula (1925).

For egg hatching bioassay, the same methodology was used, but in this case, 20 fertile eggs (2 days old) were placed in each plastic jar. Untreated eggs were used as control. The same concentrations were tested. Each concentration was replicated three times. Hatched and non hatched eggs were counted. Exposure periods were 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 h. The hatching rate was calculated as follows:

$$\frac{\text{Number of hatched larvae} * 100}{\text{Total number of eggs}}$$

RESULTS

Chemical composition
A total of 99.06% from the constituents of *E. transcontinentalis* essential oil were identified. Mainly components were 1,8-cineole (82.82%) and α-pinene (7.96%).
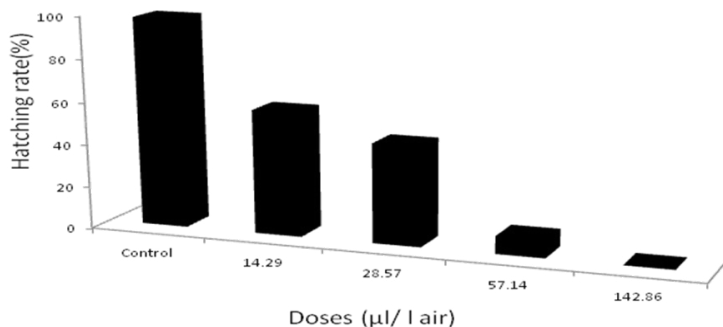
Toxicity on eggs
Fig. 1 illustrates the evolution of hatching rate of *E. ceratoniae* eggs at different doses of *E. transcontinentalis* essential oil after 10 days of exposure. At the concentration of 142.86 µL L^{-1} air, hatching rate was 0% against 98.33% for the control. Thus, *E. cereratoniae* eggs are very susceptible to *E. transcontinentalis* essential oil.

Fig. 1- Hatching rate of *E. ceratoniae* eggs exposed to different dosages of *E. transcontinentalis* essential oil after 10 days of exposure.

Effect on adult mortality
Results of the insecticidal activity of essential oil from *E. transcontinentalis* against carob moth adults were shown in Fig. 2.

At the highest concentration (142 µL L^{-1} air), 100% mortality was obtained after 2 h of exposure.

Probit analysis showed that the LC$_{50}$ and LC$_{95}$ were respectively 12.94 and 27.4 µL L^{-1} air after 24h of exposure.

CONCLUSION

Based on this study, we can conclude that the essential oil of *E. transcontinentalis* was rich in 1,8-cineole (82.82%). Insecticidal activity results suggested that this oil could be used for the development of new natural "bio-fumigant" for control of the carob moth in storage.
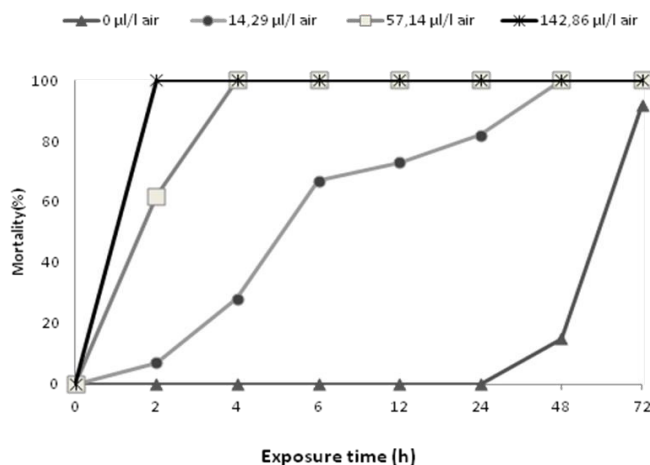
Fig. 2- Percentage mortality of *E. ceratoniae* adults exposed to various concentrations and periods to *E. transcontinentalis* essential oil.

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SiO$_2$ DUST AGAINST STORED PRODUCT INSECTS

Gennady Zakladnoy, Anton Fernas

GNU VNIIZ Rosselhosakademii, Russia
*Corresponding author's e-mail: *tonic_87@mail.ru*

ABSTRACT

Some experimental investigations were fulfilled to find out response of *Sitophilus granarius* and *S. oryzae* adults on the presence of SiO$_2$ dust in grain mass. The dosages of dust form 0,05 kg to 5 kg per metric ton were tested. Adult mortality time was observed after beetles contact with testing grain during 8-20 days. The mortality time depended on dosages as well as grain moisture content. Dust introducing in grain resulted to some reducing of grain moisture content. SiO$_2$ dust is able to be methyl bromide alternative.

Keys words: SiO$_2$, dust, grain, insects, mortality.

SESSION 4

Novel fumigants and application technologies

Chairpersons:
Karuppiah Alagusundaram, India
Gurler Tan, Turkey
Cem Hisarli, Turkey

NOVEL FUMIGANTS AND HEAT TREATMENTS FOR FLOUR MILLS

Paul G. Fields

Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd, R3T 2M9, Winnipeg, Manitoba, Canada
* Corresponding author's e-mail: *paul.fields@agr.gc.ca*

ABSTRACT

The efficacy of methyl bromide fumigations in mills (6 mills) was compared to heat treatments (3 mills), sulfuryl fluoride (ProFume®) (4 mills) and phosphine (ECO$_2$FUME®), heat and carbon dioxide combination treatment (2 mills). The efficacy of treatments was estimated in three ways: *Tribolium castaneum* adults and eggs in vials during treatment, pheromone traps and rebolt sifter tailings counts.

Almost all treatments were effective in killing 100% of adult *T. castaneum* put out in vials. There was more survival of eggs. In the sulfuryl fluoride treatments, egg mortality ranged from 35 to 99.6%. The other treatments had egg mortalities over 98%.

Insect populations in the mills were estimated using pheromone traps. In methyl bromide treatments, the range of times that it took insect populations to rebound to pre-treatment levels was between 3 weeks or over 30 weeks. For sulfuryl fluoride the rebound took from as little as 1 week to never rebounding within the 18-week study. Phosphine combination treatment saw populations rebound within seven to 29 weeks. In all three heat treatments, none of the populations returned to the original levels by 19 weeks post-treatment.

Adult and larva flour beetles were monitoring in the tailings from rebolt sifters. For the most of the mills, there is a good correlation between insects found in the pheromone traps and insects found in the rebolt sifter tailings. However, on several occasions pheromone traps were not a good predictor of insect numbers in the rebolt sifter tailings. In methyl bromide treatments, the rebound of insect populations to pre-treatment levels occurred between 13 and over 31 weeks. For sulfuryl fluoride, the rebound occurred between 9 and over 18 weeks. The phosphine combination treatment saw populations rebound within 1 to 33 weeks. In all three heat treatments, the mills either did not sample rebolt sifter tailings or no insects were found in the tailings.

Key words: Sulfuryl fluoride, phosphine, carbon dioxide, heat, red flour beetle, *Tribolium castaneum*

INTRODUCTION

Methyl bromide (MB) is a very effective broad spectrum fumigant. It is used around the world to control a wide variety of pests (pathogens, nematodes, weeds and insects) in diverse substrates (soil, food, museum artefacts, buildings, equipment, aircraft). It has been used in flour mills since the 1930's, and it has become the major tool to control insects in food processing facilities, such as flour mills, pasta production plants and breakfast cereal plants.

In 1992, methyl bromide was recognized as a significant ozone depletor and its

consumption was frozen at 1991 levels starting in 1995 (Banks, 2002; Fields and White, 2002). In 1997 Parties of the Montreal Protocol on Substances that Deplete the Ozone Layer agreed to phase out methyl bromide starting in 2005 with interim reductions along the way. These dates are ten years later for developing countries. Given that methyl bromide is such a widely used fumigant, in 1997 Parties also agreed to allow critical and emergency use exemptions for very specific uses of methyl bromide. Users must demonstrate that there are no technically and economic alternatives to methyl bromide, and they are actively trying to find alternatives to methyl bromide. This Critical Use Exemption (CUE) has been granted for flour mills in Canada, USA, Europe and Australia. This project examined the efficacy of IPM (Integrated Pest Management), heat, sulfuryl fluoride, phosphine combined with heat and carbon dioxide, and compares them with methyl bromide fumigations.

Heat treatments have been used as early as the 16th century to control stored-product insects (Fields, 1992). Heating flour mills and food processing facilities to control insects in the USA and Canada has been used since 1910 and continues to this day (Fields and White, 2002). Steam, propane and electric energy sources have been used to power the heaters. In general, the building is heated to at least $50^{o}C$ for 24 hours. Extra fans are used to distribute the heat within the structure. This project worked with two heat providers, Temp-Air and Armstrong-Hunt.Temp-Air uses the same heaters that are designed for temporary heating of construction sites and sporting events and modified for heat treatments. They have been doing heat treatments to control insect pests since 1999 (Johnson and Danley, 2003). Currently they heat treat approximately twenty-five locations a year, with most of these locations receiving two heat treatments a year. The size of the facilities range from 17,000 to 1,300,000 m^3 and include flour mills, food processing facilities and malting plants. Heaters use either propane, as is the case with these trials, or natural gas. Armstrong-Hunt has been manufacturing heavy-duty steam heaters for insect pest management since 1990. Currently they have provided portable equipment and permanently installations in over twenty-five locations. The plant sizes range from 300 to 113,000 m^3, including flour and corn mills, pasta plants, food processing plants and pharmaceutical plants.

Sulfuryl fluoride (SF or SO_2F_2) has been proposed as a replacement for methyl bromide in the fumigation of flour mills and other structures (Bell et al., 1996; Banks, 2002,). Sulfuryl fluoride was originally registered for termite control in 1961, under the trade name Vikane®. Since 1995, Dow AgroSciences has been expanding the use pattern of sulfuryl fluoride for use in flour mills, under the trade name ProFume® (Schneider and Hartsell, 1999). Currently it is registered in USA, France, Switzerland, Germany, Italy, Belgium, United Kingdom, Mexico and Australia.

Phosphine has been used extensively as a fumigant in bulk grain. However, it is rarely used in empty flour mills, because it can cause corrosion of copper and other metals and requires more time than methyl bromide to control insects. Using phosphine in combination with carbon dioxide and heat mitigate these problems (Mueller, 1993). This phosphine combination treatment has been used successful in the USA to control insects in food processing facilities in 24 h without corrosion. It requires extra sealing. For example, some electrical boxes are sealed, a pressurized hose attached and a small amount of air bled into the line to prevent phosphine from entering into equipment where corrosion is a concern. ECO$_2$FUME®, manufactured by Cytec Canada Inc. is an effective way to deliver the phosphine for this combination treatment for two reasons, good control of the phosphine concentration and rapid release of gas. ECO$_2$FUME is 2% phosphine in 98% carbon dioxide held under pressure in gas cylinders.

MATERIALS AND METHODS

Treatments

There were two trials with propane-fired heaters (Temp-Air) and one trial with portable steam heaters (Armstrong International Inc.), four trials with sulfuryl fluoride (ProFume®), two trials with phosphine (ECO$_2$FUME®), heat and carbon dioxide combination treatment and six trials with methyl bromide (Table 1). For full details of the treatments see Harrison (2007).

Dome traps

Dome traps (Trece Inc.) that are specific for trapping flour mill insects were placed on the roll stand floor and the sifter floor, five to ten traps/floor and the insects removed and counted each week. The traps were in the mill 3-9 weeks before the control treatments and for at least 18 weeks post-treatment. The traps were baited with a pheromone for the confused and red flour beetles (*Tribolium confusum* Jacquelin du Val and *Tribolium castaneum* (Herbst)) and a vegetable oil attractant. The vast majority of insects caught in the traps were flour beetles, and those data are reported here. There were two measures of efficacy. The first was time taken to return to 100% pre-treatment level. The pre-treatment populations were estimated by the number of insects/trap/day (mean of weekly captures before treatment). The second was time taken until the insects were detected three consecutive weeks in both roll stand and sifter floors or in tailings. Mill 4 and Mill 6 were followed for more than one treatment, the initial pre-treatment population estimates were used for all treatments. There was no estimate of insect populations in the immediate area outside the mills, as was done in other studies (Campbell et al., 2002).

Rebolt sifter tailings

In Mills 1 and 3, no insects were seen in the rebolt sifter tailings. In Mill 4, 1 kg samples were taken 5 d/week and the number of live and dead adults was counted. In Mill 5, all insects were counted for rebolt sifter tailings three times each 24 h. In Mill 6, 2-kg samples were taken from the rebolt sifter tailings barrel in the mill and in the packing plant. An estimate of the total flour in the tailings barrel is made to calculate the total number of insects in the barrel. Both of these rebolt sifters pull flour from the same bins. In Mill 8, tailings samples were taken immediately after every load and inspected. Samples for the entire week were summed. Insect counts were divided by the number of days of sampling and the daily average calculated for the pre-treatment period, and all numbers divided by this to express the insect count data as a percentage of pre-treatment levels.

Bioassays

Tribolium castaneum (Steinbach strain), was used as a test insect. They were reared on white wheat flour with 5% brewer's yeast at 30°C, 60% r.h. Twenty unaged adults of unknown sex were placed in 16 g of culture medium in plastic vials, 4-8 d before the treatment, and held at 20-30°C, 60% r.h. So at the time of the treatment there were 20 adults per vial and an unknown number of immatures, most would be eggs. Eight vials were used as controls. They were treated as the insects exposed to the treatment, but they were not held in the mill during the treatment. Twenty-five vials were placed thorough-out the mill a few hours before the treatment and retrieved a few hours after the end of the treatment. About half of the vials were placed in the middle of the mill and half of them near windows or doors. In some of the treatments, a group of vials were placed at one location, and pulled at regular intervals during the treatment. Dataloggers (Hobo dataloggers, Onset Computers Inc.) were placed with each vial, and the temperature recorded every 15 minutes.

Table 1. Summary of treatments to control insects in Canadian flour mills, heat treatments with external propane fired heaters, heat treatments with steam-powered portable heaters, sulfuryl fluoride (SF), phosphine combined with carbon dioxide and heat, methyl bromide (MB).

Treatment	Mill	Plant	Start Date (month/year)	Mill Preparation Time (h)	Duration of Treatment (h)	Duration of Post-treatment (h)	Plant Shut Down Time (h)
Heat, propane	1	mill	08/06	25.5	29.3	2.25	64
Heat, steam	3	mill	06/06	5	24	2	33.5
Heat, propane	7	mix plant	09/06	19	28.3	12.3	79.5
SF	4A	old mill	12/04	36	27	6	96
SF	4B	new mill	12/04	34	29	6	96
SF	4A	old mill	10/05	15	29.5	28.5	96
SF	4B	new mill	10/05	13.5	13.5	46	96
SF	5	mill	08/06	24.5	22	12.5	67
SF	8	mill	06/06	30.8	24.3	6	104
PH$_3$ combo	6B	packing	11/05	6.5	26.5	12	53.75
PH$_3$ combo	6A	mill	07/06	51	26.5	4	92
MB	4A	old mill	11/03	33	33.5	9.5	94.5
MB	4B	new mill	11/03	33	33.5	9.5	94.5
MB	4A	old mill	06/04	10	32	7	72
MB	4B	new mill	06/04	10	32	7	72
MB	4A	old mill	05/05	10	32	7	72
MB	4B	new mill	05/05	10	32	7	72
MB	6A	mill	11/05	40.8	28.3	12	101
MB	6B	packing	07/06	23.5	25	5	60
MB	6A	mill	11/06	37	24	3.5	74
MB	6B	packing	11/06	21	24	3.5	56
MB	7	mill	06/06	6	25	11	46

A summary of the conditions for the treatments is given in Table 1, and a summary of the efficacy is given in Table 2.

Insect bioassays

There were three treatments with heat, two (Mills 1 and 7) that used external propane-fired heaters (Temp-Air), and one (Mill 3) that use internal portable-steam-powered heaters (Armstrong International Inc.). For Mill 1 (propane heaters), the average time above 50°C was 24.4 h and average maximum temperature was 65°C. For Mill 7 (propane heaters), the average time above 50°C was 26.4 h and average maximum temperature was 57.8^{o}C. For Mill 3 (steam heaters) the average time above 50°C was 24.9 h and average maximum temperature was 56.5^{o}C.

All insects, both adults and eggs, were dead in the two propane-fired heat treatments in Mill 1 and 7. In Mill 1, in the timed sequence, all insects were dead by 10 h after the heating began and the final temperatures had reached 57°C. In Mill 7, in the timed sequence, all insects were dead by 5.5 h after the heating began and the temperature had reached 53°C.

In Mill 3, the steam heat treatment, most bioassay insects were all dead except at four of the twenty-five locations. In the timed sequence, all insects were dead by 21 h after the heating began and the temperatures had reached 53-57°C.

The only damage caused by the heat treatments was in Mill 1, one sprinkler head was activated because during the start-up one of the fabric ducting was not properly anchored and hot air was directed at sprinkler head. The duct was rerouted and anchored to overcome the problem. Mill 3 and 7 exhibited no damage to the building, processing equipment or electronics.

The Canadian sulfuryl fluoride label did not allow for the treatment of food products. So the mills were emptied of all food products such as; wheat, feed, milled products and additives. In Mills 5 and 8, silos within the mill containing finished flour were sealed and not exposed to sulfuryl fluoride. Sulfuryl fluoride has a range of times and doses that is adjusted in relation to the life stage and species targeted. *Tribolium castaneum*, the insect used in the bioassay and a common pest of flour mills in Canada, has a relatively high tolerance to sulfuryl fluoride. Eggs are the most tolerant life stage. In general, there are two doses, a high dose that will kill all life stages, and a lower dose that will kill all post-embryonic life stages and some but not all of the eggs. The sulfuryl fluoride doses used in these trials, range from the dose to kill all post-embryonic life stages (target ct Product (CTP) = 428 gh/m^3; Mill 4, December 2004), to the dose to kill all life stages (target CTP = 645-965 gh/m^3; Mill 4, October 2005; Mill 5, August 2006; Mill 8, June 2006, target CTP was adjusted for temperature).

For all sulfuryl fluoride fumigations, there was 100% mortality of the bioassay adults. In the first fumigation with SF in Mill 4 in December 2004, the average mortality of bioassay immatures (mostly eggs) was 35% in Mill 4A and 63% in Mill 4B, with a CTP of 457-490 gh/m^3. This was expected, as the dose chosen was the low dose targeting post-embryonic stages. The other three later fumigations used the high dose with CTs from 832 to 1280 gh/m^3 and gave average control of bioassay immatures from 94.5 to 99.9%.

The average temperature in Mill 4 during the December 2004 fumigation was 19°C in Mill 4A and 23°C in Mill 4B, which is cooler than fumigations done with sulfuryl fluoride in the USA. As with most fumigants, higher temperature improves efficacy. There was a strong correlation between temperature and mortality (Fig. 1).
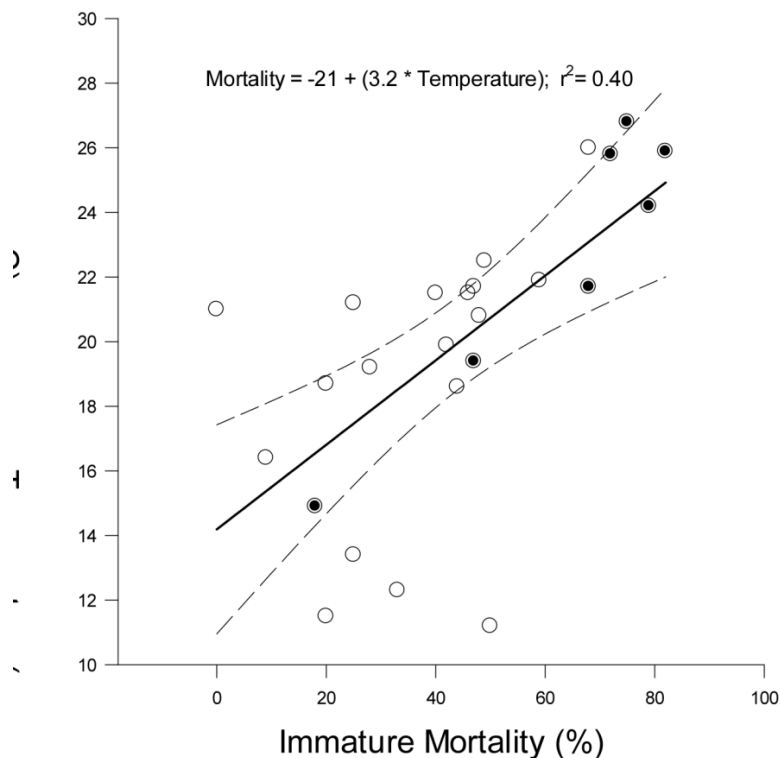
Fig. 1- The mortality of *T. castaneum* immatures (mostly eggs) during sulfuryl fluoride fumigations with CTP of 457-490 gh/m^3 in Mill 4A (○) and Mill 4B (●) on December 2004.

During the second sulfuryl fumigation an effort was made to raise the mill temperature by turning up the heat for the comfort heaters and using propane-fired heaters before the fumigation began. This increased the average temperature to 26°C. The other mills that used sulfuryl fluoride did the fumigations during the summer, did not use heaters, and had average temperatures of 24.3^{o}C (Mill 5) and 29.5^{o}C (Mill 8).

Some of the insect bioassays were removed from the fumigation after different durations. In Mill 5 for *T. castaneum*, all adults were dead after 2 h (118 gh/m^3), all immatures were dead after 16 h (1150 gh/m^3). In Mill 8 for *T. castaneum*, all adults were dead after 4 h (259 gh/m^3), all immatures were dead after 12 h (803 gh/m^3). In Mill 8 for *T. confusum*, all adults were dead after 4 h (259 gh/m^3), all immatures were dead after 8 h (529 gh/m^3).

The treatment with phosphine, heat and carbon dioxide was unique in the trials in that a methyl bromide treatment was done on the same date as the alternative treatment, but in a different part of the facility. The first fumigation was done in November 2005, the packing plant was fumigated with phosphine and the mill fumigated with methyl bromide. For the second fumigation, the packing plant was fumigated with methyl bromide and the mill with phosphine, heat and carbon dioxide.

All adults in the bioassays were killed with the phosphine combination treatment on both of the dates. The average immature mortality in the phosphine combination treatment was 98.6% for the 2005 fumigation and 99.5% for the 2006 fumigation.

The phosphine did cause corrosion on the copper strips placed in the packing plant. The copper plates were blackened (Harrison, 2007), whereas the controls and the methyl bromide fumigated strips did not. Some equipment that was sealed was exposed to phosphine gas. Each piece of equipment that was sealed had a line for delivering compressed air and a second line for gas sampling. In the 2005 fumigation, nine sealed boxes had phosphine from 25 to 115 ppm, due to a malfunction in the delivery of the compressed air. However, none of these pieces of equipment had problems after the fumigation. After the 2005 fumigation, one light at the top floor of the mix plant needed a ballast replaced. This may have been unrelated to the fumigation. After the 2006 fumigation there was no failure of the equipment at start-up.

There were several methyl bromide fumigations that were part of this project; in Mills 4, 6 and 7. All adults in the bioassay were killed in all the fumigations. For the immatures in the bioassay, occasionally there was a small amount of survival.

Rebound of insect populations

It is difficult to estimate the populations of insects in flour mills. Flour beetles are small, cryptic and the populations are not uniformly distributed throughout the mill. Also, insect populations change rapidly, at 30°C, 70% r.h., *T. castaneum* populations increase 70-fold per month.

For the three mills that used heat treatments, none of the insects returned to the pre-treatment levels with the 20 weeks of pheromone sampling. Mills 1 and 3 had very low levels throughout the post-treatment sampling. Mill 7, which used propane-fired heaters, consistently had insects in the pheromone traps (insects found on both floors for three consecutive weeks) 2 weeks after heat treatment. Mills 1 and 3 never have had significant numbers of insects in rebolt sifter tailings before or after treatments. Mill 7 does not regularly check for insects in rebolt sifter tailings.

For the three mills that used sulfuryl fluoride there was a wide variation in rebound of insects after fumigation. The low concentration (457 gh/m^3), combined with the cool temperatures during the fumigation (18.9^{o}C) were probably contributing factors to the rebound in Mill 4A. Insects were regularly found in pheromone traps after 16 weeks, the rebound to pre-treatment levels (over 100%) occurred after 13 to 18 weeks. Insects were consistently found in the tailings after 21 weeks, and at very high levels (Table 2).

A second fumigation with sulfuryl fluoride at the high concentration (1096 gh/m^3) and at higher temperatures (26.5^{o}C), still saw insects regularly being caught in pheromone traps 11 weeks after the fumigation, although the time to rebound to pre-treatment levels occurred after 17 to 25 weeks. The major difference with the second fumigation in Mill 4A was that the insects in the rebolt sifter tailings remained low until 26 weeks after the fumigation.

Mill 8 used similar concentrations (836 gh/m^3) as the 2005 fumigation in Mill 4A. However, populations quickly rebounded. Insects were regularly found in pheromone traps 5 weeks after fumigation, populations returned to pre-treatment levels after 3 to 8 weeks. Insects were regularly found in the rebolt sifter tailings 3 weeks after fumigation and returned to pre-treatment levels after 12 weeks. The insects in the roll stand floor were not consistently found before the fumigation, so the sifter floor and basement probably give a better indication of the population trends before and after the fumigation. Some flour remained in the mill silos. It was sealed off from the rest of the mill and was not exposed to sulfuryl fluoride.

Table 2. Summary of efficacy of treatments to control insects in Canadian flour mills, heat treatments with external propane-fired heaters, heat treatments with steam-powered portable heaters, sulfuryl fluoride (SF), phosphine combined with carbon dioxide and heat, methyl bromide (MB).

Method	Mill	Start Date (month/year)	Temp. Inside (°C)	Temp. Outside (°C)	Gas CT (gh/m³)	Gas Half Loss Time (h)	Bioassay Adult Mortality (%)	Bioassay Immature Mortality (%)	Trap catches Rebound Time (wks)			Tailings Rebound Time (wks)	
									Regularly Found[1]	To 100% Levels Roll Stand[2]	To 100% Levels Sifter[2]	Regularly Found[1]	To 100% Levels[2]
Heat, propane	1	08/06	56.5	27.5	-	-	100	100	20+	20+	20+	-	-
Heat, steam	3	06/06	49.7	23.7	-	-	94	99.6	20+	20+	20+	-	-
Heat, propane	7	09/06	51.1	18.8	-	-	100	100	2	19+	19+	-	-
SF	4A	12/04	19.8	2.6	457	6.3	100	35	16	13	18	21	9
SF	4B	12/04	24.2	2.9	490	2.9	100	63	-	-	-	-	-
SF	4A	10/05	27.6	7.0	1096	5.8	100	96	11	17	25	26	26
SF	4B	10/05	27.0	4.6	1180	5.3	100	92	-	-	-	-	-
SF	5	08/06	29.5	20.8	1280	6.0	100	99.7	18+	18+	1	23+	18+
SF	8	06/06	31.7	24.3	832	4.7	100	99.6	5	3	8	3	12
PH₃ combo	6B	11/05	33.2	3.7	-	-	100	98.6	29	29	29	23	33
PH₃ combo	6A	07/06	33.4	25.1	-	-	100	99	10	17+	7	5	5
MB	4A	11/03	16.4	-5.1	365	5.7	100	-	30+	30+	30+	31+	31+
MB	4B	11/03	24.2	-5.1	108	1.2	100	-	30+	30+	30+	-	-
MB	4A	06/04	-	-	365	6.3	-	-	25+	25+	25+	14	16
MB	4B	06/04	-	-	279	12.0	-	-	25+	25+	25+	-	-
MB	4A	05/05	24.3	13.4	443	4.9	100	99.8	17	22	22+	14	15
MB	4B	05/05	28.9	13.4	273	5.3	100	100	-	-	-	-	-
MB	6A	11/05	27.3	3.6	-	-	100	100	25	19	29	22	25
MB	6B	07/06	40.0	25.1	210	4.3	100	100	9	2	3	4	18+
MB	6A	11/06	27.6	5.1	-	-	100	100	-	-	-	-	-
MB	6B	11/06	29.9	5.1	-	-	100	100	-	-	-	-	-
MB	7	06/06	24.7	13.2	242	3.5	100	100	25	33+	33+	-	-

[1] 3 consecutive weeks of insects detected in both roll stand and sifter floors or in tailings
[2] Weeks for populations to return to 100 % pre-treatment levels.
- Initial numbers too low to estimate rebound, or samples not taken
+ Insects were not found 3 consecutive weeks in a row, or had not reached 100% of pre-treatment during study

One reason that the populations rebounded so quickly in Mill 8 after the fumigation could be that these unfumigated silos served as a source of insects that quickly reinfested the mill.

Mill 5 used the highest concentration tested in these trials (1280 gh/m^3). Although insects were found in pheromone traps regularly on the sifter floor for 4 weeks right after the fumigation, these numbers quickly dropped and remained low the remaining 14 weeks post fumigation sampling. One suggestion is that the insects found in the traps were on the window sills that were covered with polyethylene and outside the fumigated area, but still inside the mill. Insects on the roll stand floor did not rebound within the 18 weeks of sampling after the fumigation. There were some insects in the rebolt sifter tailings right after the fumigation. This mill did not divide live and dead insects, and these insects were probably dead insects that were being flushed from the system after the fumigation.

In the December 2004 fumigation in the packing plant with the phosphine combination treatment, the insects were consistently found in the traps after 29 weeks, the same length time it took the insects to the pre-treatment levels. Insects in the rebolt sifter tailings were regularly found after 23 weeks, and reached pre-treatment levels after 33 weeks.

In the July 2006 phosphine fumigation in the mill, the insects were regularly found in the traps after 10 weeks and returned to pre-treatment levels in the sifter floor after 7 weeks and never returned to original levels in the roll stand floor. Insects in the rebolt sifter tailings were regularly found after 5 weeks, and reached pre-treatment levels at 5 weeks after treatment. Rebolt sifter tailings should be interpreted with caution because although one rebolt sifter is located in the mill and the other in the packing plant both pull flour from the same bins.

The efficacy of methyl bromide fumigations were assessed in Mills 4, 6 and 7. In Mill 4A the two methyl bromide fumigations were done at the upper level of the label rate Mill 4A (November 2003: 38-50 g/m^3, 377 gh/m^3; May 2005: 24-67 g/m^3, 447 gh/m^3) The label rate for methyl bromide is 16 to 48 g/m^3. For the fumigation in November 2003 with methyl bromide, insect populations remained low both in the traps and in the rebolt sifter tailings for over 31 weeks. For the fumigation in June 2004, the insects were not consistently found in the traps in the 22-week sampling after the fumigation. Insect levels in the tailings rebounded after 16 weeks. For the fumigation in May 2005, the insects were consistently found in the traps after 17 weeks, and had returned to the pre-treatment levels after 22 weeks on the roll stand, but did not on the sifter floor. Insect levels in the tailings rebounded after 15 weeks. The new mill, Mill 4B, after the first fumigation with methyl bromide in November 2003, insects caught in traps remained at low levels for the rest of the study, so it is impossible to determine the efficacy of the subsequent fumigations.

The methyl bromide fumigations in Mill 6 were conducted at the same time as the phosphine combination fumigations. In the December 2005 fumigation in the mill plant with methyl bromide the insects were consistently found in the traps after 25 weeks (vs. 29 weeks with phosphine), the insects returned to pre-treatment levels in 29 weeks (vs. 29 weeks with phosphine). Insects were consistently found in rebolt sifter tailings after 22 weeks (vs. 23 weeks with phosphine), the insects returned to pre-treatment levels in 25 weeks (vs. 33 weeks with phosphine). In the July 2006 fumigation in the packing plant, the insects were regularly found in the traps after 9 weeks (vs. 10 weeks with phosphine) and returned to pre-treatment levels after 2-3 weeks (vs. 7 to over 17 weeks with phosphine). Insects were consistently found in rebolt sifter tailings after 4 weeks (vs. 5 weeks with phosphine), the insects never returned to pre-treatment levels in 18 weeks of sampling (vs. 5 weeks with phosphine).

Although the two buildings, mill and packing plant, are well isolated from a fumigation perspective, they are not from a product flow perspective. Flour and any insects in the flour

would flow from the mill to the packing plant, but little product flows from the packing plant to mill. The insect Dome trap that caught over 50% of the insects trapped in the packing plant were from one trap on the first floor, close to the barrels that hold the tailings from the rebolt sifter. Hence, a large insect population in the mill could increase the insects caught in traps in the first floor packing plant. Also for the rebolt sifter tailings, the two sifters pull flour from the same bins.

Finally, the fumigation with methyl bromide in Mill 7 saw insects consistently in the traps after 25 weeks, but populations had not reached pre-treatment levels when the study ended after 33 weeks.

DISCUSSION

For several reasons it is difficult to make direct comparisons between treatments because they were carried out at different mills or the same mill treated at different times. Mills differ in age of the building, age of equipment, cleaning practices, location, which effects outside temperatures and humidity, grain milled and product produced. All these factors affect the pest pressure that a facility faces. A good example is Mill 4A and Mill 4B. Both mills were fumigated at the same time. Mill 4A has older equipment in an old building with wooden floors and insect populations are difficult to control. Mill 4B has new equipment in a new building and after the initial fumigation in December 2003, insects populations remained very low throughout 2.5 years of the study.

There is limited replication with regard to treatments. There were three heat treatments, done in two different ways. There were four sulfuryl fluoride treatments, one at the low dose and three at the high dose. There were two phosphine combination treatments. There were seven methyl bromide fumigations. Even treatments done in the same mill are done at different times, so pest pressures may be different from one treatment to another. For example, the pest pressure will be much greater in the summer than the winter. One would expect that all things being equal, the rebound of insect populations from a treatment in the fall would be slower than a treatment done in the spring. There were only four fumigations with sulfuryl fluoride, each with a different concentration or in a different mill. Sulfuryl fluoride does not yet have food tolerances in Canada, so some of the bins contained flour, but were isolated from the gas to prevent food contact. This may have been the reason that insects populations rebounded quickly in one mill that used sulfuryl fluoride. In the USA and other countries, food products can be treated with sulfuryl fluoride and do not need to be removed from the facilities. Whereas, methyl bromide can be used to treat flour remaining in the mill. The phosphine, heat and carbon dioxide had only two treatments, and there are problems using the twinned methyl bromide for comparisons.

Despite the limitations, this project has provided Canadian flour millers and pest control operators with many opportunities to test several alternatives to methyl bromide in their facilities. Sulfuryl fluoride, heat and phosphine combination treatment (phosphine, heat and carbon dioxide) can control insect populations in flour mills for over 18 weeks.

For any of the fumigants tested, methyl bromide, sulfuryl fluoride or phosphine, improved sealing in Canadian flour mills before fumigation, would allow for effective fumigations with less gas. Many flour mills in the USA are much better sealed than the Canadian mills we tested. The American mills have Half Loss Times twice that found in mills in this study. Higher temperatures would also improve the efficacy of any of the fumigants. For example, the CTP for sulfuryl fluoride needed to control *T. castaneum* eggs is 1768 gh/m^3

Table 3. Stage specific toxicity for various fumigants.

Fumigant	Temp. (°C)	Time (h)	Insect	CTP to kill 95% of the population (gh/m³)[1]				Reference
				Egg	Larvae	Pupae	Adult	
sulfuryl fluoride	27	16	*T. confusum*	1125	-	-	55	Kenga, 1957
	27	16	*Sitophilus granarius* (L.)	794	14	14	15	Kenga, 1957
	25	24	*T. confusum*	498				Bell et al., 1999
	25	24	*T. castaneum*	1368				Bell et al., 1999
	25	24	*T. castaneum*	1768				Bell et al., 1999
	30	24	*T. castaneum*	1154				Bell et al., 1999
methyl bromide	25	-	*T. confusum*[2]	-	-	90	60	Heseltine & Thompson, 1974
	15	-	*T. confusum*[2]	96	255	255	-	Bell et al., 1988[3]
	25	-	*T. confusum*[2]	65	98	251	-	Bell et al., 1988[3]
	27	2	rice weevil	37	10	44	27	Krohne & Lindgren, 1958
phosphine	25	48	*Plodia interpunctella* (Hübner)[2]	77	1	1	-	Bell, 1976
	27	16	*T. confusum*	2	0.1	0.3	0.3	Lindgren & Vincent 1966

[1] g/m³ = mg/L= ounces/1000 ft³
[2] complete kill
[3] methyl bromide at 4.9 g/L

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at 25^oC, but only 1154 gh/m^3 at 30^oC (Table 3, Bell and Savvidou, 1999). Comfort heaters could be used during the summer to increase mill temperatures, or additional heaters could be used for fall fumigations.

ACKNOWLEDGMENTS

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PROPYLENE OXIDE AS POTENTIAL QUARANTINE FUMIGANT FOR INSECT DISINFESTATION OF DRIED FIGS

Ali A. Isikber[1*], Serdar Oztekin[2], K. Sinan Dayisoylu[3], Ahmet Dogan Duman[4]

[1]Plant Protection Department, Agriculture Faculty, Kahramanmaras Sutcu Imam University, 46100 Kahramanmaras-Turkey,
[2]Faculty of Agriculture, Agricultural Machinery Department, Çukurova University, 01330 Adana-Turkey
[3]Agriculture Faculty, Food Engineering Department, Kahramanmaras Sutcu Imam University, 46100 Kahramanmaras-Turkey
[4]Agriculture Faculty, Food Engineering Department, of Mustafa Kemal University, Hatay-Turkey
*Corresponding author's e-mail: *isikber@ksu.edu.tr*

ABSTRACT

In this study, propylene oxide (PPO) at low pressure (100 mm Hg) was tested for rapid disinfestation of dried figs as replacement for methyl bromide by evaluating its toxicity to Indian meal moth, *Plodia interpunctella* (Hübner) and the fig moth, *Ephestia cautella* (Walker), in absence and presence of dried figs, its sorption and residue on dried figs. The complete mortality of all life stages of *P. interpunctella* and *E. cautella* was achieved at a Ct product of 45.5 and 53.2 mg h l^{-1} for empty space fumigation (without commodity) respectively. It required a dosage of 11.4 and 13.3 mg l^{-1} for empty space fumigation and 32.4 for and 30.2 mg l^{-1} for fumigation in presence of dried figs to kill 99% of the larvae of *P. interpunctella* and *E. cautella* respectively. Thus, 2.85-fold and 2.27-fold higher dose of PPO required for PPO fumigation in presence of dried figs to obtain the complete mortality of the larvae of *P. interpunctella* and *E. cautella*, respectively. Sorption of PPO by dried figs after 5 h exposure time was relatively high by an average of 58% reduction of initial concentration. The PPO residue in dried figs was a maximum average of 85 ppm at 0-1 day after termination of aeration, which all was much lower than the 300 ppm maximum tolerance. Based on these data, the combination of PPO with low pressure can be a potential as fumigant for replacing methyl bromide for quarantine purposes in dried figs.

Key words: Propylene oxide, dried fig, quarantine fumigation, toxicity, sorption, *Ephestia cautella*, *Plodia interpunctella*

INTRODUCTION

Turkey is one of the most important dried figs producing and exporting country. The annual Turkish dried fig production is around 55 to 60 000 tones and comes from a single cultivar, (*Ficus carica* Sarilop (Calimyrna)), grown in the western Aegean Region. Nearly 90% of the production goes to the export market, the main period of marketing being between end of September and December (Aksoy et al., 2008). The fig moth (*Ephestia cautella* (Walker),

Lepidoptera: Pyralidae), the Indian meal moth (*Plodia interpunctella* Hübner, Lepidoptera: Pyralidae), the dried-fruit beetle (*Carpophilus* spp., Coleoptera: Nitidulidae) and the fig mite (*Carpoglyphus lactis* (L.), Acari: Carpoglyphidae) are main storage pests that play important roles in the quality and consequent trade volume of dried fig (Turanlı, 2003). Storage pests infesting fruit especially during over-ripening, drying and storage period may create cause significant problems in dried fig sector. The fig moth reduces fruit quality by feeding and damaging the fruit and contaminating by leaving its excretions and other residues as silky net weaves (Damarlı et al., 1997). Large populations can develop before being discovered and severe damage may occur (Jarratt, 2001).

Methyl bromide (MeBr) has been used as a gas fumigant for many years to fumigate the commodities that have become infested with stored-product insect pests, since it kills the insects rapidly, has a wide spectrum of activity and relatively low-cost (Fields and White, 2002). However, it has been banned in developed countries since 2005 and scheduled for worldwide withdrawal from routine use as a fumigant in 2015 under the directive of the Montreal Protocol on Substances that Deplete Ozone Layer (Schneider et al., 2003) except quarantine, laboratory and pre-shipment purposes. Various alternatives as integrated pest management, some chemicals (phosphine, carbonyl sulfide, sulfuryl fluoride, ozone, cyfluthrin) and non-chemical treatments (modified atmospheres, high pressure, heat/cold treatments, sanitation, radio frequency, long-wave energy, and irradiation) were tested (Zettler et al., 1999; Johnson et al., 2000; Fields and White, 2002; Schneider et al., 2003). Although there are a large number of suggested potential chemical and non-chemical alternatives to MeBr, each has limitations in terms of efficiency, cost, penetration or residues that prevent it from being a direct replacement for MeBr in all its current uses.

The dried-fig industry in Turkey has continued to rely on phosphine for post-harvest insect infestation. Phosphine is also under attack because of pest resistance (Bell and Wilson, 1995; Zettler and Cuperus, 1990) and its requirement of long exposure period (5 d or longer), which makes the chemical unsuitable for quarantine fumigations.

Propylene oxide (PPO) is a liquid fumigant under normal temperature pressure (NTP) with a boiling point of 35ºC and a noticeable ether odor. As a fumigant, PPO has reduced environmental risks compared with MeBr. It is not an ozone depleter and degrades into nontoxic propylene glycol in the soil and in the human stomach. PPO is commonly used as a sterilant to reduce bacteria, mould and yeast contamination on processed spices, cocoa and processed nutmeats except peanuts. A disadvantage of PPO is that it is flammable from 3% to 37% in air and therefore, to avoid flammability it should be applied under low pressure or in a CO_2-enriched atmosphere. Several studies reported by Creasy and Hartsell (1999), Isikber et al. (2006) and Navarro et al. (2004) have shown that PPO has insecticidal properties under vacuum conditions as a fumigant by killing all stages of various stored-product insects within a short exposure time. These reports on insect toxicity indicated that PPO would be an effective replacement for methyl bromide in some postharvest situations (Creasy and Hartsell, 1999; Isikber et al., 2006; Navarro et al., 2004).

The loss of methyl bromide could have a significant negative impact on the dried fig industry, particularly because of non-availability of alternatives to methyl bromide currently exist for rapid disinfestation of dried figs. Thus, there is a critical need to develop new fumigants for quarantine purposes. PPO is considered here for rapid disinfestation of the dried fig as a replacement for methyl bromide by evaluating its toxicity against major insect pests of dried fig, and its sorption and residue on the dried fig.

MATERIALS AND METHODS

Test insects
Toxicity tests were carried out on all life stages of the most common insect pest, *Plodia interpunctella* (Hübner) (Indianmeal moth) and *Ephestia cautella* (Walker) (Fig moth). All stages were obtained from laboratory cultures reared at 26±1 °C and 70±5 % r.h. using standard culture techniques (Donahaye, 1990). Eggs for exposure to treatments were transferred into "pits" drilled into Perspex exposure slides, each slide containing 50 pits. When filled, the slides were covered with a cover glass to retain the eggs. Two slides containing 100 eggs aged 1-2 days were exposed to each treatment. Two days old pupae, 17-19 d old larvae and newly emerged adults were removed from culture jars and exposed to the treatment.

Commodities
Sun-dried fruit of Sarılop (Calimyrna) fig variety with a m.c. of of 21±1 %, harvested in 2011 season and delivered to TARİŞ, Farmers' Sales Cooperative (İzmir, Turkey), was used in the tests.

Fumigation chambers
Test chambers consisted of 3 liter glass jar, each metal lid capped with a ground-glass stopper equipped with entry and exit tubing. A magnetic stirrer placed in the bottom well beneath a wire-mesh disc served to mix the air with the fumigant. Two pieces of rubber tubing, 5 cm long, 6.2 mm ID, were attached to the tubing and sealed with pinch-clamps.

The fumigant
The fumigant was 99% pure liquid PPO that was withdrawn from a sealed vial fitted with a rubber septum, using a gas-tight syringe.

Dosing and fumigation procedures
Propylene oxide was introduced as a liquid into the test chamber using 50 or 250 µL gas-tight syringes. Pressure in each glass jar was measured using a 0 to 800 mm Hg vacuum gauge (Celesco SE-2000, U.S.A.). The 100 mm Hg measure referred to herein is absolute pressure, with 760 mm Hg considered as atmospheric pressure. Prior to each test, 50 larvae, pupae or adults were confined, separately, inside 3 cm diameter by 8 cm long wire-mesh cages. For eggs, two exposure slides each holding 50 eggs were used per fumigation. For fumigations at low pressure, the insects were first placed in the empty test chamber and then, prior to the introduction of the required PPO concentration, 100 mm Hg of low pressure was obtained by evacuating air. PPO at 100 mm Hg was tested at four to five dosages varying from 1 to 20 mg l^{-1}. Each test was replicated at least three times. The Exposure time was 4 h for all the experiments. The gas mixtures in the test chambers were stirred for at least 20 min. The r.h. and temperature were maintained at 65±5 % at atmospheric pressure and 26±1 °C respectively.

For PPO fumigation in presence of the commodity only larval stage which was found to be the most tolerant stage to PPO was used. Each test chamber was filled up with 1.5 kg of dried figs and then 50 larvae confined separately inside the wire-mesh cages were placed into the commodity. For fumigations at low pressure, prior to the introduction of the required PPO concentration, 100 mm Hg was obtained by evacuating air. PPO at 100 mm Hg was tested at

four to five dosages varying from 5 to 35 mg l^{-1}. Each test was replicated at least three times. The exposure time was 4 h for all the experiments. The r.h. and temperature were maintained at 65±5 % at atmospheric pressure and 26±1 °C respectively.

Measurement of sorption and residue in the commodity

Dried figs weighing 1.5±0.2 kg were placed into the fumigation chamber and the lids of each fumigation chamber were tightly closed. The fumigation chambers before treatment were held for two or three hours for preconditioning of the commodities at 26°C. Sorption profiles of PPO were determined for dried fig at a dose of 68.7 mg l^{-1} applied over a 5 h period. The calculated volumes of PPO were introduced as a liquid into the desiccators containing the commodities using 50 µL gas-tight syringes. Controls consisting of sealed, empty fumigation chambers were also dosed to determine the "chamber effect" on fumigation concentrations to see any loss or reduction of gas concentration. All exposures were conducted at 26±1 °C and 60±5 % relative humidity, ambient conditions. PPO was sampled from the free-space of each chamber to determine the decrease in fumigant concentration due to sorption. The gas concentration of PPO was measured using a Shimadzu 17A GC fitted with an FID (Flame Ionization Detector) and an ECTM–WAX capillary column (30 m length x 0.25 mm ID x 0.25 µm Film Thickness) run at 170 °C isothermal. The PPO residues in dried figs were measured after 5 h fumigation at 26°C at a sole dose of 112 mg l^{-1} PPO. The levels of PPO residue on dried figs were determined at the end of the fumigation and following a 3-day aeration period. The levels of PPO residue in the commodities were determined by analytical laboratory service of Kahramanmaras Sutcu Imam University following the analytical method that was a modification of the ASTA analytical method of the Official Methods of Analysis of the AOAC (Anonymous, 2000).

Data processing and analysis

After each treatment, larvae, pupae, and adults were transferred to 200-mL jars containing food medium and were held at 26±1 °C and 65±5 % r.h. until examined for mortality. Mortality counts for all life stages of the insects were made after each treatment. Zero dose control and dose-mortality responses were subjected to probit analysis by the POLO-PC computer program (LeOra Software, 1987) to determine LC_{50}s, LC_{99}s and their respective 95% confidence intervals. Required concentrations x time (Ct) products to obtain 50 % and 99 % mortality of all insect stages of each insect were calculated using the LC_{50} and LC_{99} concentrations derived from probit analyses.

RESULTS AND DISCUSSION

PPO at 100 mm Hg was toxic to all life stages of *P. interpunctella* and *E. cautella*. Eggs and larvae of *P. interpunctella* by LC_{99} values of 12.24 and 11.37 mg l^{-1} repectively were more tolerant than the adults and pupae by LC_{99} values of 4.65 and 6.98 mg l^{-1}, respectively (Table 1). On the other hand, larvae and pupae of *E. cautella* LC_{99} values of 13.31 and 8.98 mg l^{-1} respectively were more tolerant than the eggs and adults LC_{99} values of 7.11 and 4.15 mg l^{-1}, respectively (Table 1). The complete mortality of all life stages of *P. interpunctella* and *E. cautella* were achieved at a Ct product of 45.47 and 53.24 mg h l^{-1} respectively. These findings may be compared with several studies of the two most commonly used fumigants, methyl bromide (MB) and phosphine, for control of *P. interpunctella*. Methyl bromide requires CT products of 21, 25 and 35 mg h l^{-1} to obtain complete mortality of eggs, larvae and pupae, respectively, at 30 ºC (Bell, 1976a), while phosphine requires Ct products of > 9.4,

0.9 and 1.3 mg h l^{-1} to achieve complete mortality of eggs, larvae, and pupae, respectively, at 30 °C (Bell, 1976b).

Table 1. Probit analysis data and Ct products (mg h l^{-1}) for propylene oxide at low pressure of 100 mm Hg for all life stages of *Plodia interpunctella* and *Ephestia cautella* resulting from 4-h laboratory fumigations at 26°C.

Life stage	n[a]	Slope[b]±SE	LC$_{50}$ (Fiducial limit)[c] (mg l^{-1})	LC$_{99}$ (Fiducial limit)[c] (mg l^{-1})	H[d]	Ct product for LC$_{99}$ (mg h l^{-1})
Plodia interpunctella						
Egg	350	4.20 ± 0.35	3.42 (3.07 – 3.75)	12.24 (10.57 – 14.89)	0.86	24.48
Larva	140	16.85 ± 3.07	8.28 (7.79 – 8.61)	11.37 (10.57 – 13.1)	0.63	45.47
Pupa	140	11.05 ± 2.21	4.41 (4.12 – 5.52)	6.98 (6.52 – 9.70)	0.70	27.92
Adult	140	4.23 ± 0.81	1.48 (1.06 – 2.02)	4.65 (3.44 – 8.15)	0.86	18.58
Ephestia cautella						
Egg	350	7.23 ± 0.62	3.39 (3.18 – 3.59)	7.11 (6.45 – 8.09)	0.32	28.44
Larva	140	4.93 ± 0.61	3.81 (3.37 – 4.21)	13.31 (9.33 – 15.31)	0.59	53.24
Pupa	140	2.85 ± 0.21	1.37 (0.69 – 1.04)	8.98 (6.58 – 14.34)	0.23	35.92
Adult	140	2.01 ± 0.15	0.29 (0.23 –0.36)	4.15 (2.69 – 7.77)	0.27	13.18

[a] Number treated, excluding controls; [b] Slope ± Standard Error; [c] Numbers in brackets give the 95% confidence range; [d] Heterogeneity factor

The LC$_{50}$ and LC$_{99}$ levels for PPO at 100 mm Hg against larval stage of *P. interpunctella* and *E. cautella* resulting from 4-h laboratory fumigations in empty space and presence of 1.5 kg of dried fig are presented in Table 2. There was a significant difference in toxicity of PPO at 100 mm Hg against the larvae fumigated in empty space and presence of dried figs. It required a dosage of 32.40 and 30.21 mg l^{-1} to kill 99 % of the larvae of *P. interpunctella* and *E. cautella* when fumigated in empty space and in presence of dried figs, respectively (Table 2). The results indicated that there were 2.85 and 2.27-fold increase in LC$_{99}$ value of PPO at low pressure respectively when *P. interpunctella* and *E. cautella* larvae were fumigated in the presence of dried figs as compared to those fumigated in the empty space. Thus, the present study indicates that a much higher dose of PPO is required for fumigation in the presence of dried figs to obtain complete mortality of *P. interpunctella* and *E. cautella* larvae. This could be due to a high sorption of PPO by dried figs. It is a well-recognized fact that a much higher dose of fumigants is required to kill an insect in a container filled with a commodity than in an empty one, owing to the sorption of the gas by the product. Just as, Punj (1969) reported that LC$_{50}$ value of different fumigants against *T. castaneum* in presence of paddy and groundnut kernels varied from 2.7 to 7.5 times as in empty space.

Sorption of PPO by dried figs after a 4-h exposure time was high with 58% of the initial concentration (Fig. 1). In all cases, there was an initial rapid decrease in concentrations of PPO during the first hour of exposure followed by a more gradual subsequent drop (Fig. 1). The drop in concentrations during the first hour for dried figs was 50% of the initial dosage

indicating a rapid sorption of PPO by dried figs. These data also support those of Zettler et al. (2002) who showed that PPO rapidly sorbed into the commodities, with 97.3, 99.2 and 98.6 % sorbed in the almonds, pecans and walnuts, respectively, at 48-h after initiation of the fumigation. The PPO residue in dried figs was a maximum average of 85 ppm at 0-1 day after termination of aeration, which was below the 300 ppm maximum tolerance determined by US FDA (Table 3). A very low 22 ppm of PPO residue was detected at 3 days after termination of aeration (Table 3). This data indicate that the PPO rapidly desorbs from the commodity at conditions of NAP and 30-35 °C. Thus, it is clear that most of the sorption of PPO by the commodity was physical. These data also support those of Zettler et al. (2002) who showed that the PPO residues among almonds, pecans and walnuts immediately after 4-h fumigations were well below the 300 ppm tolerance and that residues could not be detected following three days aeration.

Table 2. Probit analysis data for propylene oxide at low pressure of 100 mm Hg for the larvae of Plodia interpunctella and Ephestia cautella resulting from 4-h laboratory fumigations with 1.5 kg of dried fig and empty spaces

Treatments	n[a]	Slope[b]±SE	LC$_{50}$ (Fiducial limit)[c] (mg l^{-1})	LC$_{99}$ (Fiducial limit)[c] (mg l^{-1})	H[d]
Plodia interpunctella					
PPO at 100 mm Hg with Dried Fig	240	21.2±3.16	19.80 (15.70 – 23.53)	32.40 (29.95 – 38.25)	0.51
PPO at 100 mm Hg with Empty Space	140	16.85 ± 3.07	8.28 (7.79 – 8.61)	11.37 (10.57 – 13.1)	0.63
Ephestia cautella					
PPO at 100 mm Hg with Dried Fig	240	19.8±3.77	21.21 (18.84– 24.66)	30.21 (26.67 – 35.60)	0.45
PPO at 100 mm Hg with Empty Space	140	4.93 ± 0.61	3.81 (3.37 – 4.21)	13.31 (9.33 – 15.31)	0.59

[a] Number treated, excluding controls; [b] Slope ± Standard error; [c] Numbers in brackets give the 95% confidence range; [d] Heterogeneity factor

Although sorption of PPO by dried figs was relatively high, the fumigation still enables a sufficient build up of gas concentrations to achieve insect mortality. Based on its high and rapid toxicity to insects, and its rapid desorption from dried figs, the combination of PPO with low pressure can become a potential fumigant for replacement of MB for quarantine purposes where rapid disinfestation of dried figs is essential. However, further research is needed to obtain data on its penetration through the mass of commodities, its phytotoxicity and its impact on commodity quality.
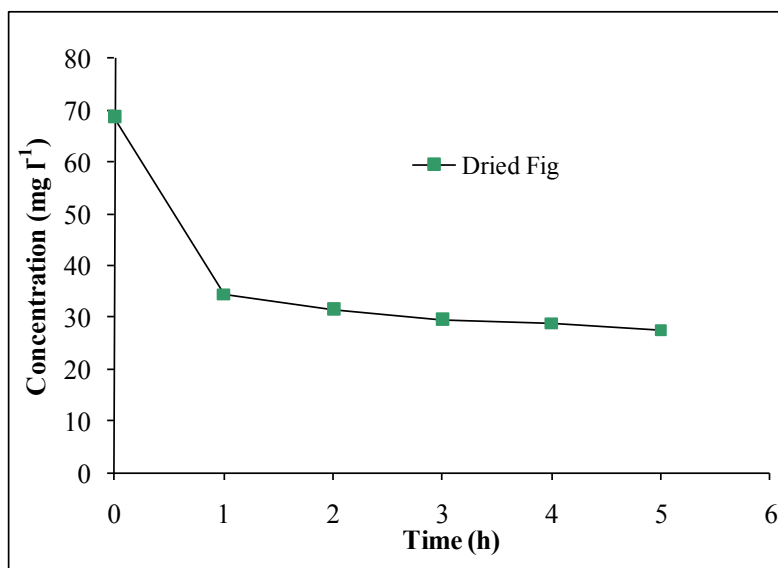
Fig. 1- Concentrations of PPO (mg l^{-1}) in fumigation chamber of 3 l during five hours of exposure after the application of PPO dose of 68.7 mg l^{-1} to 1.5 kg of dried fig at 26°C and 60±5 % relative humidity.

Table 3. PPO residues (ppm) on dried figs at 0-1 day and 3 days after termination of aeration when exposed to 4-h fumigation at 26º C and atmospheric pressure with a dose of 112 mg l^{-1} PPO

Commodity	Average PPO Residue (ppm) in sample during aeration	
	0-1 day	**3 days**
Dried fig	85	22

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ACTIVE CELL PHOSPHINE GENERATOR SGF-M2 AND FUMIGATION TECHNOLOGIES WITH ITS USE

Gennady Malushkov*, Dmitry Prokopov, Lyudmila Tikhonova

NPF SCARABEY LTD. (http://www.fumigaciya.ru)
*Corresponding author's e-mail: *info@fumigaciya.ru*

ABSTRACT

Phosphine Generator SGF-M2 is an independent physico-chemical system. The fumigant gas, phosphine, produced by the generator, is injected into the object of fumigation through tubing as a result of the chemical reaction energy and generated pressure. The total time of phosphine gas (80 g) development is 2 hours for each generator. Fumigation of grain in a silo with SGF-M2 was carried out without grain transfer using the natural convectional airstreams, driving the so-called "fumigation wave" - a high phosphine concentration region produced by the generator. The "fumigation wave" method allows reduction of the fumigant gas use and shortening the exposure time. Fumigation of grain in hopper cars with SGF-M2 takes 15 hours only.

Key words: phosphine generator, the fumigation wave method, fumigation without grain transfer, silos, hopper cars, insects.

INTRODUCTION

Traditional technologies of grain fumigation in silos are mainly based on application of tablet form preparations directly into the grain mass during the process of grain transfer from one silo bin to another. Such application procedure is labour-intensive, the gas development is slow, and the transfer operation damages the grain. The NPF SCARABEY's team has developed the new technical tool – the phosphine gas generator, SGF-M2, – and fumigation technologies for its use. The SGF-M2 generator consists of two interconnected chambers containing raw chemical components. When the reactants are brought into contact, parallel chemical reactions start, resulting in fumigant gas development, being a mixture of phosphine and carbon dioxide. At the result of the energy produced by these chemical reactions drives the fumigant gas into the object of fumigation through plastic delivery tubing. The approximate time of the fumigant gas development by one SGF-M2 generator is 2:00 hours with a yield of 80 g phosphine.

Fumigation technology based on the SGF-M2 use allows treatment of large volumes of grain without grain transfer. This technology allows simultaneous application of fumigant gas into all silo bins (including metal bins) possible. The fumigant gas rises through the grain layers by the convective airflow, ensuring layer-wise insect eradication, thus reducing both the gas usage rate and the exposure time.

Temperatures and humidities typical in hopper cars or ship's holds can result in slow decomposition of tablet formulations, which often result in low efficiency, high fire risk,

demurrage and other financial losses. With SGF-M2 generators use, the fumigant gas can be applied within 2 hours.

MATERIALS AND METHODS

Three series of trials are reported here. In the first series, studies were conducted to determine the "fumigation wave" output peak time and value as determined by the grain height in a concrete silo bin. Wheat grain was stored in three 30 m high concrete silo bins, with a cross-sectional area of 9 m^2. Silo bins were filled with grain to 15, 12 and 10.5 m high. The fumigant gas was applied from SGF-M2 generators to the bottom of each bin through the injection tubing at the dosage of 3 g m^{-3} (Fig. 1). The phosphine concentration in the grain mass was measured at 0.5 m depth from the surface of the grain, i.e. at approx. 14.5, 11.5, and 10 m distance from the gas injection point. The grain temperature during the study period averaged 21 - 22°C and the air temperature ranged from 9°C (night) to 15°C (day).

Fig.1- Application of SGF-M2 generators to silo bins

In the second series, fumigation was carried out in non-gastight metal silos with 27 m high walls, 3.5 m high cone bottom, 30 m diameter, containing 5000 tonnes of malt barley, occupying 5900 m^3 of volume. Temperatures: air - 10-12°C (day), 3-4°C (night), grain - 17-22°C. The fumigant gas was applied through tubing to the air duct during the trial period of 5 days. Total of 160 generators (12.8 kg PH$_3$, 2.15 g m^{-3} dosage) were used. Phosphine concentration measurements were taken at a depth of 1, 12, 24 m from the surface of the grain

at 1.5 m and 15 m distance from the bin walls. The fumigation efficiency was determined by the mortality rate of *Sitophilus granarius* L. in bioassays located at check points.

In the third series, studies were conducted to determine the time and efficiency of wheat (68 t) fumigation with generators SGF-M2 in a hopper car (Fig. 2). Two plastic tubes diam. 8 mm were introduced into the grain (through loading hatches #1 and #3) at approx. 1.8 m depth from the grain surface (Fig. 2). The injection time of 160 g of gas was 2:00 hrs. Grain temperature was 15 ° C. The air temperature ranged from 4°C (night) to 10°C (day). The exposure time was 15 hours. The time of passive degassing was 7 days. The fumigation efficiency was determined by mortality rate of *S. granarius* in bioassays located at check points.

Fig. 2- Application of SGF-M2 generators to a hopper car through loading hatches #1 and #3

RESULTS AND DISCUSSION

The results for the first series showed the "fumigation wave" peak time changed, depending on the height of the grain mass. Concentration readings, Table 1, show the peak at 36 hours for 15 m, 28 h for 12 m and 26 h for 11 m. The maximum phosphine concentration at the "fumigation wave" peak is 7.3 g m^{-3} at 15 m, 7.1 g m^{-3} at 12 m and 6.1 g m^{-3} at 10.5 m.

In the second series, 20 hours (five generators used, 400 g PH_3 added, average dosage 67 mg m^{-3}) after the first generator started, the phosphine concentration found at 1.5 m distance from the wall and at 1 m depth was 10 mg m^{-3}, at 12 m - 60 mg m^{-3}, at 24 m - 100 mg m^{-3}, at 15 m distance from the wall at 1 m depth - 20 mg m^{-3}, at 12 m - 90 mg m^{-3}, at 24 m - 140 mg m^{-3}. In 32 hours (eight generators use, average dosage 107 mg m^{-3}) at 1.5 m distance from the wall at 1 m depth the concentration was 50 mg m^{-3}, at 12 m - 90 mg m^{-3}, at 24 m - 110 mg m^{-3}, at 15 m distance, and 1 m depth - 90 mg m^{-3}, at 12 m - 250 mg m^{-3}, at 24 m - 400 mg m^{-3}. The time of passive degassing was 30 hrs.

Table 1. The "fumigation wave" peak dependence on the height of grain mass in silo bins

Silo bin number	Grain mass height (m)	PH_3 applied (g)	Check number	Exposure time (h)	PH_3 concentration (g m^{-3})	PH_3 max. concentration (g m^{-3})
1	15	315	1	15	0	7.3
			2	20	0.1	
			3	25	0.9	
			4	30	3.6	
			5	32	5.2	
			6	34	5.9	
			7	35	6.8	
			8	36	7.3	
			9	37	7.0	
			10	38	6.1	
2	12	270	1	15	0.3	7.1
			2	20	1.3	
			3	25	5.4	
			4	26	5.9	
			5	27	6.6	
			6	28	7.1	
			7	29	6.7	
			8	30	6.7	
3	10.5	225	1	15	0.6	6.1
			2	20	2,8	
			3	22	4,2	
			4	23	5,0	
			5	24	5,5	
			6	25	5,8	
			7	26	6,1	
			8	27	5,7	
			9	28	5,3	

The research results for non-gastight metal silos fumigation show the ability to maintain the necessary phosphine concentration the grain mass during the process of fumigation. The fumigation efficiency, as bioassayed, was 100 %.

The observations in the third series are presented in Table 2. They show uniform phosphine distribution in the grain mass of the hopper car. The fumigation efficiency (bioassay) was 100%.

Table 2. Phosphine distribution in the grain mass in hopper car, showing fumigation efficiency on *Sitophilus granarius* mortality.

PH$_3$ check point and bioassay locations	Check time after first generator start (h)	PH$_3$ concentration, (g m^{-3})	*S. granarius* mortality, %	
			Before	After
Grain mass at 0.1m, loading hatch #2 area	1	0		
	1.5	0.05		
	2	0.15		
	3	0.3		
	4	0.7	0	100
	5	0.8		
	9	0.8		
	12	0.8		
	14	0.8		
	15	0.8		
Grain mass at 0.1m loading hatch #4 area and 0.1m from the wall	1	0		
	1.5	0.01		
	2	0.4		
	3	0.8		
	4	0.8	0	100
	6	0.8		
	9	0.8		
	12	0.8		
	14	0.8		
	15	0.8		

THE USE OF GASEOUS PHOSPHINE BY ONSITE MIXING

Robert. F. Ryan[1][*] and William. P. Shore[2]

[1]VAPORFAZE, , PO Box 4, Sans Souci NSW 2219, Australia.
[2]GasApps, 6/20 Valediction Rd, KINGS PARK NSW 2148, Australia
*Corresponding author e-mail: *robert.ryan.consultant@gmail.com*

ABSTRACT

The use of liquid carbon dioxide (CO_2) as a solvent / propellant resulted in the development of a nonflammable compressed gaseous phosphine (2% PH_3) in liquid CO_2 mixture which progressed to the onsite mixing of pure flammable 99% PH_3. Prior to compressed gaseous PH_3 in industrial gas cylinders, metallic phosphide "solid phosphine" (e.g. AlP) was the only PH_3 source. Issues of flammability, OH&S and disposal cost of the spent AlP formulations has seen a continuing growth in the use of compressed gaseous PH_3 (currently some 40 tonne/year is used in Australia). The early PH_3/CO_2 mixture has been overtaken by the use of pure gaseous PH_3 mixed with CO_2 or air on-site. The onsite mixing of PH_3/CO_2 eliminates flammability issues and currently fumigated some 2 million tonne/year of grain in Australia. The on-site mixing technology can treat grain storage that is not gastight using the SIROFLO® flow through technique. This flow through technology maintains a low concentration of PH_3 over an extended period of weeks (e.g. 140 ppm x 21 days exposure). The external control of dispensing the gaseous PH_3 outside the grain storage eliminates the need for confined space entry to apply fumigant or retrieve spent solid waste. The precise control and instant dispensing of gaseous PH_3 from a high pressure industrial gas cylinder are reasons for its continued acceptance. The use of gaseous PH_3 also avoids the time and cost involved in disposal of the spent AlP formulations. Proprietary dispensing equipment has been developed that is capable of 24/7 dispensing a PH_3/CO_2 mixture unattended for a period of over 4 weeks.

Keywords: non-flammable, liquid CO_2 fumigant mixture, gaseous PH_3, metallic phosphide, cereal grain fumigation, on-site mixing, non gastight storages, gas dispensing equipment, long exposure period

INTRODUCTION

"Golden Age of Malathion and Tin Sheds":
The quality standard for stored grain has undergone a quantum leap over the last 50 years. In Australia prior to 1963 insects were accepted components of stored grain. The issues were "visible" insects (approximately 2000 insects/tonne which at 30°C can multiply to 200,000 insects/tonne in one month [Rees, 1998]) or if significant losses from heating or mould occurred. Because of excessive financial penalties paid in compensation for infested grain, the Australian Government promulgated the Export (Grain) Regulations in 1963 which prohibited the export of grain from Australia unless it was found to be free from insect pests. Initially the insect-free standard was achieved by the use of liquid insecticide "grain

protectants" sprays and this was the treatment for most grain stored in Australia. The grain was sprayed using a number of insecticides including malathion and during this "golden age of malathion and tin sheds" period insect-free status was achieved using residual pesticides, however, eventually concern of pesticide residues contamination was raised as important marketing issue.

Pesticide residue-free grain is achieved using gas fumigation to eliminate insect infestations however sealed storage is required. The solution adopted to treat the large number of non-gastight storage was SIROFLO®, the flow through fumigation system developed by CSIRO's Stored Grain Research Laboratory (Winks, 1986).

Phosphine (PH$_3$) – the fumigant of choice:
There has always been a need to control insects in grain and foodstuffs to prevent food losses and to satisfy marketing requirements. The traditional preferred fumigants were methyl bromide (MeBr) and phosphine (PH$_3$) with the latter the fumigant of choice because of cost considerations, superior efficacy and environmental acceptance (MeBr is listed on the Montreal Protocol as an ozone depletor). PH$_3$ is a naturally occurring gas, albeit short lived because it reacts with atmospheric air forming phosphoric acid - an acid used extensively as a food additive. The PH$_3$ releasing metallic phosphide ("solid phosphine") formulations have been commercially available for almost eighty years and have made significant contributions to grain protection. The original "solid" PH$_3$ fumigation patent was lodged in Germany on Nov 6, 1934 and in the USA on May 10, 1938 (US patent 2,117,158: "Method of exterminating Corn Beetles and other Vermin"). The PH$_3$ gas is generated from metallic phosphide formulations on their exposure to moisture in atmospheric air. These formulations minimised the flammability hazard by slowly releasing the flammable PH$_3$ over days to allow dilution with the surrounding air to avoid ignition and fires. While a very low cost PH$_3$ source there are other issues with the "solid" PH$_3$ formulations (e.g. unreacted powder residues; disposal costs and long exposure times). Over time the phosphine dose has been dramatically reduced from the 10,000 ppm in early recommendations to the current recommendation as low as 100 ppm. With residue levels of 0.001 ppm (Scudamore and Goodship, 1986) PH$_3$ was the preferred fumigant to attain insect-free and residue-free foodstuffs; however PH$_3$ is an extremely flammable gas with a lower explosion limit in air of 1.6%. Discounting its flammability hazard, PH$_3$ is a very effective fumigant being some fifty times more toxic to insects than MeBr. After a lapsed Fruit Fly project in1976 involving Gosford Postharvest Horticultural Laboratory, CIG Ltd (now BOC/The LINDE Group) patented PHOSFUME® [now ECO$_2$FUME®] a non-flammable gaseous mixture of 2 wt% PH$_3$ in liquid CO$_2$ (Ryan and Latif, 1989). In August 1999, the gaseous PH$_3$ products ECO$_2$FUME (2% PH$_3$/CO$_2$) and VAPORPH$_3$OS (99% PH$_3$) were sold to CYTEC and the associated dispensing equipment to support this business was carried on by GasApps Australia Pty Ltd (formerly BOC Gases R&D Workshop). Although more expensive, the non-flammable compressed gaseous PH$_3$ has many benefits over the traditional metallic phosphide formulations:

- Mixing with CO$_2$ eliminates PH$_3$ spontaneous flammability hazard
- Compressed PH$_3$ allows accurate control to maintain the required PH$_3$ concentration
- Quick gas release reduces the long exposure times of the "solid" PH$_3$ formulations.
- Gaseous PH$_3$ allows quick distribution in the grain mass without disturbing the grain;
- Regulated PH$_3$ allows controlled flow for long periods e.g. 28 days SIROFLO exposure
- Piped gas system contains the PH$_3$ and minimises OH&S concerns.

- Compressed PH_3 eliminate handling and disposal of the "spent" metallic phosphide tablets
- Cylinder gas avoids fires associated with the tablets;
- Dispensing of the gas can be automated - "solid" PH_3 distribution is very labour intensive.

Fumigation of non gas-tight storages:
Fumigations to be effective should be carried out in gastight storages which are validated using a decaying pressure test (SCA Technical Report, 1980). Many Australian grain storages fail this test however they can be fumigated using the SIROFLO flow through fumigation technique.

SIROFLO is a flow-through fumigation technique that maintains a small positive pressure throughout the grain mass to ensure a uniform low concentration of PH_3 and can control phosphine-resistant insect's strains in non-gastight storage (Winks and Ryan, 1990). The low PH_3 concentration (~100 ppm) is maintained for a period (up to 25 days) sufficient to kill all stages of insects in non gastight storages that can be effectively "sealed" in critical areas. The small positive pressure is calculated to overcome the forces that would otherwise lead to air ingress with consequent loss of gas and failure of the fumigation. This flow through fumigation technique provides a method for fumigating grain in leaky storage and has made many old silos useful storage facilities again. Advantages of gaseous PH_3 flow through fumigation include:
- enables the fumigation of "leaky" (non gastight) storages;
- achieves pesticide residue-free and insect-free status for grain in non gastight storage;
- improves efficacy using low concentrations for long exposure periods;
- greater control over fumigant dosage (both concentration and time);
- increases workers safety, low emissions to the environment and low cost of treatment.

Global Technology Transfer:
China:
A fumigation demonstration installation at the Beijing Grain Centre (Nov 1997) led to the largest onsite mixing of PH_3 and CO_2 to date installed by Grain Tech Systems at the Xizui Grain Import and Export Terminal, Dalian, China [150 x 3000 tonne + 20 x 30,000 tonne = 1.05 million tonne storage].

The Xizui onsite mixing of PH_3 and CO_2 is carried out using a unique and very simple mixing system developed by GasApps Pty Ltd, Australia which incorporates no moving parts. Liquid CO_2 was delivered by bulk road-tanker and stored at low temperature in a 5 tonne cryogenic tank. The PH_3 was dispensed from 50 L industrial gas cylinders (holding up to 22 kg of the liquefied PH_3 gas). A small PLC (programmable logic controller) controls the opening and closing of solenoid valves for the release of the two gases for passage through the mixer, making the mixing process a safe and automated operation.

The main advantage of on-site mixing is that it saves cost by avoiding the transport, storage and handling of hundreds of cylinders of pre-mixed gas. One 22 kg cylinder of PH_3 contains the same quantity of PH_3 as 35 x 31 kg cylinders of the PH_3/CO_2 premix.
Cyprus:
Following the installation of PH_3/CO_2 demonstration unit at Larnaca, the Cyprus Grain Commission installed flow through fumigation SIROFLO technology in all steel vertical grain silos. In addition demonstration PH_3/CO_2 trials were carried out New Zealand, Bahrain, Qatar, Vietnam, Thailand and Indonesia.

Dispensing Equipment

Pre-Mix PH_3/CO_2 dispensing equipment:

Specialised PH_3/CO_2 dispensing equipment was developed to satisfy the requirements of SIROFLO flow through technology. Innovations include: regulation of the high pressure (70,000 kPa) liquid PH_3/CO_2 mixture and dispensing vaporised gas mixture (100 L/min / 12 kg/h) for periods up to a month; "Spider" manifold using dual 3 mm SS tubing allow purging to eliminate polymer formation and avoid PH_3 diffusion associated with traditional SS Teflon-lined flexible hoses; Auto/Manual motorised Flow Control Metering Valves; higher capacity PH_3/CO_2 vaporiser for global customers. Customised PH_3/CO_2 dispensers have been the outcome of joint development between GasApps Australia and Bulk Handling Companies.

On-Site Mixing PH_3/CO_2 dispensing equipment:

The development of onsite of PH_3 and CO_2 was made possible by the innovation of novel mixing equipment. The initial trials used a purpose built piston mixer which incorporated a pressure equaliser to ensure each piston received the exact amount of gas. The pressure equaliser was itself further developed into a simple low cost mixer, and further developed into the high-pressure construction used in the 1.1 million tonne grain storage facility at Dalian, China.

The development of specialised equipment for the dispensing of gaseous PH_3 continued to evolve. The range was extended from the non-flammable PH_3/CO_2 mixture to include the flammable 99% PH_3 which can be mixed onsite with CO_2 to produce a non-flammable mixture [2.6 v/v% PH_3/CO_2].

MATERIALS AND METHODS

The onsite mixing of PH_3 and CO_2 eliminates flammability issues and is used to fumigate some 2 million tonne/year of grain in Australia. The on-site mixing technology can treat grain storages that are not gastight using the SIROFLO flow-through technique. The treatment of unsealed grain storage fitted with a SIROFLO flow through fumigation system requires GasApps to supply/connect gas dispensing/mixing equipment to the installed air blower and fumigant gas distribution pipe work. This pipe work directs the dispensed $PH_3/CO_2/Air$ mixture at a calibrated low pressure to the individual grain storage. Orifice plates installed in the pipe work meter the diluted PH_3/CO_2 in air mixture to ensure the individual storage or sections of the same storage receives the target PH_3 concentration. The SIROFLO system is designed to ensure the treated storage has approximately one air volume change each day to ensure the PH_3 concentration is maintained for the duration of the fumigation exposure period (up to 25 days).

GasApps supply the required quantity of PH_3 and CO_2 in high pressure industrial gas cylinders. These gases are connected to the dispensing/mixing equipment and adjustments made to deliver the gas flows required to achieve the specified PH_3 concentration 24/7 for exposure periods up to 25 days. Once everything is connected the system is activated and the blower provides the Air carrier flow to dilute and dispense the PH_3 and CO_2 mixture through the storage.

The external control of dispensing the gaseous PH_3 outside the grain storage eliminates the need for fumigation space entry. The precise control and instant dispensing of gaseous PH_3 from a high pressure industrial gas cylinder are reasons for the continued acceptance of gaseous PH_3. Proprietary dispensing equipment has been developed that is capable of 24/7 dispensing a PH_3/CO_2 mixture unattended over 4 week's exposure period.

RESULTS AND DISCUSSION

The current ongoing requirement of delivering insect and pesticide free grain for export continues to be achieved using PH_3 fumigation even in non gastight grain storage. The insect free status is independently verified. Government regulations mandate that export grain from Australia is "insect-free" and this is enforced by thorough inspection at grain export terminals. Grain in non gastight storages is fumigated using the SIROFLO flow through technique. The types of storage vary with a mixture of vertical and horizontal storage.

The cost of treatment varies with the storage type, as vertical storage is much cheaper to fumigate than horizontal storage – a greater height of grain is treated with the same gas flow in a vertical storage. Costs also vary with exposure time as longer exposure time allows lower PH_3 concentration (*ct*-product). The other significant variable is labour and consumable costs. The fumigation cost of flow through fumigation are also more expensive than "one-shot" fumigation however the horrendous cost of modification or replacement of existing non gastight storage is not a financial option in the short term. Taking into account all the expenses the fumigation cost of non-gastight storage is in the range of AUD 0.5 to AUD 2/tonne (treatment cost of PH_3 fumigation of gastight storage can be as low as AUD 0.1/tonne).

The PH_3 dosage for flow through fumigation has increased fourfold (4x) from the dose recommended in 1996 (Ryan, 1997) this is due to the ongoing increases in insect tolerance to PH_3. A major concern of bulk grain handlers is the increase in insect resistance to PH_3. The immediate problem is the PH_3 resistant *Cryptolestes* [flat grain beetle] in NE Australia where existing PH_3 label rates are not effective.

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ECO$_2$FUME AND VAPORPH$_3$OS PHOSPHINE FUMIGANTS - GLOBAL APPLICATION UPDATES

Justin Tumambing[1], Mike Depalo[2], Jean Paul Garnier[3], and Remil Mallari[1]

[1]CYTEC AUSTRALIA HOLDINGS PTY LTD, P O Box 7125, Baulkham Hills, NSW 2153 Australia
[2]CYTEC Canada Inc. 9061 Garner Road, Niagara Falls, Ontario L2E6S5, Canada
[3]CYTEC Industries France
*Corresponding author's e-mail: *Justin.Tumambing@cytec.com*

ABSTRACT

ECO$_2$FUME®(2% phosphine, 98% CO$_2$ by weight) and VAPORPH$_3$OS®(99.3% phosphine average by weight) are cylindered gas formulation of phosphine which have realized significant growth in commercial application for the disinfestation of food and non-food commodities. These two cylindered phosphine fumigants offer numerous advantages over traditional fumigants like methyl bromide and metal phosphide products such as enhanced worker safety, fast action, high effectiveness, ease of application and dose control, absence of waste, absence of residues and preferred environmental profile.

This paper presents the history and recent developments of the different applications of ECO$_2$FUME®and VAPORPH$_3$OS®as fumigants for food commodities such as stored grains, oilseeds, nuts, pulses, fruits and vegetables, animal feed and feed ingredients and non-food commodities such as tobacco, cut flowers, foliage and structural fumigation. ECO$_2$FUME®was first introduced in Australia in 1988 for stored grains, oilseeds and nuts both for unsealed and sealed vertical silos and horizontal sheds using the SIROFLO®, SIROCIRC® and SIROFUME® fumigation methods. ECO$_2$FUME®was also used in Australia and New Zealand for export cut flowers and foliage. In North America, ECO$_2$FUME®started its commercialization in 2000 in sealed storages for grains, nuts, dried fruits, tobacco, flour, processed foods, feeds and structural fumigation.ECO$_2$FUME®is now expanding in several countries in Asia and in the Middle East for grains, pulses, dried fruits, tobacco, cut flowers, nursery trees, structural fumigation, fresh fruits and vegetables. VAPORPH$_3$OS®was first introduced in China in 2000 at Dalian Grain Export Terminal using the on-site mixing with CO$_2$technology. With the development of the Horn Diluphos System™ for safe on-site mixing of VAPORPH$_3$OS®with air in 2002, VAPORPH$_3$OS®became an increasingly popular phosphine fumigant as a practical and convenient approach for large scale fumigation of grains, oil seeds, nuts and fresh fruits and vegetables in Australia, New Zealand, USA and South America.VAPORPH$_3$OS®is also being applied as replacement to ECO$_2$FUME®for SIROFLO® applications in Australia.

Key words: phosphine, ECO$_2$FUME®, VAPORPH$_3$OS®, history, recent developments.

INTRODUCTION

Phosphine is regarded as the world's most cost effective and widely used fumigant for stored product protection against insect pests. The usual disadvantages associated with the solid phosphine formulation of slow acting, self-igniting when exposed to air and the need for deactivation and disposal of unspent residue have been overcome with the introduction of ECO$_2$FUME$^®$ and VAPORPH$_3$OS$^®$ cylinderized phosphine fumigants.

ECO$_2$FUME$^®$ is a non-flammable and ready to use liquefied gas mixture of 2% phosphine and 98% carbon dioxide (CO$_2$) by weight. It comes in high pressure aluminum or steel cylinders with a net fumigant weight of 31 kg and containing 620 grams of phosphine. It requires simple dispensing equipment designed to deliver the fumigant as quickly or slower as required by each individual application. VAPORPH$_3$OS$^®$ is 99.3% phosphine by weight and is designed for use with approved blending equipment for on-site dilution with CO$_2$ or air in non-flammable proportions. It comes in steel cylinders with a net fumigant weight of 22 kg. VAPORPH$_3$OS$^®$ is most suitable for larger volume applications where it is not practical to store, handle or transport large numbers of cylinders, price sensitive applications such as grains and for locations that conduct frequent fumigations.

ECO$_2$FUME$^®$ and VAPORPH$_3$OS$^®$ offer the advantages of being safer, greener and faster. Safer because it is applied externally to the fumigation structure which eliminates confined space entry, reduces worker exposure and eliminates retrieval of partially spent fumigant, ECO2FUME$^®$ being also non-flammable. It is greener because there is no waste product or residue that requires waste deactivation or disposal. Phosphine vented into the atmosphere will react with oxygen in the air and in the presence of sunlight will readily convert to phosphoric acid. It is environmentally friendly as it is non-ozone depleting and does not contribute significantly as a greenhouse gas. It has non-phytotoxic property to sensitive commodities such as cut flowers, fruits and vegetables. The required fumigation time is relatively faster than the solid phosphine formulation since it is easily applied as a gas mixture to quickly distribute and achieve uniformly the target concentration. There is more effective control of target insects due to better gas distribution and maintenance of target concentration by safe and quick top-up which leads to decreased amount of phosphine applied.

Applications in Australia and New Zealand

ECO$_2$FUME$^®$ (originally known as Phosfume) was first commercially applied in Australia in 1988 by BOC Gases Australia which produced and patented the phosphine/CO$_2$ blend and developed special dispensing equipment for fumigating grains and oil seeds in unsealed and well-sealed silos and horizontal sheds. This was in conjunction with the CSIRO patented fumigant application technologies called SIROFLO$^®$, SIROCIRC$^®$ and SIROFUME$^®$. To date, there are over 250 million tons of grains and oil seeds that have been fumigated with ECO$_2$FUME$^®$. In 1999, Cytec Industries Inc. acquired the ECO$_2$FUME$^®$ global fumigant business from BOC Gases including all patents, trademarks, registrations and pending registrations. The SIROFLO$^®$ dispensing equipment was further improved during the early 2000's for extra safety and less maintenance. The improved versions were developed by GasApps Australia, Viterra and Cytec Industries Inc.

SIROFLO® is a continuous slow addition of ECO$_2$FUME® in an air stream such that phosphine concentration is diluted from 26,000 ppm to about 90 - 160 ppm before introducing it into the bottom of the silo or shed and exit at the top of the grain for an exposure period of 14- 28 days (Fig. 1). The bottom and the walls of the storage should be reasonably gas-tight to ensure that with the low positive pressure of the gas mixture the fumigant permeatesoutward throughout the storedcommodity, and there is only minimum ingress of air that could locally dilute the fumigant concentration. The long exposure period to low phosphine concentration will allow for the killing of all stages of insects including the less susceptible egg and pupae stages. With the development of increased resistance of some insects species (lesser grain borer and rice weevil) a new set of fumigation protocols was established in 2004 covering minimum phosphine concentration of 70 – 700 ppm for 3 – 21 days at 15 - >30°C. There has been in recent years the emergence of strong resistant flat grain beetle particularly in areas with warmer temperature and high relative humidity which render the current fumigation protocols insufficient to achieve complete control. Experimental studies are currently being conducted by postharvest grain protection team of Queensland Department of Employment, Economic Development and Innovation to establish the new phosphine fumigation protocols for complete treatment of strong resistant flat grain beetle.
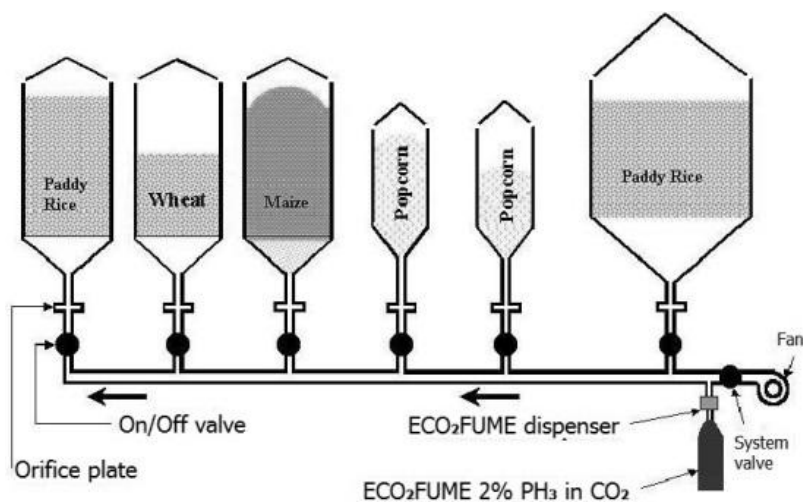
Fig.1- Schematic of SIROFLO fumigation ECO$_2$FUME®system for unsealed silos.

SIROCIRC® is similar to SIROFLO® except insofar as it includes a recirculation duct connected between the storage roof and the fan inlet. This allows the recovery of phosphine from the headspace above the grain and its recirculation through the grain mass. At least 90% of phosphine can be recycled in a reasonably well sealed storage. While SIROFLO® is a set-and-leave operation; SIROCIRC® requires a reduction in the fumigant flow-rate once phosphine begins to recycle back from the top of the storage. This can be done manually, but control is facilitated by the use of an automatic electronic controller that intermittently adjusts the fumigant flow to generate a near-constant phosphine concentration in the delivery duct.

Large storages such as big silos in grain terminals and horizontal sheds have employed the use of on-site mixing of VAPORPH$_3$OS® with CO$_2$. GrainCorp's large storage facilities in

Queensland, NSW and Victoria have used on-site mixing equipment developed by CYTEC and GasApps Australia.

SIROFUME® differs from the other two in being a "one-shot" technique wherein gaseous phosphine is dumped into the head space of a sealed storage. Nowadays, this fumigation approach is mostly used with VAPORPH$_3$OS® using on-site phosphine/air mixing equipment.

ECO$_2$FUME® is also used in Australia and New Zealand for pre-shipment treatment of exported cut flowers and foliage. At normal atmospheric pressure, the protocol used is 700 ppm of phosphine for 15 hours at minimum temperature of 15°C. In New Zealand, a shorter exposure of 3-4 hours is adopted at 700 ppm and minimum 15°C with the use of a vacuum chamber at 70 mm Hg absolute pressure. ECO$_2$FUME® is used to a relatively limited extent for quarantine treatment of imported grains, flours, oil seeds and nuts that come in shipping containers.

With the development and commercialization of the Horn Diluphos System (HDS) (a Cytec Industries Inc. approved phosphine/air on-site mixing equipment) in 2004, VAPORPH$_3$OS® became an increasingly popular fumigant for cost effective, flexible and convenient way of fumigating grains and oil seeds in sealed storages (vertical silos, horizontal sheds and bunkers). The HDS fumigation equipment is manufactured and supplied by Fosfoquim SA in Chile. The HDS comes in four size models (HDS CF/30 – 0.06 – 0.36 kg phosphine/hr, HDS 80 – 1.2 kg phosphine/hr, HDS 200 – 3 kg phosphine/hr and HDS 800 – 12 kg phosphine/hr) which cater to a wide range of storage capacities ranging from 1,000 to 300,000 tons (Fig. 2).
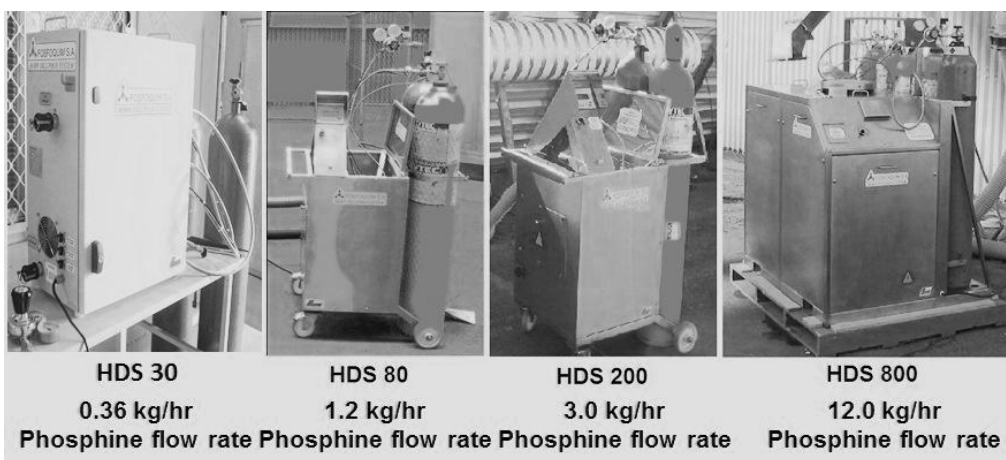
Fig. 2- The four models of the HDS fumigation equipment and corresponding capacities

The VAPORPH$_3$OS® phosphine fumigant in combination with the HDS fumigation equipment is now widely used by the three Australian bulk handling companies (GrainCorp, Viterra and CBH Group) which handle over 90% of harvested grains and oilseeds in the country. The HDS CF/30 model is progressively being adopted by GrainCorp and Viterra in replacing ECO$_2$FUME® with VAPORPH$_3$OS® for SIROFLO® application mainly due to the

ability to simplify the cylinder handling issue. Figs. 3 – 4 show examples of current major applications of VAPORPH$_3$OS$^®$ with the HDS.

Fig. 3- The 300,000 ton capacity sealed horizontal shed at CBH Kwinana Grain Terminal Western Australia with insert HDS 800/VAPORPH$_3$OS fumigation setup.

Fig. 4- A 20,000-ton bunker with the HDS 800/VAPORPH$_3$OS fumigation setup at GrainCorp Nhill grain storage center in Victoria Australia.

In New Zealand, VAPORPH$_3$OS$^®$ is currently used with the HDS for fumigation of cereal grains in sealed silos, chicken sheds and fruits such as kiwi. The phosphine fumigation protocol for kiwi is 3,000 ppm for 36 hours at 1- 6°C against armored scales.

VAPORPH$_3$OS$^®$ application for apples is currently in the fine tuning stage required prior to commercial application.

Application in Asia

China was the first country to commercially apply VAPORPH$_3$OS$^®$ at Dalian Xizui Grain Terminal by on-site mixing with bulk CO_2 in 2000. The on-site mixing of VAPORPH$_3$OS$^®$ with bulk CO_2 produced the ECO$_2$FUME$^®$ blend which was introduced for fumigation with the SIROCIRC$^®$ fumigation system developed by GasApps Australia and constructed by Grain Tech System Pty Ltd. This grain terminal had a 1 million ton grain capacity divided into a block of 144 x 3000 ton sealed silos and another block of 20 x 30000 ton sealed silos both equipped with a SIROCIRC$^®$ fumigation system. The fumigation system was composed of 1) a 5-ton bulk liquid CO_2 tank and VAPORPH$_3$OS$^®$ cylinders storage, 2) on-site mixing system (ECO$_2$FUME$^®$ mixer), 3) ECO$_2$FUME$^®$ delivery pipe work, and 4) SIROCIRC$^®$ system. Some components of the fumigation system are shown in Fig. 5. During fumigation, a phosphine concentration of 100 ppm was maintained throughout the grain mass for a period of 18 days - enough to kill all stages of insects.

Fig. 5- The on-site mixing of VAPORPH$_3$OS$^®$ with bulk CO_2at Dalian Phase-2 silo block of 20 bins by 30,000 ton capacity each.

ECO2FUME$^®$ is used in the Philippines for structural fumigation of flour mills, feed mills and food processing plants such as dairy powder and cheese factories. The dose can vary from $100 - 500$ ppm for $1 - 3$ day at 30°C or higher. All the corrosion sensitive components such as PLCs and other electronic controls are well covered with totally impermeable film for easier setup and better protection against corrosion. Other applications are in tarpaulin and container fumigation of cereal grains (rice, corn, seeds)and other commodities (flour, sugar) requiring disinfestation. Efficacy trials are currently being conducted for applications to fruits such as export mango, banana, pineapple and avocados.

In Korea, ECO$_2$FUME$^®$ fumigation protocols were established for quarantine fumigation of export paprika, cherry tomato, strawberry and nursery trees. The effective doses vary from $700 - 1,400$ ppm phosphine for 24 hours depending on the temperature of $2 - 15$°C. For cut flowers, the effective dose is 1,400 ppm for 24 hours at 8°C.

In Indonesia, fumigation protocols were established for strong resistant strain of cigarette beetle that infest tobacco. The suggested fumigation protocols for achieving 100% efficacy for all stages of strong resistant cigarette beetle in Indonesia are 1,000 ppm for 5 days, 700 ppm for 8 days and 350 ppm for 12 days at average temperature of 28°C or higher.

In Thailand, the largest rice exporter in the world, the established fumigation protocols for complete treatment against major rice insect pests are 1,000 ppm for 36 hours, 700 ppm for 48 hours (2 days) and 350 ppm for 96 hours (4 days) at temperature range of 26 – 35°C. Fig. 6 shows a setup of tarpaulin fumigation of rice in jumbo bags with ECO$_2$FUME$^®$ in Thailand.

Fig. 6- Setup of tarpaulin fumigation of rice in jumbo bags in one of the rice mills in Thailand.

Applications in North America

Commercial applications of ECO$_2$FUME$^®$ in the USA began in the fourth quarter of 2000 after full registration both for food and non-food uses was granted in August 2000. ECO$_2$FUME$^®$ is applied into the sealed fumigation structure by direct injection using simple and quick dispensing equipment with variable fumigant flow rates.

Tobacco fumigation was among the first commercial applications of ECO$_2$FUME$^®$ in the USA. Tobacco bales stored inside large warehouses were fumigated by first sealing the warehouse and injecting ECO$_2$FUME$^®$ from a bank of cylinders in manifold located outside the warehouse. A phosphine concentration of 250 ppm was maintained for a period of 96 hours to achieve successful fumigation. Among the different commercial applications of ECO$_2$FUME$^®$ and VAPORPH$_3$OS$^®$ in the USA is either methyl bromide or solid phosphine formulation replacement as below. Fig. 7 shows some of the applications.
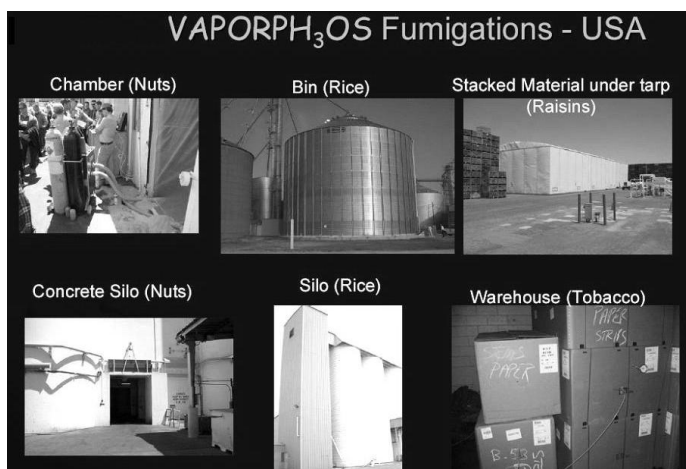
Fig. 7- Methyl bromide replacement applications with VAPORPH$_3$OS for fumigation in the USA.

1. In-transit fumigation of flour and rice in rail cars using ECO$_2$FUME[®]
2. Rice, wheat, corn and other grain fumigation in sealed vertical bins using ECO$_2$FUME[®] or VAPORPH$_3$OS[®]
3. Fumigation of almonds, walnuts, and pistachio nuts with VAPORPH$_3$OS[®] that were previously fumigated with methyl bromide in fumigation chambers/containers and with metal phosphide in metal storage bins
4. Bagged and bulk seed in cold storage warehouses with ECO$_2$FUME[®]
5. Fumigation of stacked raisins boxes under tarp and other dried fruits using ECO$_2$FUME[®] or VAPORPH$_3$OS[®]
6. Structural fumigation (e.g., flour mills and empty warehouses) using ECO$_2$FUME[®] in combination with heat and CO$_2$.
7. Bunker storage using VAPORPH$_3$OS[®]

The dosage recommendation for ECO$_2$FUME[®] and VAPORPH$_3$OS[®] in the USA varies in phosphine concentration of 200 – 1,000 ppm for 36 hours to 6 days at 0 to above 26°C depending on the commodity, target insects and sealing degree of the fumigation structure. There is also a protocol with a shorter exposure period of 24 hours at above 26°C using a phosphine concentration of 500 – 1,000 ppm. Rodents and other vertebrate pests in storages may be controlled with short-term fumigations within 1 to 4 hours after achieving distribution of phosphine throughout the structure.

Only ECO$_2$FUME[®] is currently used in Canada for a similar range of applications but using the same dosage rate of 200 – 1,000 ppm phosphine concentration for an exposure period 2 – 14 days and temperature range of 0 – 16°C or above.

Applications in Latin America

Chile was the first country in the world which has commercially applied VAPORPH$_3$OS[®] (TK Gas brand name in Chile) for fumigation of export fruits and vegetables. Fosfoquim SA developed the Horn Diluphos System (HDS) fumigation machine to safely mix

VAPORPH$_3$OS$^®$ and air and deliver the phosphine air mixture at lowto high flow rates into different sized sealed fumigation structures. Fosfoquim SA has also formed a fumigation company that provides fumigation services to fruit exporters. A fleet of fumigation vans and trained fumigators provide mobile fumigation service to all customers in Chile (Fig. 8).

Fig. 8- The HDS 800 in a fumigation van with a two-hose connection to a fruit fumigation chamber.

Fumigation services provided by Fosfoquim include fruit and vegetable fumigation accounts for a major portion of the total fumigation services.

There are many advantages of using VAPORPH$_3$OS$^®$ in combination with the HDS for fruit and vegetable fumigation as follows:

- Phosphine eliminates the target pests in fruits.
- No changes in taste, smell, texture, color or shelf life of the fruit, if fumigation has been conducted at low temperature.
- It is not necessary to heat fruits up before fumigation with subsequent shelf life extension.
- There are no need for deactivation and disposal of residues after fumigation.
- Cylinderized phosphine does not produce ammonia and it is, therefore, not phytotoxic.
- The fumigation can be done in the same cooling chambers where the fruit is stored prior to shipment.
- There is no need to fumigate at the port of arrival, since the fumigation can be done at the processing plant before shipping.
- The fruit can be delivered immediately upon arrival at the port.
- This fumigation technique has no environmental problems, since phosphine is readily deactivated by sunlight upon release into the atmosphere.
- The fumigation is operator friendlier than methyl bromide in terms of minimizing exposure to workers due to better gas concentration monitoring and control.
- There is uniform gas distribution even in largest fruit fumigation cool houses which takes less than one hour and followed by one hour and a half of venting the gas by aeration.

- As the method permits applying the gas from outside the facility, the gas concentration can be changed at any time during the fumigation.
- The gas can be applied to a totally sealed structure without increasing the pressure due to safe recirculation of inlet mixture of air and gas.
- The HORN DILUPHOS SYSTEM allows flexibility and control in gas dispensing with no corrosion issue on cooling system.

In Trinidad and Tobago, ECO$_2$FUME® is used for cargo container fumigation, shiphold fumigation, warehouse fumigation, storage silo treatment, and fumigation of grains on pallets and in silos. ECO$_2$FUME® is dispensed using simple and quick dispensing equipment into a sealed structure.

Applications in Europe, the Middle East and Africa

In Turkey, ECO$_2$FUME® fumigation protocols for complete treatment of major insect pest of stored grains, dried legumes and tobacco were established as part of the registration label. The recommended dosages are 1,000 ppm phosphine for 3 days, 2 days and 1 day at temperatures of 17°C, 23°C and 28°C, respectively.

In Egypt, ECO$_2$FUME® recommended protocols for tarpaulin fumigation of stored grains are 600 ppm phosphine for 3days at 20°C or higher for non-porous ground surface and 700 ppm phosphine for 3 days at 20°C or higher for porous ground surface. Fig. 9 shows a setup of fumigating bagged wheat in tarp using ECO$_2$FUME®.

Fig. 9- Setup of fumigation of bagged wheat in tarpaulin plastic sheet in Egypt.

In the Sultanate of Oman, dates had been successfully fumigated with ECO2FUME using a dosage of 1,000 ppm phosphine for 36 hours at 28°C or higher (Fig. 10).

Fig.10- Setup of container fumigation of dates with ECO$_2$FUME$^®$ in the Sultanate of Oman.

Summary

ECO$_2$FUME$^®$ and VAPORPH$_3$OS$^®$ are cylinderized phosphine fumigants that offer safer, greener and faster advantages for disinfestation of food commodities such as stored grains, oilseeds, nuts and beans, fruits and vegetables, animal feed and feed ingredients, and non-food commodities such as tobacco, cut flowers and nursery trees, tires and structural fumigation.

DISCLAIMER

NDIR BASED SO$_2$F$_2$ DETECTOR FOR FUMIGATION MONITORING

R. C. Naik[*] and R. D. Shroff

Uniphos Envirotronic Pvt. Ltd., A United Phosphorus Group Company, India
[*]Corresponding author's e-mail: *naikrc@uniphos.com*

ABSTRACT

Sulfuryl Fluoride (SO$_2$F$_2$) is being considered as one of the preferred fumigants to replace Methyl Bromide (MBr) which, owing to its large Ozone depletion potential, is being partially phased out as a fumigant. Fumigation of commodities with SO$_2$F$_2$ in closed enclosures can last over 24 hours, during which period, fumigant concentration is to be continuously monitored as a measure of process control. SO$_2$F$_2$ concentration during such fumigation can range from a few hundred ppm to several thousand ppm. For the detection of SO$_2$F$_2$ in the said concentration range, instruments based on interferometry and thermal conductivity are available. However, both the above techniques have their own limitations. For example, thermal conductivity based detector fails to have specificity when other interfering gases (apart from normal constituents of air) are present along with SO$_2$F$_2$.

This paper presents a new instrument for the detection of SO$_2$F$_2$ in the concentration range of 500-30000 ppm. The instrument is based on NDIR (Non Dispersive Infrared) technique which is highly specific to the target gas. The instrument measures the absorbance of SO$_2$F$_2$ molecules in the IR region. The SO$_2$F$_2$ molecule has several absorption bands in the IR region, out of which, the absorption at 6.64 µm having no interference from possible other co-existing gases has been chosen as a fingerprint of the molecule. Using a specially designed NDIR absorption cell, the absorbance of SO$_2$F$_2$ at 6.64 µm is measured and related to its concentration.

The instrument is microprocessor based and has the capability of storing the measured concentration data. The details of operation, calibration and other features of the instrument are described in the paper.

Key words: Fumigation, Fumigant, Sulfuryl Fluoride, Infrared Absorption, NDIR.

INTRODUCTION

There is a constant search for substitutes in place of methyl bromide as a fumigant which is known to be an Ozone depletor and its use is banned for certain products and in certain countries. Ideally, a fumigant should pose no environmental hazard, penetrate quickly into porous materials, cause no undesirable changes or odors in the material treated, and dissipate rapidly during its removal by aeration. It should also be effective against a wide variety of insects and other pests. With these criteria in mind, the fumigation industry is narrowing down to sulfuryl fluoride (SO$_2$F$_2$) as a fumigant. It is non-corrosive, highly penetrating, is stable in air and practically insoluble in water. Unlike methyl bromide, SO$_2$F$_2$ does not deplete the atmospheric Ozone layer when released into the atmosphere. These desirable

properties, along with its marked toxicity to all types of insect pests, have made SO_2F_2 a recommended fumigant for a wide variety of closed structures, furnishings, food and other non-food products [1, 2].

NDIR (NON DISPERSIVE INFRARED) TECHNOLOGY

All diatomic and polyatomic molecules have one or several vibrational modes which can absorb radiation in the infrared region. Taking advantage of this absorption by molecules, instruments can be built for the detection of gas molecules. There are mainly two techniques available for the measurement of gas concentrations using IR technology. One is the dispersive technique and the other is the non-dispersive technique. In dispersive technique the light from a continuum source is dispersed in wavelength using prisms or diffraction gratings and the required wavelength is selected and its absorption is related to target gas concentration. Alternatively, in non-dispersive infrared approach, an optical filter can be used to select the transmission of only the absorption wavelength of the target gas molecule. It has been shown that non dispersive techniques using wavelength selective filters are typically 1000 to 100,000 times more sensitive than dispersive technique using gratings or prisms as no slits are used in the non-dispersive technique [3]. NDIR technique is also found to be cost effective, simple and rugged with no compromise on the performance.

NDIR BASED SO_2F_2 ANALYZER

Construction
The NDIR based SO_2F_2 analyzer described in this paper consists of an IR absorption cell and the signal conditioning electronics as shown in figures 1 & 2. The absorption cell is a 120 mm long cylindrical aluminium tube of 10 mm internal diameter with a highly polished inner wall (Fig.1). It has gas inlet and outlet ports. The cell is sealed with two CaF_2 windows on both open ends. On one end of the cell a ceramic IR radiation source is placed which emits IR radiation in the range of 2-16 μm and on the other end a detector module is placed. The module consists of two pyroelectric detectors fitted with two filters which serve as two channels, viz. reference and gas sensing channels. The sensing channel detector is fitted with a filter whose centre wavelength is 6.64 μm which coincides with the chosen IR absorption peak of SO_2F_2. The other detector is fitted with a filter whose centre wavelength lies at 3.9 μm which is not absorbed by SO_2F_2. This channel serves as the reference channel.
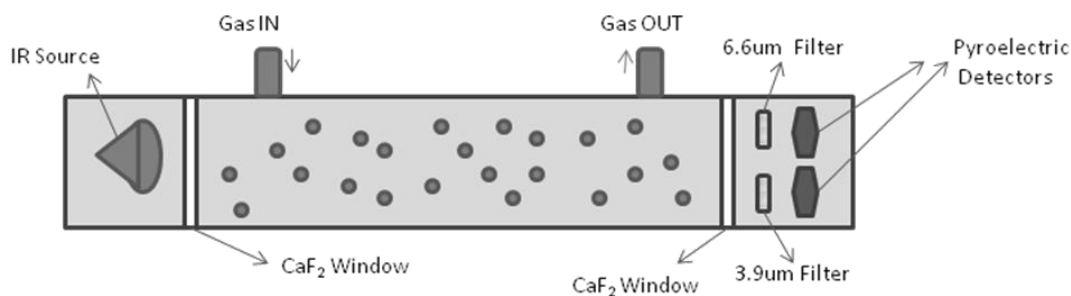
Fig. 1- SO_2F_2 IR absorption cell

The continuum radiation from the IR source passes through the gas cell and falls on the two detectors. However the filter on the sensing detector allows only 6.64 μm and the reference filter allows only 3.9 μm IR radiation to fall on detectors. If the cell is filled with SO_2F_2, the sensor signal decreases because of the absorption, and the reference signal is unaffected. This change in the signal with respect to the reference signal, caused due to the absorption by SO_2F_2 is calibrated with known standards to read the gas concentration. The reference signal serves to correct for any variation in source intensity and other parameters affecting absorption of SO_2F_2. The CaF_2 windows on the two sides not only transmits IR radiation but also serve to isolate the IR source and detectors coming in direct contact with SO_2F_2.
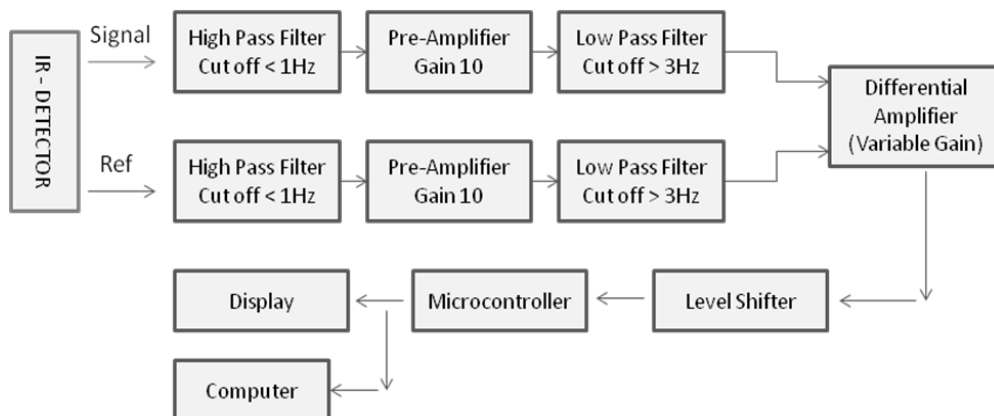
Fig. 2- Signal conditioning module

The instrument is specially designed for monitoring the gas concentration in the fumigated silo or container. The air sample is brought to the sensor by means of the built-in air-sampling pump of the instrument. A dust filter is placed in the inlet of the sampling line to prevent dust particles entering the absorption cell. The instrument works on built-in 14.8 V Li-Ion rechargeable batteries.

Performance & Calibration

The analog signals from both the channels of the detector are pre amplified and fed to the differential amplifier which gives a signal equal to the differences in the peak-to-peak amplitudes of the two signals. This differential signal is further amplified and fed to the microcontroller which gives the digital counts which are related to the gas concentration.

The instrument is calibrated in the range of 0-30,000 ppm using different SO_2F_2 gas concentrations. The instrument has two different ranges of measurement viz. 0-5000 ppm and 3000-30,000 ppm. The Concentration Vs Digital Counts is linear in the lower concentration range and is found to be exponential for higher concentrations of SO_2F_2 as shown in figures 3 & 4 respectively. For lower range the R-Square value was found to be 0.997 and for higher range, the R-Square value was found to be 0.999.

Operation

Once the instrument is turned ON the instrument goes through a series of prompts such as model number, monitor name, operating ranges, etc.. After the initialization, the instrument is automatically driven into purging mode for 3 minutes and the internal air sampling pump gets activated to draw in fresh air in order to purge the absorption cell. After purging, the instrument enters into measurement mode upon selection of suitable range for measurement. As soon as the instrument enters the measurement mode the internal air sampling pump will be activated & will draw the air/gas sample from the fumigation chamber/silo. By default the pump will be on for 3 minutes. Once the pump stops, the unit goes into the data processing mode for about 10 seconds. After completion of the data processing it gives the reading of the SO_2F_2 gas concentration on the LCD panel. Being a microprocessor based unit it has data logging facility and it can store data such as gas concentration, silo/container number etc. with date and time. There is also provision to download the stored data on a computer or a serial printer.
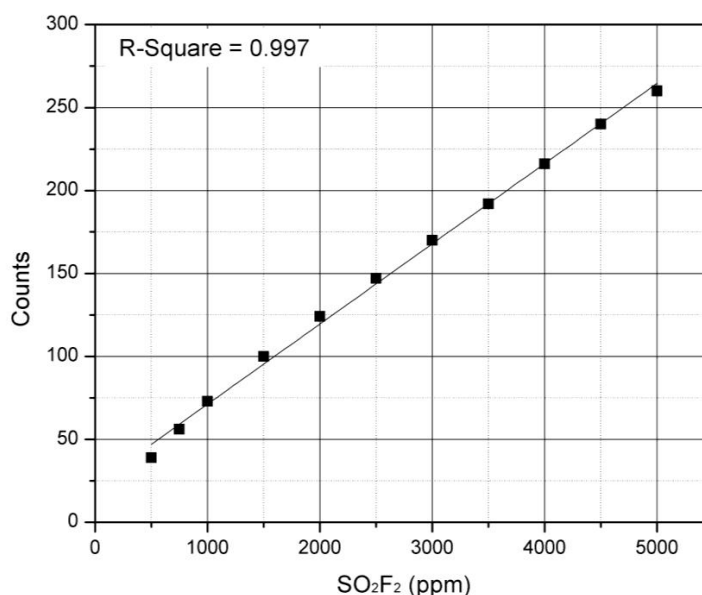
Fig. 3- Low range (0-5000 ppm)

CONCLUSION

SO_2F_2 is becoming popular as a fumigant of choice for closed structure fumigation, furnishings, etc. NDIR based instruments provide a cost effective, accurate and reliable measurement system for monitoring of SO_2F_2 concentration during fumigations. It is endowed with high specificity and sensitivity with no interference from other coexisting gases.
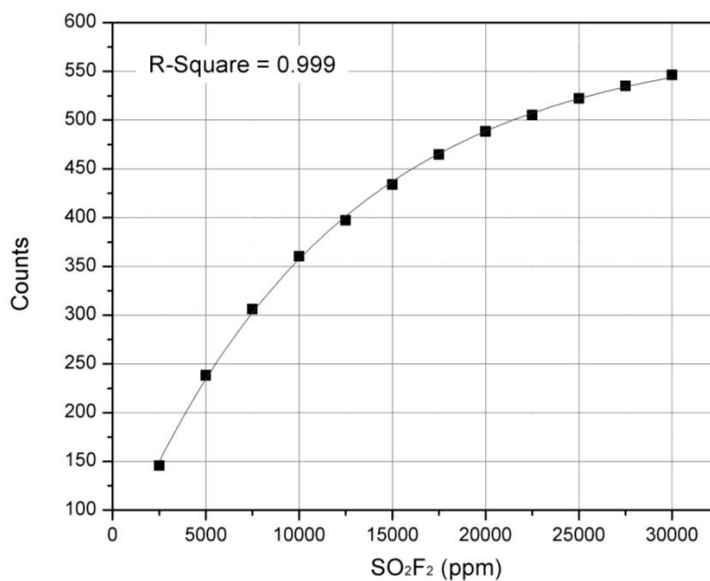
Fig. 4- High range (3000-30000 ppm)

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POSSIBILITIES OF PROFUME® GAS FUMIGANT FOR THE COMMERCIAL FUMIGATION OF STORED COCOA BEANS IN EU

Herlinde Noppe*[1], Stanislas Buckley[2], Benno Ruebsamen[3]

[1]Dow AgroSciences B.V. Prins Boudewijnlaan 41, 2650 Edegem, Belgium
[2]Dow AgroSciences S.A.A. Marco Polo B-790 Avenue Dr Donat, B.P. 1220, 06254 Mougins Cedex, France
[3]Dow AgroSciences GmbH, Truderinger Straβe 15, 81677 München, Germany
*Corresponding author's e-mail: *hnoppe@dow.com*

ABSTRACT

Cocoa beans, shipped to EU warehouses, are stored in bulk or stack at major ports until needed for processing. They are often fumigated before transport to the processing factory to ensure disinfestation of common stored-product insects. Insect infestation usually originates in the country of production where the raw cocoa has been stored, but may also occur during transport or storage in the warehouse. Since the phase out of methyl bromide in 2000 in EU, ProFume® gas fumigant (99.8 % sulfuryl fluoride [SF]), has been seen as an alternative for methyl bromide within the commercial cocoa fumigation world. ProFume is a broad spectrum, non-ozone depleting gas fumigant developed and manufactured by Dow AgroSciences LLC. Fumigation of cocoa beans with ProFume in the United States, trial fumigations in Germany and The Netherlands and commercial fumigations in Belgium and the Netherlands have shown that fumigation with ProFume is fast and effective, without any adverse effects on the quality of the cocoa beans and their processing. The overall goal of this report was to develop experience in commercial fumigation of cocoa beans stored in stacks and in bulk.

Key words: Postharvest systems, cocoa fumigation, sulfuryl fluoride

INTRODUCTION

ProFume® gas fumigant (99.8% sulfuryl fluoride [SF]) is a broad spectrum, non-ozone depleting fumigant developed and manufactured by Dow AgroSciences LLC for the control of rodents, insects and other invertebrate pests. ProFume® was developed in response to post-harvest industry requests for an alternative to methyl bromide (MeBr). ProFume® has been registered for use in food handling establishments (e.g., pet food facilities, bakeries, food production facilities, mills, warehouses, etc.), stationary transportation vehicles (railcars, shipping containers, trucks, etc.), temporary and permanent fumigation chambers, and storage structures. ProFume® is relatively non-reactive as a gas and does not cause off-flavors. It is an odorless, colorless inorganic gas, and as such, does not form unpleasant odors. Due to its higher vapor pressure and lower sorption characteristics, ProFume® compared to MeBr penetrates commodities more effectively, reaching target pests faster for optimal control (Thoms et al., 2008). Furthermore, it has been proven that the sorption of SF is much lower than that of MeBr under identical conditions (Phillips et al., 2000).

The fumigant of choice for control of common stored-product pests during transport and storage of cocoa historically has been MeBr. However, with the adoption of the Montreal protocol, MeBr was phased out in the EU in 2000 due to its ozone depleting properties and the search for replacements and alternatives to MeBr has begun. Now, trials and experience have proven that ProFume® is a successful post-harvest fumigant for cocoa and a valuable alternative for MeBr both in efficacy and cost (Bookout and Milyo, 2006; Buckley, 2008; Adam et al., 2010). The commercial treatment of cocoa beans with ProFume® was first described in United States by Bookout and Milyo (2006), who confirmed the effectiveness, the absence of sensory effects and the customer acceptance of sulfuryl fluoride. ProFume® was granted registration for the control of stored-product insect pests in cocoa beans in EU in Belgium in 2011 and the Netherlands in 2010. In addition, after zonal re-registration under 1107/2009/EC approval for treatment of cocoa with ProFume will be also granted authorisation in Germany in the near future for cocoa bean treatment.

Cocoa (*Theobroma cacao* L.) is an internationally traded commodity with estimated annual production of over 3 million tons and a global market value of $5.1 billion (World Cocoa Foundation, 2012). Cocoa beans, shipped to EU warehouses, are stored in bulk or stacks at the major ports (Amsterdam [Netherlands], Antwerp [Belgium], and Hamburg [Germany]) until needed for processing. At arrival, they are often infested with common stored-product pests. New shipments enter the warehouses and are stored adjacent to other cocoa beans. There is no segregation of new and old shipments. As such, they are subject to re-infestation during their storage in the warehouse. Warehouse operators hold cocoa beans for numerous cocoa brokers and traders. Chocolate manufacturers typically purchase whole or partial shipments of cocoa beans from various brokers weekly. Their choice of cocoa beans depends on quality attributes, price and condition of the cocoa beans, production needs, country of origin, and other factors (Van Meijel et al., 2010; Bookout and Milyo, 2006).

In EU, cocoa fumigation can be performed in different ways: fumigation of bulk or bagged (e.g. stacked) cocoa beans in the warehouse or fumigation of bagged cocoa beans in containers. This study provides a description of the development of effective and practical methods for fumigation of cocoa on commercial scale by evaluating the current methods for fumigation with ProFume, which are acceptable to the fumigators and the cocoa industry in terms of exposure time, efficacy, costs and safety. Trial cocoa fumigations have been carried out to demonstrate that cocoa fumigation inside a warehouse can be undertaken without causing concentrations of ProFume in air that should be of concern. Additionally, current practices for fumigation with ProFume have been evaluated and adapted for fumigation of cocoa stored in stacks or bulk and gaps in knowledge on cocoa fumigation have been identified. This information is being used to establish a specific approach for the commercial fumigation of cocoa beans in EU with ProFume.

MATERIALS and METHODS

For this study, several trial fumigations in Hamburg, Germany and Amsterdam, The Netherlands have been carried out in port warehouses between 2007 and 2009. In Germany and the Netherlands, one 120 m³ stack and two 500 and 600 m³ stacks of cocoa beans were fumigated respectively. Based on these trials, commercial scale fumigation of cocoa beans (bulk stacks within the range of 3000 m³) has been evaluated.

All fumigations have been carried out according to the Dow AgroSciences' guidelines for fumigation of cocoa, now included on the national label of ProFume. According to these guidelines, cocoa in hessian bags or in bulk piles, held on a fumigant resistant foundation (e.g.

puncture-resistant tarpaulin, asphalt or concrete) have to be covered with a material resistant to fumigant penetration (such as vinyl coated nylon or polyethylene sheeting of at least 150 micron (µm) in thickness. The tarpaulin needs to be supported to create a gas expansion dome of sufficient dimensions, such as approximately 0.5 m above the items to be fumigated and at least 0.3 m around the sides, to allow gas diffusion. The edge of the tarpaulin has to be sealed by weighting the edges with sand or water 'snakes' or the equivalent.

Introduction tubes have to be directed in an open air space under the tarpaulin to avoid direct contact with the cocoa beans. Fans need to be used to mix the fumigant with the air and to aid effective penetration into the commodity. Introduction needs to be done slowly (e.g. at a rate of 0.5-2.0 kg/min) to prevent excessive cooling of air. The maximum dosage is 1500 g-h/m^3 for 48 h, with a maximum concentration of 128 g/m³. Nonetheless, the dosage is usually reduced according to planned exposure time and temperature of the stack, as applicators follow guidelines provided by proprietary software, the ProFume Fumiguide™.

Cocoa beans can be aerated using passive or active ventilation to lower the concentration of ProFume in the fumigated area and risk zone to 3 ppm (bystanders) or 1 ppm (workers). (Initial) exclusion zone can be extended when necessary according to the instructions of the fumigation leader. Monitoring, essential for dosage accuracy and calculation of the actual half loss time (HLT), needs to be done by appropriate monitoring devices (e.g. Fumiscope[1] or SF-ReportIR[2]) as required by the Dow AgroSciences Stewardship. To confirm the concentration of ProFume, which does not exceed permissible exposure limits where workers may be present during aeration, detection devices with sufficient sensitivity need to be used such as the Interscan[3] GF-1900 or SF-ExplorIR[4].

All trial and commercial fumigations described in this study were carried out according to the safety instructions related to the use of ProFume, as described in the national label on the ProFume bottle. For evaluation of efficacy of ProFume for common stored-product insects during fumigation of cocoa, bio-assays with different target pests have been distributed within the fumigated space during the trial fumigations. During the trial fumigation in Germany, bio-assays with all life stages of Red rust flour beetle (*Tribolium castaneum* Herbst), Flat grain beetle (*Cryptolestes ferrugineus* Stephens), Merchant grain beetle (*Oryzaephilus mercator* Fauvel), Rice moth (*Corcyra cephalonica* Stainton), Indian meal moth (*Plodia interpunctella* (Hübner)) and cocoa moth (*Ephestia elutella* (Hübner)) have been evaluated. For the trial fumigation in the Netherlands, bio-assays with confused flour beetle (*Tribolium confusum* Jacquelin Du Val) were assessed.

RESULTS and DISCUSSION

Challenges have been identified based on the results of the trial fumigations of cocoa in Germany and in the Netherlands and the first commercial fumigations in Belgium and the Netherlands of both bagged and bulk cocoa beans. Based on the experiences with the trial and commercial fumigations concerning sealing of the stack, most difficulties were encountered with the sealing of the stack itself and the creation of the headspace of bulk cocoa beans. In Belgium and the Netherlands, bulk cocoa beans are stored in concrete bunkers. Gas-tight tarping of bunkers is difficult due to the large size of the beans piles, the bunker construction

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[1] Trademark of Key Chemical & Equipment Co
[2] Trademark of Spectros Instruments
[3] Trademark of Interscan Corp.
[4] Trademark of Spectros Instruments

with cracks between the panels of adjacent bunkers, and the location of the bunkers (f.e. against the walls of the warehouse). Depending on the circumstances where the fumigation took place, gas tight sealing was obtained by putting a tarpaulin under the cocoa beans before loading beans in the bunker or nailing or attaching the tarpaulin to the bunker walls. For creation of the headspace above the heap or stack a tightened cable to create a kind of tent above the heap was seen to be useful.

The method of introduction of the gas is important to achieve effective and uniform gas distribution within the stack or bulk of cocoa beans. For this, different methods of introduction were evaluated: introduction at ground level under pallets of bagged cocoa beans (German trial); at the top with pulsed introduction and three fans; at the bottom; at the side (the method used in US); at ground level in the aisle between two stacks of bagged cocoa beans (Dutch trial); in the headspace or in the tubing of the recirculation system for fumigation of bulk cocoa beans (commercial fumigations). Based on these experiences, it has been shown that slow introduction from the top of the stack of cocoa beans, or in the aisle between two stacks is most effective (Van Meijel, 2010). Above this, it has been shown that despite the good penetrating properties of SF and the different methods of introduction, it is difficult to obtain a uniform distribution of SF throughout the stack or bulk cocoa beans, unless provision is made for forced circulation of the fumigant. This can be achieved by using fans or introduction in the tubing of a recirculation system. This is due to the physical properties of SF which is heavier than air and initially drops within the stack or bulk cocoa beans if active circulation is not used and the fumigant is introduced rapidly. The recirculation system that can be used for fumigation of cocoa beans is based on the US J-system, designed to avoid fumigant distribution problems in large bulk grain held in silos for the storage. This system consists of a perforated tube placed under the bulk cocoa beans before loaded into storage, while another perforate tube (if there is no large headspace possible) is placed on top of the bulk, connected with a recycling fan. It has been observed that fumigant introduction without an additional fan resulted in tarps depressed against the bulk cocoa beans due to the lower temperature of air. Dow AgroSciences recommends to introduce ProFume in the headspace above the stack or bulk cocoa beans, or in the tubing of the recirculation system. If no recirculation system is available, an additional fan can improve circulation and uniform distribution of SF within the stack or bulk cocoa beans. Based on the current experience, it is concluded that a larger headspace and a recirculation system accelerate fumigant equilibrium.

Bagged, stacked cocoa beans can be efficiently monitored using standard monitoring hoses. For bulk cocoa beans, fluctuating monitoring results were observed using standard monitoring hoses. Based on these experiences during commercial fumigations, Dow AgroSciences recommends inserting modified, perforated tubes (e.g. 1 m of PVC tube melted at one end and connected to the monitoring hoses) into bulk cocoa beans to facilitate penetration. Since the density of bulk cocoa beans is quite high, an additional pump within the monitoring system is advised.

Based on the commercial fumigations, it has been shown that a circulation system enables efficient, controlled aeration of cocoa. The circulation system can be used to ventilate fumigant from bulk cocoa beans using powerful fans. If no circulation system is available, a pre-positioned chimney, attached to a fan and venting through an opening in the roof of the warehouse, can be used to initially draw the gas out of the stack or bulk cocoa beans, followed by progressive removal of the tarpaulin.

Field trials in United States have been conducted using insect bioassays to test for optimum dosages, performance and verification that those dosages can be consistently attained and maintained. Assessments have also been made of the penetration, dispersion and

aeration characteristics of ProFume under typical conditions for commercial cocoa bean fumigations. One important outcome of the trials has been the determination that in terms of efficacy only one application rate is needed for stacks of cocoa beans fumigation under tarps (Brookout and Milyo, 2006). Tests with bio-assays with common storage pests showed that 1500 g-h/m^{3}controls all life stages (eggs, larvae, pupae). This was also confirmed by assessment of the bioassays distributed in the stacks during the trial fumigations in EU. However, the importance of a good equilibrium and penetration of SF in the bulk cocoa beans needs to be emphasized. As eggs are the least susceptible life stage, it is important that the dose within the bulk or stack cocoa beans is sufficient high on top as well as at the bottom. Besides that, the good equilibrium and penetration is also important to reach infestation of the sacks and the fabric of bagged cocoa beans.

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PENETRATION CAPACITY OF VAPORMATE[TM] INTO COMMERCIALLY PLASTIC WRAPPED CARDBOARD BOXES FILLED WITH SULTANAS FOR CONTROLLING STORED PRODUCT PESTS

Swaminathan Thalavaisundaram* and John Roynon

BOC limited (Member of LINDE Group) 10 Julius Avenue, North Ryde
NSW 2113, Australia
*Corresponding author's e-mail: *Swaminathan.Thalavaisundaram@boc.com*

ABSTRACT

Stored product pests are a serious threat to the grains and dried fruit industry. These pests damage the produce and reduce the commercial value. Dried fruit is often packaged in smaller cardboard containers and then shrink wrapped. This packaging can be difficult to treat while many traditional fumigants also leave residues that require significant ventilation, with holding periods and residue testing. The study was conducted using 5 types of multilayer cardboard boxes wrapped with plastic sheet containing sultanas placed inside a fumigation tent. Mixed culture of saw toothed grain beetle (*Oryzaephilus surinamensis* L.) and red flour beetle (*Tribolium castaneum* (Herbst) were placed inside packed commodities and treated with Vapormate[TM] at 420 g m-3 at or above 15 ^{0}C for 24 h. Sampling lines were placed in each packed box and the gas samples were taken at regular time intervals to monitor the penetration capacity of Vapormate[TM] through out the treatment period. The study found that Vapormate[TM] penetrated into the multilayered cardboard wrapped with plastic sheet, providing complete mortality of both adult and immature stages of stored product pests. Vapormate[TM] is an effective fumigant alternative to methyl bromide and other fumigants while being a fumigant that can be managed safely as a result of its relatively high TLV. Vapormate[TM] could also be used as a phosphine resistance breaker in countries where resistance exists. The dried fruit and grains industries will benefit from an alternative fumigant that penetrate effectively into the packed commodities and controls all life stages of stored product pests

Key Words: stored product insects, dried fruits, ethyl formate, fumigation, generally regarded as safe, methyl bromide alternative, phosphine resistance

INTRODUCTION

Ethyl formate is a fast acting, flammable liquid that has been traditionally used for fumigation of dried fruit since 1927 (Simmons and Fisher, 1945) for controlling stored product insects (Hilton and Banks, 1997). Ethyl formate and its break down compounds are naturally present in food commodities (Desmarchelier, 1999) and have Generally Recognised as Safe (GRAS) status (US FDA, 1984). Ethyl formate has low mammalian toxicity. Vapormate[TM] contains 16.7 % (by weight) of ethyl formate in liquid carbon dioxide (10.7 % by volume of ethyl formate in gaseous carbon dioxide). The addition of carbon dioxide enhances the toxicity of ethyl formate towards the target pests. Studies have shown that Vapormate[TM] has been effective in controlling adult and immature stages of storage pest of cereal grains, dried fruits

and nuts. These studies have been conducted by exposing the stored pests directly to the fumigant (Krishna et al., 2002). However in commercial situation, insects are concealed between the food commodities and are unlikely to directly expose to Vapormate™. For example, sultanas are packed in plastic card board boxes placed inside the tent and treated with Vapormate™. In this situation, Vapormate™ should penetrate into the cardboard boxes and kill the target pest before transporting to local or overseas markets. Therefore this study has been designed to determine if the registered target dose rate (420 g m-3 for 24 h) penetrates through cardboard boxes and achieves complete control of immature and adult stages of saw toothed grain beetle (*Oryzaephilus surinamensis* L.) and rust red flour beetle (*Tribolium castaneum* (Herbst).

MATERIALS AND METHODS

Fumigation was conducted in a tent to reflect the current practice followed by the customer. The internal volume of the tent was 38 m^3. The loading factor was 68 %. The tent was made of a tarpaulin that was secured with the ground using a sand snake to prevent the leakage. Sultanas were packed in a range of boxes used for commercial sales as shown below in Table 1. Some boxes were tightly warped with polythene sheet.

Table 1. Dried fruits stored in different type of boxes used for Vapormate™ fumigation

No.	Pack Format	Pack Weight (kg)	Packs / Layer	No. Layers	Total Packs	Total Weight (kg)	Carton Type	Wrap
1	Bulk Australian - 4 Crown	12.5	12	7	84	1050	2 Piece	Solid
2	Wooden Pallet Bins	400	1	3	3	1200	Open box	Open
3	Bulk Australian - 3 Crown	12.5	12	7	84	1050	2 Piece	Perforated
4	Import (Plain Carton)	12.5	12	7	84	1050	1 Piece	Solid
5	Multi Packs	5.76	16	8	128	737.28	1 Piece	Solid

STORED PRODUCT INSECTS

Both adult and immature stages of saw toothed grain beetle *O. surinamensis* and rust red flour beetle *T. castaneum* were placed inside a livestock tube (100 mm x 25 mm). The open end of the livestock tube was covered with sieve plastic lid to allow the gas to pass through and prevent the insects escaping during the trial. The control insects were used and not treated with Vapormate™.

FUMIGATION

Cardboard and wooden boxes containing dried fruits were loaded into the tent. The livestock tube containing target pests were marked and randomly placed in some of the boxes and tent. The recommended product (15.60 kg, 420 g m-3) was applied using a vaporiser into the tent.

The Vapormate^{TM} level inside the tent and the cardboard box was measured using a portable monitoring device. At the end of the fumigation period (24 h), the remaining fumigant was safely vented into the atmosphere. G460 Multigas monitor was used to measure ethyl formate and carbon dioxide. Gas samples were collected from the tent and sampling boxes. Each sampling line was colour coded to avoid confusion (Table 2). The sampling lines were placed deep into the box.

Table 2. Penetration of ethyl formate and carbon dioxide into the box

Number	Sampling location	Sample Line colour	Ethyl formate (g/m^3)*#	Carbon dioxide (g/m^3)*#
1	In the tent- Back Middle	Yellow black	24.58a	189.03ab
2	In the tent- Middle-top	Green black	24.78a	189.49ab
3	In the tent- Font- Bottom	Blue black	25.32a	190.40ab
4	Sample 1	Green	15.37bc	157.91b
5	Sample 2	Blue	24.08ab	188.80ab
6	Sample 3	White	17.80ab	218.32a
7	Sample 4	Yellow	6.24c	147.98b
8	Sample 5	Blue/White	18.30b	170.49b

*Values followed by the same letter within a column are not significantly different (P ≤ 0.05). # Mean of ethyl formate and carbon dioxide levels for 24 h

MORTALITY ASSESSMENT

After fumigation treatment, the live stock tube containing the target pests were assessed for mortality. The adult mortality was assessed 24 h after treatment, removed and the remaining mixed age cultures incubated between 20 and 25 ^{0}C. Subsequent emerging adults were counted 7, 14 and 28 d after treatment. Control adults and immature insects were assessed at the same assessment dates.

STATISTICAL ANALYSIS

The levels of ethyl formate and carbon dioxide found inside the tent and different boxes was analysed using a one way ANOVA followed by a test for Fishers least significant differences The analyses were conducted using MINTAB version 15.

RESULTS AND DISCUSSION

During Vapormate^{TM} treatment, the required temperature (above 15 ^{0}C) was maintained for 24 h inside the Vapormate^{TM} treated tent. The recommended dose rate was applied into the tarp (420 g m-3) and at the end of the fumigation period (24 hours), the tarpaulin sides were opened and the ventilation fan was switched on for 1 hour. After ventilation, ethyl formate (<100 ppm) and carbon dioxide (<5000 ppm) levels were below the safe level and the boxes were safely unloaded. There were no significant difference between the level of ethyl formate

found in the tent compared with sample 2 (wooden pallet bins) and sample 3(Bulk Australian - 3 crown) however the level found in sample 1 (Bulk 4 crown-solid wrap) sample 4 (Import plain carton solid) and sample 5 (multipacks solid) was significantly different to the level found in the tent (Table 2). This is not surprising as sample 2 was not plastic wrapped and sample 3 had perforation therefore the penetration and ethyl formate level would be higher than the tightly wrapped samples. Ethyl formate penetrated into the tightly wrapped boxes but the levels of penetration varied in sample 1, 4 and 5(Table 2).

All the adult insects placed at various locations within the tent and boxes were killed 24 h after treatment. Only 3 adults of *O. surinamensis* and 2 of *T. castaneum* were killed in control treatment. No adults were emerged from the mixed culture 7, 14 and 28 d after Vapormate[TM] treatment whereas there were emergences from the control treatment (Table 3). Complete mortality of adults and mixed age culture of *O. surinamensis* and *T. castaneum* placed inside the various boxes reflects the fact that the dose rate used for the trial penetrated deep into the plastic wrapped dried fruit box and provided 100 % control. Previous studies conducted in 20ft shipping container loaded with unprocessed sultanas with 34 g m-3 of ethyl formate for 48 h treatment period (206 g m-3 of Vapormate[TM]) provided 100 % mortality of mixed population of the stored product pests *T. confusum* and *P. interpunctella* (Tarri et al., 2007). Although the treatment time was longer the dose rate was half compared with our study (420 g m-3 for 24 h).

Table 3. Insects emerging from livestock after treatment with 480 g/m^3 Vapormate[TM] for 24 h

Insects species	Vapormate[TM] treatment	24 h after treatment		Mortality (in %)	7 days after treatment Number of adults emerging	14 days after treatment Number of adults emerging	28 days after treatment Number of adults emerging
Saw toothed grain beetle		Live	Dead				
O. surinamensis	Sample box-1	0	112	100.00	0	0	0
	Sample box-4	0	193	100.00	0	0	0
	Sample box-5	0	201	100.00	0	0	0
	In the tent	0	242	100.00	0	0	0
	Control	212	3	1.42	16	12	13
Red rust flour beetle							
T. castaneum	Sample box-2	0	81	100.00	0	0	0
	Sample box-3	0	94	100.00	0	0	0
	In the tent	0	74	100.00	0	0	0
	In the tent	0	76	100.00	0	0	0
	Control	112	2	1.79	14	16	6

This study demonstrates that Vapormate[TM] @ 420 g m-3 for 24 h treatment penetrates into the multilayer cardboard plastic wrapped boxes and provide complete control of the target insect species tested. Based on this result, Vapormate[TM] is a potential replacement fumigant for methyl bromide application in dried fruits.

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FUMIGANT ACTIVITY OF SOME ESSENTIAL OILS AGAINST FOUR MAJOR INSECT PESTS OF STORED GRAINS

Waqas Wakil[1*], Tahira Riasat[2], Nadia Saeed[1], Misbah Ashraf[1], Muhammad Yasin[1]

[1] Department of Agricultural Entomology, University of Agriculture, Faisalabad, Pakistan
[2] Department of Wildlife and Fisheries, G.C. University, Faisalabad, Pakistan
*Corresponding author's e-mail: arid1972@yahoo.com

ABSTRACT

Essential oils extracted from *Acacia nilotica*, *Calotropis procera*, *Dodonaea viscosa*, *Cassia fistula*, *Ocimum basilicum*, *Adhatoda vasica* and *Ziziphus jujuba* were tested for their fumigant activity against adults of *Tribolium casatneum* (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (Coleoptera: Bostrychidae), *Cryptolestes ferrugineus* (Coleoptera: Laemophloeidae) and *Liposcelis paeta* (Psocoptera: Liposcelididae). Essential oils from all plant species were obtained by Clevenger-type water distillation and were evaluated using fumigation method. Adult mortality was recorded after 24, 48 and 72 h of exposure under laboratory conditions. The results revealed that the adult mortality increased with the exposure period and *L. paeta* was the most while *T. castaneum* was the least susceptible than the remaining two insect species. Insecticidal activity also varied with essential oils and *O. basilicum* and *D. viscosa* oils were highly effective against *L. paeta* and *C. ferrugineus* with highest mortality after 24 h of exposure. The results of the current studies suggested that the use of essential oils in stored grain insect management program offer them as environment-friendly insecticides to decrease the injurious effects of synthetic insecticides.

Key words: Fumigation, essential oils, *T. casatneum*, *R. dominica*, *C. ferrugineus*, *L. paeta*, stored grains

INTRODUCTION

Stored products of agricultural and animal origin are infested by more than 600 species of beetles, 70 species of moths and approximately 355 species of mites which cause qualitative and quantitative losses to the produce (Rajendran, 2002). In order to keep these stored-products infestation free, fumigation is one of the major chemical methods to control the stored-product insects. Phosphine and methyl bromide are the broad spectrum fumigants being extensively used against stored grain insect pests (Emekci, 2010). But, the use of methyl bromide is phased out because of its potential hazards to deplete the ozone layer (MBTOC, 1998). On the other hand, the future of phosphine as grain protectant is also endangered by the development of resistant insect strains (Daglish and Collins, 1999).

Various alternatives have been experienced to replace these chemical fumigants with the eco-friendly and natural products for stored grain protection. Consequently, some plants are receiving worldwide attention hence their secondary metabolites have been explored as

botanical pesticides against insect pests. The plant based control measures possess low mammalian toxicity and do not leave toxic residues in the environment (Duke, 1985).

The stored product insects differ in their vulnerability to plant compounds (Rajendran and Sriranjini, 2008). The insecticidal potential of several essential oils from various plant species has been evaluated against stored-product insect pests in numerous studies (Negahban and Moharramipour, 2007; Rajendran and Sriranjini, 2008).

In the present studies, we evaluated the fumigant toxicity of *Acacia nilotica* (L.) (Fabaceae: Fabales), *Calotropis procera* (Aiton) (Asclepiadaceae: Gentianales), *Dodonaea viscosa* Jacq. (Sapindaceae: Sapindales), *Cassia fistula* L. (Fabaceae: Fabales), *Ocimum basilicum* L. (Limiaceae: Lamiales), *Adhatoda vasica* (L.) (Acanthaceae: Lamiales) and *Ziziphus jujuba* (L.) (Rhamnaceae: Rosales) essential oils against adults of most damaging insect pests *Rhyzopertha dominica* (F.), *Tribolium castaneum* (Herbst), *Cryptolestes ferrugineus* (Stephens) and *Liposcelis paeta* (Pearman) at different exposure intervals under laboratory conditions.

MATERIALS AND METHODS

Insect culture
The adults of four species used in the bioassays were obtained from infested stored grain samples maintained at 28 ± 1°C and $65\pm5\%$ rh in the IPM Laboratory, Department of Agricultural Entomology, University of Agriculture, Faisalabad, Pakistan. *T. castaneum* and *C. ferrugineus* were reared on clean, infestation free wheat flour and for *T. castaneum* 5% brewer's yeast was also added (Vayias and Stephou, 2009). Whereas, *R. dominica* was reared on whole wheat (Kavallieratos et al., 2006) and *L. paeta* was maintained in 97% cracked wheat, 2% rice krispies and 1% brewer's yeast in jars (Athanassiou et al., 2009). One week old adults of each insect species were used in the bioassays.

Plant materials
The stems, branches and leaves of all selected plants; *O. basilicum, D. viscosa, A. nilotica, C. procera, C. fistula, A. vasica* and *Z. jujuba* were collected separately in polythene bags from different areas of University of Agriculture, Faisalabad, Pakistan. The plant materials were dried on laboratory worktable at room temperature for 6-7 days and then grounded to powder. The dehydrated materials were stored separately at 24ºC until required and then hydrodistilled to extort their essential oils.

Extraction of essential oils
Extraction of essential oils from the plant samples was carried out by using a Clevenger-type apparatus where 50 g of dehydrated plant samples were gone under the process of hydrodistillation at 1:10 plant materials per water volume ratio for 4 h. The extracts were dehadrated by the use of anhydrous sodium sulphate and then the extracted oils from seven plants were stored in a refrigerator at 4ºC separately till use in the bioassays.

Fumigant toxicity

Whatman No. 1 filter papers (Whatman Inc., Clinfton, N.J.) were cut into pieces of 2 cm diameter and impregnated with oils separately to determine the fumigant toxicity of the oils of *O. basilicum, D. viscosa, A. nilotica, C. procera, C. fistula, A. vasica* and *Z. jujuba* at dose rate calculated to give corresponding fumigant concentration 8 uL/L in air. The impregnated filter papers were fixed to the caps of glass vials which were used separately for each insect species and the oil type. 30 adults of each insect species were released in each vial and the caps of the vials were tightened firmly. There were five replicates for seven oils and one control for each insect species. In this way, there were 40 vials (8 treatments × 5 Reps) for each insect species. The mortality of all adults was calculated after 24, 48 and 72 h.

Data analysis

The adult mortalities were corrected by using Abbott's formula (Abbott, 1925) and data were analyzed with Minitab 13.2 (Minitab, 2002 Software Inc., Northampton, MA) by One-way Analysis of Variance (ANOVA) where means were compared with Tukey Kramer test at 5% level of significance (Sokal and Rohlf, 1995).

RESULTS

In all cases, significant differences in adult mortality rates for all four insect species with different essential oils were observed (Table 1). Insecticidal activity varied with different essential oils and increased with the extending exposure intervals. All oils showed different toxicity levels in the following order: *O. basilicum >D. viscosa >A. nilotica >C. procera >C. fistula >A. vasica >Z. jujuba.*

Table 1.   ANOVA parameters for main effects and associated interactions for the mortality levels of *R. dominica*, *T. castaneum*, *C. ferrugineus* and *L. paeta* when treated with different essential oils (total $df = 419$)

Sources of variation	df	F	P
Insect species	3	124.01	<0.01
Plant species	6	111.30	<0.01
Intervals	2	95.75	<0.01
Insect species x plant species	18	0.51	0.95
Insect species x intervals	6	8.77	<0.01
Plant species x intervals	12	1.71	0.06

O. basilicum and D. viscosa was more effective against *L. paeta* and *C. ferrugineus* than *R. dominica and T. castaneum* even after 24 h (Figure 1). Both *O. basilicum* and *D. viscosa* yielded 100% mortality of *L. paeta* on all three (24, 48 and 72 h) exposure intervals.

While all adults of *C. ferrugineus* were found to be dead only after 48 and 72 h exposure to *O. basilicum* and *D. viscosa*. The mortalities of adults of *R. dominica* and *T. castaneum* were increased with the increasing exposure intervals (Figure 1, 2 and 3). The lowest mortality rates were achieved with essential oil of *Z. jujuba* for all four testes insect species. But here again, 60% of all exposed adults of *L. paeta and C. ferrugineus* were recorded dead after 24 h of treatment which was just 37% in case of *T. castaneum*. However, after 72 h of exposure, the highest mortality rate for *L. paeta* was 75% while it was just 62% for *T. castaneum*.
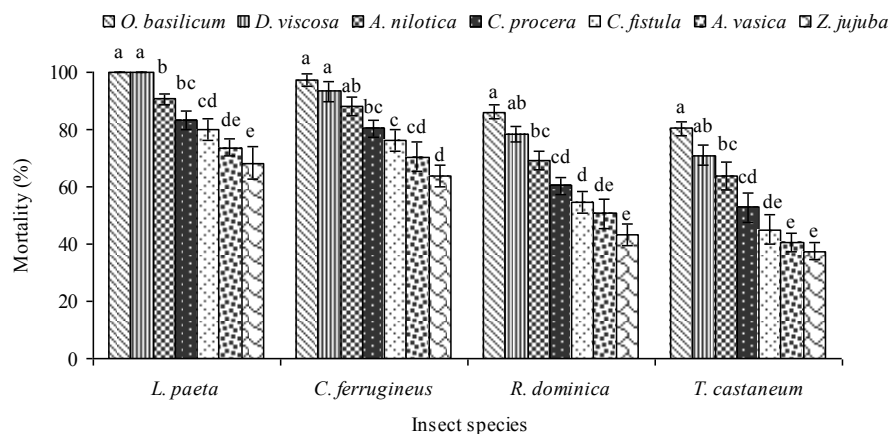
Fig. 1- Mortality (% ± SE) of *L. paeta, C. ferrugineus, R. dominica* and *T. castaneum* 24 h post exposure to seven different essential oils (means having same letters within each essential oil are not significantly different from each other; HSD test *P*<0.05).
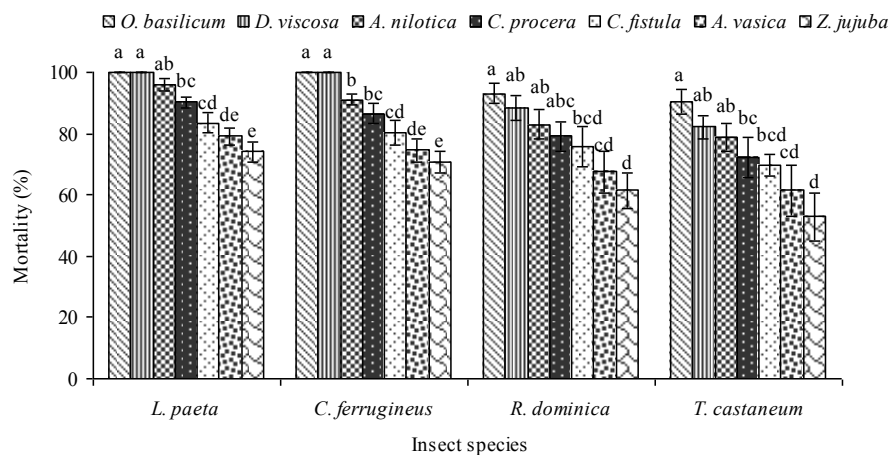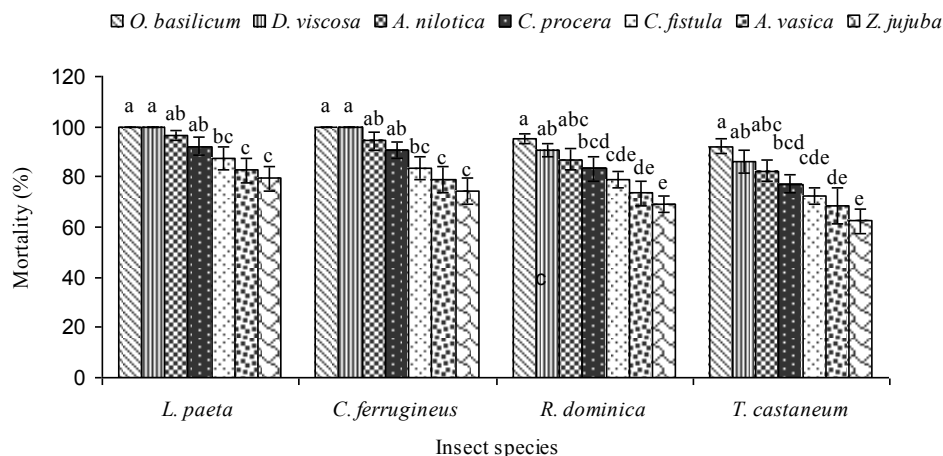

Fig. 2- Mortality (%± SE) of *L. paeta, C. ferrugineus, R. dominica* and *T. castaneum* 48 h post exposure to seven different essential oils (means having same letters within each essential oil are not significantly different from each other; HSD test *P*<0.05).

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Fig. 3-   Mortality (% ± SE) of *L. paeta, C. ferrugineus, R. dominica* and *T. castaneum* 72 h post exposure to seven different essential oils (means having same letters within each essential oil are not significantly different from each other; HSD test *P*<0.05).

DISCUSSION

A wide range of essential oils extracted from various spices and herbs have been screened for their action against several stored-product insect pests (Tunç et al., 2000; Cosimi et al., 2009; Pérez et al., 2010). The toxicity of various essential oils and their constituents, monoterpenes, have been evaluated against stored product insects. Klingauf et al. (1983) studied the fumigant toxicity of 16 essential oils against *Acanthoscelides obtectus* as Rosmarin and caraway oils were most effective and 100% mortality was obtained after 3 h at 31.4 pi/l air. El-Nahal et al. (1989) evaluated the toxicity of essential oil from *Acorus calamus* against various species of stored products. The essential oils investigated in the present study are generally used as pharmaceuticals and in food flavoring, therefore, they are considered to be less harmful to humans than most conventional insecticides. Furthermore, studies have shown that these products are readily biodegradable (Baysal, 1997) and less damaging to non-target organisms than insecticides (Yeğen et al., 1998).

In the present study *L. paeta* and *C. ferrugineus* were the most susceptible towards all the seven essential oils than *R. dominica* and *T. castaneum* for all exposure intervals. Among the tested essential oils, *O. basilicum* and *D. viscosa* showed more toxic effect against tested insect species. The insecticidal constituents of many plant extracts and essential oils are mainly monoterpenoids (Ahn et al., 1998; Cosimi et al., 2009) which due to their high volatility, have fumigant action that might be of importance for the control of stored-product insects. El-Nahal et al. (1989) stated that the period of exposure appears to be more important than the dosage in influencing the efficacy of essential oils against the adults of five stored-product insect species. The use of essential oils is considered as a safe alternative to synthetic insecticides being used for the control of stored grain insect pests, because the oils are derived from natural assets. Such oils could function as a contact repellent, toxic, antifeedant, fumigant and oviposition inhibitor (Stefanazzi et al., 2006). Numerous experiments have verified that essential oils have cytotoxic, phototoxic, neurotoxic and mutagenic activities in different organisms (Bakkali et al., 2008); moreover, essential oils act at many levels in

insects, therefore the risk of resistance development is improbable (Pérez et al., 2010). For these reasons, it is suggested that essential oils should be measured as a natural alternative for the control of stored grain insect pests.

CONCLUSION

The use of essential oils is a safe approach to control insects in stored grains as a sustainable alternative to synthetic chemicals and other fumigants. Our experiment added to the literature available on the adulticidal activity of the essential oils but further work has to be done especially to formulate a plant based product to save stored grains.

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INSECTOR® SYSTEM TO MONITOR INSECT ACTIVITY AND DENSITY DURING GRAIN STORAGE AND FUMIGATION

Fuji Jian[1], Vellaichamy Chelladurai[1], Digvir S. Jayas[1*], Noel D. G. White[2]

[1]Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, R3T 5V6. Canada.
[2]Agriculture and Agri-FoodCanada, Cereal Research Centre, 195 Dafoe Road, Winnipeg, MB, R3T 2M9. Canada.
*Corresponding author's e-mail: Digvir_jayas@umanitoba.ca

ABSTRACT

In this study, 4800 adults of rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae), were introduced in a 4.7 m diameter and 8 m high hopper-bottom bin holding 48 t of wheat at 9.6±0.3 % moisture content (wet basis) and at 22 to 27ºC. Twenty Insectors® (pitfall traps that electrically count captured insects and estimate the insect densities) were installed inside the bin at four layers. The grain was fumigated by adding dry ice after six week storage. Carbon dioxide (CO_2) concentrations at these Insectors® locations were measured every week during the grain storage period and every day during fumigation period. Insect activity at each Insector® location was monitored and their densities were measured by the Insector® system. It was found that adults moved down in the first week after they were introduced. Their distribution followed the temperature gradient with most adults being captured from the warmer layer. Descendants of the introduced adults followed the temperature gradients except in the top layer which had warmer grain. Adults did not produce enough CO_2 to be detected by the CO_2 analyzer. Adults were captured in 84 h after high concentration CO_2 were introduced into the bin.

Key words: Insect activity, insect density, fumigation, Insector®, *Cryptolestes ferrugineus*

INTRODUCTION

Spatial distribution is one of the most important ecological properties of a species. There has been some effort to describe the spatial distribution of *Cryptolestes ferrugineus* (Stephens) in stored grain; however, the published studies are based on either small sample units of grain (<2.75 kg) or small containers (less than 1.5 t grain). Their distribution inside the grain was also studied in a short time period (say less than one month). Therefore, the distribution of the descendants of the introduced insects inside stored-grain bins is not known.

Fumigation by CO_2 is one of the methods of disinfestation (Mann et al., 1997). Controlled laboratory experiments have shown that a lethal environment for the rusty grain beetle can be achieved by elevating the CO_2 concentration. To create an environment lethal to insects, >40% CO_2 concentrations must be maintained for at least 4 d (White et al., 1990; 1993). This requirement poses a problem because most stored bins used in the world are not

airtight and CO_2 can leak out even in a well-sealed bin (Mann et al., 1997). Therefore, monitoring insect activity during fumigation might be an effective way to verify whether the pests are eradicated.

Traps to detect insects in stored grain are effective and sensitive tools for grain management. Sampling rusty grain beetles using modified pitfall traps is well documented (Loschiavo, 1974; Toews and Phillips, 2002). A version of an electronic pit-fall trap, produced and known as the Insector® (OPI Systems Inc. Calgary, Canada), is commercially available. The Insector® system also identifies species to groups and measures insect densities of the captured insects.

The aim of this study was to monitor insect activity and distribution of the introduced adults and their descendants inside a farm-sized bin during storage and fumigation periods by using the Insector® system.

MATERIALS AND METHODS

Experiment was conducted in a metal hopper bin (4.7 m diameter, 8 m high, and 4.0 m cylinder part) filled with about 48 t wheat (hard red spring No 1) (Fig. 1). There were 20 Insectors® and 20 CO_2 tubes installed inside the bin (Fig. 1). One extra CO_2 tube was located inside the head space of the bin. The wheat had less than 0.7±0.4% dockage which included 0.6±0.1% smaller than wheat determined by sieving using 1.651 mm opening sieve. Test weight of the wheat was 846.7±3.3 kg m^{-3}. The moisture content of the wheat was 9.8±0.1%. The hopper bin was located inside a building and the room temperature was 25±5ºC. To prevent the insects from climbing out of the bin, the vent, loading hole, and the aeration duct were taped during experimental period. The manhole was closed except during the sampling period.

Cryptolestes ferrugineus were reared at 30±5°C and 75±5% r.h. on cracked wheat plus wheat germ (95:5 wt/wt), and were held in the dark during rearing and experiments. Eggs of *C. ferrugineus* hatch in 4–5 d and the complete life cycle takes about 3 weeks at optimum conditions of 35°C and 70% r.h. (Smith, 1965). *Cryptolestes ferrugineus* have an average life span of 6–9 mo. Adults of mixed sex were 1 d to 2 mo at the start of the experiment. Before introducing beetles into the grain bin, 4800 adults were selected using a gentle vacuum and were kept inside four 4-L glass bottles with about 3 kg of wheat in each bottle. The bottles were kept inside the bin for at least 24 h to allow insects to acclimate to the experimental conditions.

To warm up the grain (using the warm room air), grain was aerated for about 6 d and temperature was recorded by the Insector® system. After the grain reached the room temperature (25±5ºC), the aeration fan was stopped and air at each Insector® location was pulled out through a CO_2 detector (Carbon Dioxide Analyzer, Model: series 9519, Alpha Omega Instruments, Rhode Island, USA) and the CO_2 concentration was measured. After this measurement, 4800 adults of *C. ferrugineus* were introduced at the top of the grain and half radii of the bin. The CO_2 concentrations at each Insector® location were measured every three or four days during the following 6 wk (the storage period).
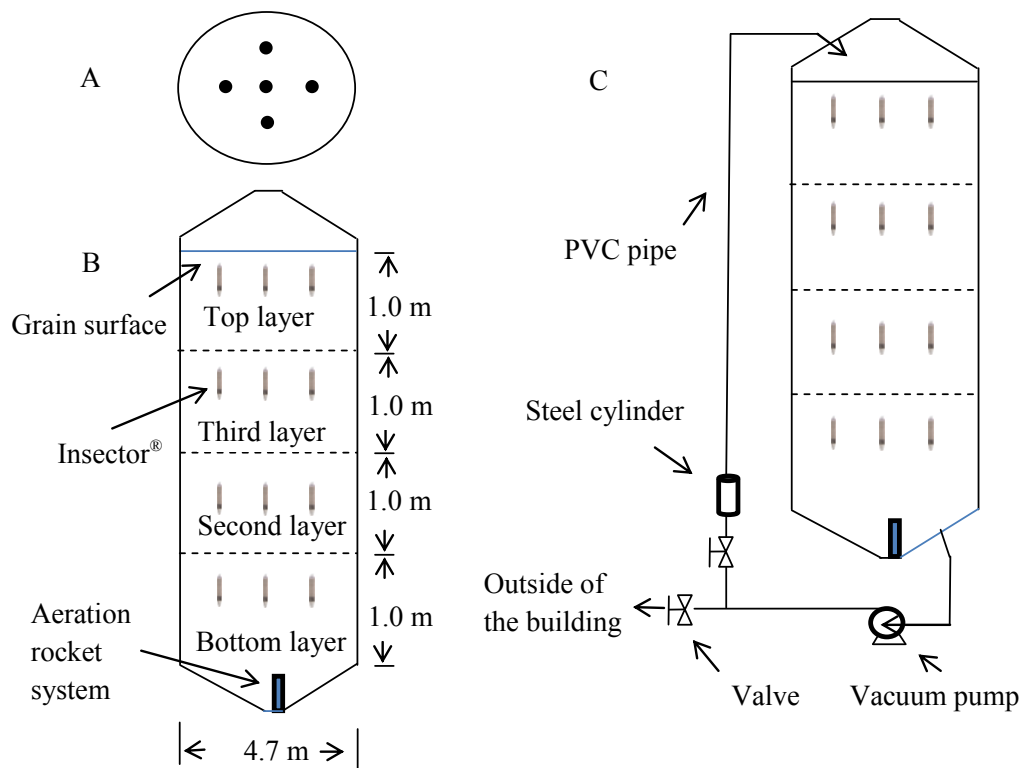
Fig.1- Locations of the Insectors®and the fumigation set up for the silo holding 48 t of wheat. Top view (**A**) shows the Insectors® at one level and side view (B) shows the Insectors® depth inside grain. Carbon dioxide tubes were fixed at the top of the Insectors®. Side view (C) shows the fumigation set up. Steel cylinder was used to hold the dry ice.

After this storage period, grain was fumigated by introducing CO_2 into the bin (Fig. 1) using the method of Mann et al. (1997). To watch the response of insects to low CO_2 concentration, CO_2 concentration inside the bin during the first fumigation period was less than 5%. During the second fumigation period, CO_2 concentration was higher than 52.9% at any location. During fumigation period, CO_2 concentration was measured every day.

Insect daily density at each Insector® location (four layers and five locations at each layer, Fig. 1) was monitored by the Insector® system. The Insector® system classified the captured insects into six groups. During testing, the density of insects (RGB group in Insector®) was measured and interpreted as the density of rusty grain beetle because there was no other insect species. These densities at each location were used to analyze the adult activity and distribution in the following time periods: the first week, four weeks (the second, third, and fourth weeks), descendant distribution (the fifth and six weeks), during the first fumigation period (the seventh and eighth weeks), and during the second fumigation period (the ninth and tenth weeks).

Grain temperature

The average temperature of the grain during the entire experimental period was 25.1±4.6ºC. In any layer, there was no difference in the temperature at any location. Grain temperature slightly changed during the entire experimental period (Fig. 2). This change was caused by the temperature gradient in the vertical direction inside the building. The temperature at the upper-layers was always higher than that at the lower-layers (Fig. 1 and 2). There was about 1.0ºC/m temperature gradient in the vertical direction inside the bin and there was no gradient in the horizontal direction (Tukey test for each layer and all the P>0.5). Temperature gradient at the beginning of the experiment between top and the third layer was about 3.0ºC/m and this gradient was gradually decreased (Fig. 2).
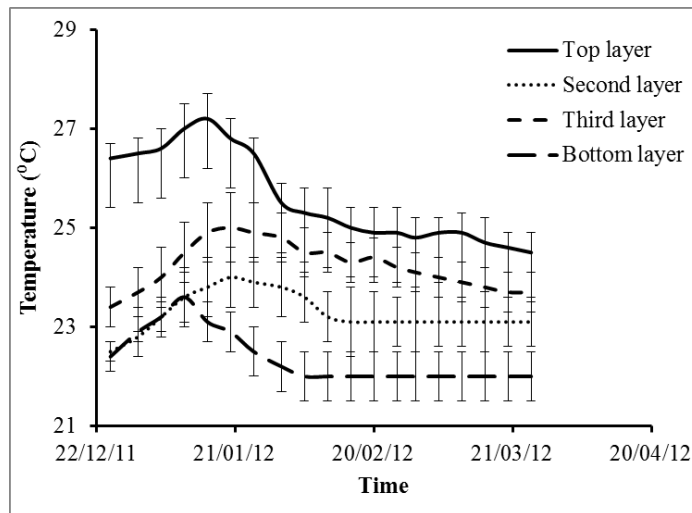
Fig. 2- Grain temperature at different layers of the bin.

Adult distribution in the first week

Adults were captured inside the entire bin in less than 24 h after they were introduced (Fig. 3). This indicated that the adults could move down more than 4 m in less than 1 d. In about 1wk of the adult introduction, adults moved down because the bottom layer had highest insect density (Fig. 3). This indicated that lots of adults did not stay at the warm top layers. The downward movement might be caused by the drift effect (Jian et al., 2009), and the crowding effect at the introduction location. This study found the same trend during that time period as has been reported in the literature that more than 70% of adults are found in the bottom half of small columns after they were introduced (Jian et al., 2009).
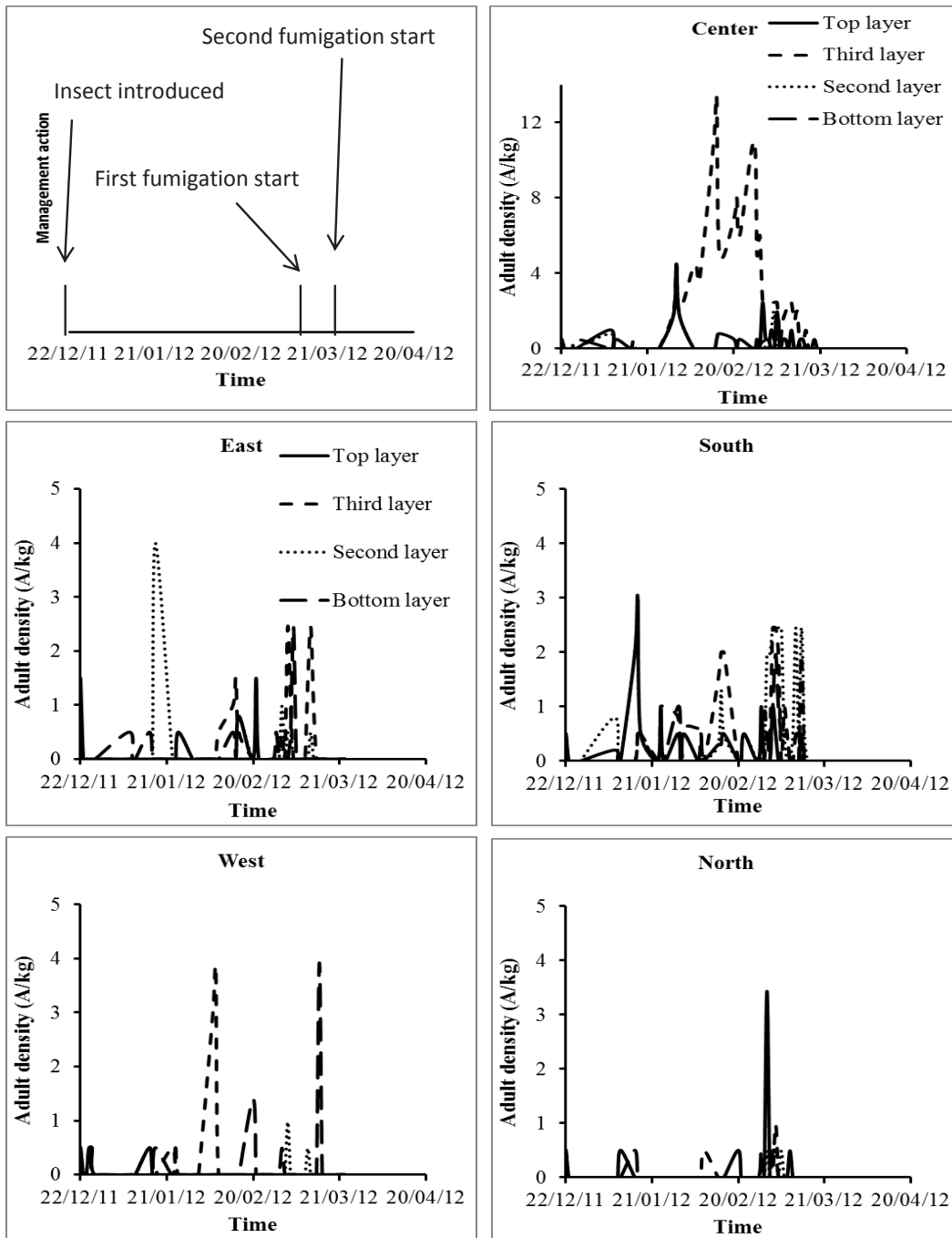
Fig. 3- Management action and adult density at each Insector® location.

Adult distribution in four weeks

Introduced adults gradually moved up because the top layer had the highest insect density in between 1 and 4 wk. Their distribution followed temperature distribution because the top grain was warmer than that in any other layer (Fig. 2 and 3). This result was consistent with the fact of that adults move to higher temperature regions inside a grain bulk in response to temperature gradients (Jian et al., 2009).

Descendant distribution

Descendants of the introduced insects were mainly found inside the third layer (Fig. 3). The insect density inside this layer during this time period was significantly higher than that inside any other layer (Tukey test and all P<0.0001). This result indicated that the distribution of the descendant adults followed temperature gradient except the top layer, which was the warmer layer, and did not move down. The descendants might also not move very often or very far because adult densities at other locations were significantly low (Fig. 3). This result indicated that the descendant adults might have different movement behavior than that of the introduced adults. If they did not move often or far away from the egg laying location, their distribution might relate to the egg laying behavior of the introduced adults.

Adult distribution in horizontal direction

There was no significant difference in insect densities at each half-radius locations in all 4 layers (Tukey test and all P>0.05). However, insect density at the center location was higher than that at other locations (Fig. 4) with more than 37% chance. The grain was loaded without using spreader and the dockage at the center location was about 8 times higher than at other locations. This high dockage at the center location might explain the higher insect densities at the center locations inside any layer (Jian et al, 2009).

Adult activity during the first fumigation period

Before grain was fumigated, CO_2 concentration was less than 0.05% which was the same as that without insect infestation. This indicated that adults of *C. ferrugineus* could not produce enough CO_2 to be detected by using the CO_2 Analyzer. During the first fumigation period, the CO_2 concentration was less than 5% at any location and there was no difference between different locations. At the end of the first fumigation, CO_2 concentration was less than 0.05% at any location. During this fumigation period, the measured insect densities at each location were significantly lower than that without fumigation (Fig.3, Tukey test and all P<0.0001). However, some adults were still moving because there were some captured adults during this time period (White et al., 1993).

Adult activity during the second fumigation period

After 44 h of the CO_2 introduction, the CO_2 concentration reached 73.4% at the center of the second layer. This was the highest concentration measured and achieved at the end of the CO_2 introduction. At the end of the CO_2 introduction, the mean of the CO_2 concentration was 60.6±2.3%. The lowest CO_2 concentration was 52.9%, which was at the center bottom. Two weeks later, CO_2 concentration decreased to 34.2±2.3%. During the second fumigation period, some adults were captured in the first 84 h. This indicated that not all adults were killed in 84 h. There were no captured adults after 4 d of the CO_2 introduction.

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EVALUATION OF CHLORINE DIOXIDE GAS AGAINST EGGS, LARVAE, AND ADULTS OF *TRIBOILUM CASTANEUM* AND *TRIBOLIUM CONFUSUM*

Lakshmikantha H. Channaiah[1,*], Chassity Wright[2], Bhadriraju Subramanyam[1], and Dirk E. Maier[1]

[1]Department of Grain Science and Industry, Kansas State University Manhattan, Kansas 66506, USA
[2]Tuskegee University, Tuskegee, Alabama 36088, USA
*Corresponding author's e-mail: *kantha@k-state.edu*

ABSTRACT

The susceptibility of eggs, young larvae, old larvae, and adults of the red flour beetle, *Tribolium castaneum* (Herbst), and confused flour beetle, *Tribolium confusum* (Jacquelin du Val), to chlorine dioxide gas was determined in the laboratory. Insect stages were exposed to gas concentrations of 248.4, 331.2, 413.9, and 496.6 g/m^3 for 1.53, 2.07, 1.80, and 1.68 h, respectively, in a specially constructed enclosure with and without food (5 g of wheat flour). These four concentrations provided gas dosages (ct products) of 380.1, 685.6, 745.0, and 834.4 gh/m^3. Wheat flour was used to simulate unsanitary conditions found in food processing facilities. Young larvae of both species succumbed to chlorine dioxide gas only at the highest dosage exposed. The presence of flour enhanced larval survival. Old larvae of both species showed 0 to 31% mortality in the presence or absence of flour. All adults of *T. confusum* died when exposed to chlorine dioxide gas dose of 745.0 and 834.4 gh/m^3. At these dosages all *T. castaneum* adults died only in the absence of flour. In general, *T. confusum* tended to be more sensitive to chlorine dioxide gas than *T. castaneum*. The presence of flour protected insect life stages from the lethal effects of the gas. These preliminary tests show chlorine dioxide gas to have potential in controlling certain life stages of these two species. Additional tests are needed under laboratory and field conditions to establish baseline dosages that provide effective control of life stages of *Tribolium* spp. and other stored-product insect species commonly associated with food processing facilities.

Keywords: Chlorine dioxide, *Tribolium castaneum*, *Tribolium confusum*, life stages, efficacy, novel fumigant

INTRODUCTION

The phase out of the fumigant methyl bromide (MB) in 2005 in the United States, except for certain critical uses, has created a huge challenge for millers, food-processors and fumigators to find effective and economical fumigant alternatives. Two fumigant alternatives to MB include phosphine as ECO_2Fume, and sulfuryl fluoride (SF). Phosphine in the United States is commonly used for fumigating bulk grains, and use by the food industry for whole structure

treatment is limited because phosphine is corrosive to metals (Bond et al., 1984). SF, registered as ProFume[TM], is less effective at temperatures below 26.7℃ (80°F), especially on eggs of stored-product insects and effective kill of eggs requires higher doses or longer exposure times (Bell and Savvidou, 1999; Hartzer et al., 2010). Concerns have been raised about the global warming potential of SF (Anderson et al., 2009). More recently, there is a debate about revoking tolerances of SF because of its contribution to fluorine residues in food and the adverse effects associated with it (http://www.fluoridealert.org/sf/index.html; http://www.fluoridealert.org/ sf/nov-2006.pdf). The US-EPA is currently reviewing data and comments from end users to decide on its future fate. A non-fumigant MB alternative is the use of elevated temperatures (Brijwani et al., 2012). Heat treatment is not suitable for all facilities, and it is more expensive than MB and may cause adverse effects to structural components of a facility if temperatures exceed 60℃. Hence, there is an urgent need to look for an alternative fumigant source to control stored product insects in food processing facilities.

Chlorine dioxide (ClO_2) is a powerful oxidizing agent being used increasingly to control microbiological growth in a number of different industries (Han et al., 2001, 2003; Du et al., 2002, 2003; Singh et al., 2002; Huang et al., 2006; Isomoto et al., 2006; Mahamoud et al., 2007). This gas has not been evaluated against stored product insects. Like MB a chemical that is effective against microorganisms as well as insects would be an ideal replacement for MB and SF. The present laboratory study was designed to evaluate the efficacy of ClO_2 gas against eggs, young larvae, old larvae, and adults of the red flour beetle *Tribolium castaneum* (Herbst) and the confused flour beetle, *Tribolium confusum* (Jacquelin du Val).

MATERIALS AND METHODS

The ClO_2 gas, which is a mixture of sodium hypochlorite and hydrochloric acid, was provided by Sterling Bridges, Palatine, Illinois, USA. Insect life stages of *Tribolium* spp. were exposed in a specially designed enclosure (113.3 m^3) made of polycarbonate plastic (Secador® Techni-Dome® 360 vacuum cabinet). The ClO_2 gas was generated by adding water to the mixture (2 ml/g of chemical), and gas concentrations were recorded with a ClO_2 sensor (Optek AF26, Optek-Danulat GmbH, Emscherbruchallee-Essen, Germany).

Eggs (1 to 2 d old), young larvae (first instars), old larvae (sixth to seventh instars), and adults of *T. castaneum* and *T. confusum*, reared on bleached wheat flour plus yeast (5% by wt) diet, at 28°C and 65% r.h. in a growth chamber (Model I-36 VL; Percival Scientific, Perry, Iowa, USA), were obtained following procedures described by Mahroof et al. (2003) for *T. castaneum* and Boina and Subramanyam (2004) for *T. confusum*. Unsexed adults of mixed ages of each species were directly collected from cultures whereas other stages were reared to a specific age in 150 ml plastic containers holding 20 g of the insect diet.

Fifty insects of each stage were exposed to ClO_2 gas dosages of 380.1, 685.6, 745.0, and 834.4 gh/m^3. Insects were exposed with and without 5 g of flour to examine the impact of food on efficacy against insects. All the fumigation experiments were carried out under the room temperatures (25 to 30℃). Life stages of *Tribolium* spp. handled similarly at 28°C and 65% r.h. but not exposed to ClO_2 served as the control treatment. Each dosage, species, and life stage combination, including the control treatment, was replicated three times. Adult mortality was assessed 24 h after exposure based on number of dead adults of the total exposed and the mortality was expressed as a percentage. The mortality of immature stages was based on rearing them to adulthood. Mortality of insects exposed to ClO_2 was corrected for mortality of insects observed in the control treatment. The mean ± SE of corrected insect

mortality responses of each species and life stage exposed to the four ClO_2 dosages were summarized in a table.

RESULTS AND DISCUSSION

Table 1 shows the mortality of *T. castaneum* and *T. confusum* life stages exposed to ClO_2 in the presence and absence of food. Mortality of *T. castaneum* and *T. confusum* life stages increased with an increase in ClO_2 dosage especially in the absence of food, and commercial kill was observed with young larvae and adults at 745.0 and 834.4 gh/m^3. Presence of food significantly decreased activity perhaps due to binding of the ClO_2 gas by food. Also, adults were more susceptible than the other stages.

Table 1. Responses of life stages of *T. casteneum* and *T. confusum* exposed to four dosages of chlorine dioxide gas.

Species	Stage	Dosage (gh/m^3)	Mean ± SE ($n = 3$) mortality (%)	
			Without flour	With 5 g flour
T. castaneum	Eggs	380.1	1.9 ± 0.9	2.7 ± 0.9
		685.6	2.8 ± 0.9	3.6 ± 0.9
		745.0	4.7 ± 1.6	3.7 ± 0.0
		834.4	9.3 ± 0.9	5.5 ± 0.9
	Young larvae	380.1	40.1 ± 2.7	6.8 ± 1.8
		685.6	60.1 ± 0.7	9.6 ± 2.4
		745.0	72.5 ± 1.3	13.7 ± 1.2
		834.4	100.0 ± 0.0	18.9 ± 1.2
	Old larvae	380.1	0.4 ± 0.4	0.7 ± 0.7
		685.6	6.0 ± 1.8	0.4 ± 0.4
		745.0	9.3 ± 1.8	0.0 ± 0.0
		834.4	18.8 ± 0.7	4.0 ± 1.2
	Adults	380.1	2.7 ± 0.7	0.0 ± 0.0
		685.6	18.7 ± 2.9	0.0 ± 0.0
		745.0	100.0 ± 0.0	77.3 ± 2.7
		834.4	100.0 ± 0.0	100.0 ± 0.0
T. confusum	Eggs	380.1	4.6 ± 0.9	1.9 ± 0.9
		685.6	7.4 ± 0.9	1.9 ± 0.9
		745.0	9.3 ± 2.4	1.9 ± 0.9
		834.4	11.1 ± 1.6	5.6 ± 1.6
	Young larvae	380.1	43.5 ± 2.7	16.3 ± 2.4
		685.6	87.1 ± 1.4	29.5 ± 1.4
		745.0	90.0 ± 0.0	38.8 ± 1.2
		834.4	100.0 ± 0.0	37.2 ± 2.3
	Old larvae	380.1	0.7 ± 0.7	0.0 ± 0.0
		685.6	12.7 ± 1.3	0.9 ± 0.4
		745.0	26.8 ± 0.7	6.7 ± 1.3
		834.4	31.3 ± 0.7	14.7 ± 1.3
	Adults	380.1	4.0 ± 1.2	0.0 ± 0.0
		685.6	29.3 ± 0.7	2.0 ± 1.2
		745.0	100.0 ± 0.0	100.0 ± 0.0
		834.4	100.0 ± 0.0	100.0 ± 0.0

Our results clearly demonstrate that the ClO_2 gas at 834.4 gh/m^3 can be used to kill 100% of young larvae and adults of *Tribolium* spp. The eggs and old larvae were most difficult to kill at the tested dosages. Exposing life stages to the same dosages over 24 h rather than 1.53 to 2.07 h may make the gas more effective. Eggs of stored product insects are the most difficult to kill using fumigants such as SF (Bell and Savvidou, 1999; Hartzer et al., 2010), and with heat (Yu et al., 2011). Additional laboratory tests with longer exposures and pilot scale field tests in a food processing facility are needed to determine viability of ClO_2 fumigant as an insect management tool.

ACKNOWLEDGEMENT

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SESSION 4

POSTERS

EFFICACY AND REGISTRATION STATUS OF ECO$_2$FUME 2 LG AGAINST *TRIBOLIUM CASTANEUM* HERBTS, *SITOPHILUS ZEAMAIS* MOTSCHULSKY AND *ORYZAEPHILUS SURINAMENSIS* LINNAEUS ON MILLED RICE IN INDONESIA

Sri Widayanti*, Idham S. Harahap and Sunjaya

SEAMEO BIOTROP, Jl. Raya Tajur Km 6 Bogor, Indonesia
*Corresponding author's e-mail: *s_widayanti@biotrop.org*

ABSTRACT

Efficacy study of ECO$_2$FUME 2 LG against *Tribolium castaneum* Herbts, *Sitophilus zeamais* Motschulsky and *Oryzaephilus surinamensis* Linnaeus on milled rice was conducted in experimental storage in SEAMEO BIOTROP, Indonesia. Using completely randomized design with four levels of dosage (i.e., 70 g/c.m. (1000 ppm), 52.5 g/c.m. (750 ppm), 35 g/c.m. (500 ppm) and 17.5 g/c.m. (= 250 ppm) and control, this study was done in 5 replications. Test insects were 200 adults of *T. castaneum, S. zeamais* and *O. surinamensis* per experimental unit. These insects were split into 5 groups and put in small plastic bottles, 40 insects per bottle. Bottles containing test insects were put randomly inside each experimental staple, including control. Parameters observed were mortality of test insects and the number of larvae that emerged from the eggs two weeks after fumigation terminated. Milled rice moisture contents were checked before and after fumigation. Fumigation was conducted for 36 hours under PVC fumigation sheets. Phosphine concentrations were checked at 2, 8, 20, 26 and 32 hours after fumigation. Upon completion of fumigation, mortality of test insects were counted and plastic bottles containing milled rice for test insects food were incubated for 14 days. Results showed that at all dosage levels tested, ECO$_2$FUME 2 LG effectively controlled *T. castaneum, S. zeamais* and *O. surinamensis*. Recommended doses based on this study were 20-70 g/c.m. (= 1000 ppm) and depend on exposure time. The longer the exposure time the lower the dose. However, for using high dose with sorter exposure time still need to be tested. The study results became the basis for the Ministry of Agriculture of Republic of Indonesia to issue a minister decree No.: 4198/Kpts/SR.140/10/2011 permitting the distribution of this fumigant in Indonesia.

Key words: ECO$_2$FUME 2 LG, *Tribolium castaneum, Sitophilus zeamais, Oryzaephilus surinamensis,* fumigant, fumigation, dosage, efficacy

INTRODUCTION

Basic management to control stored-product insects requires a combination of fumigation and surface spraying or fogging. In the past, fumigants available for this purpose in Indonesia are methyl bromide and phosphine. However, since Montreal Protocol took effect in 1995 and methyl bromide was phased out for grain storage in Indonesia in 2008, the only fumigant available for commodity maintenance in storage is phosphine.

Improper management to control stored-product insect pests causes high economic damages. Application of phosphine needs longer exposure time than methyl bromide. Fumigators, especially field workers, do not usually follow correct exposure time due to their impatience causing the failure of the control program and rendering the insects to become resistant to the fumigant used. Phosphine formulation that can be applied with shorter time could overcome this problem.

ECO$_2$FUME is a liquefied gas mixture of 2% phosphine and 98% carbon dioxide (CO$_2$) (by weight) packaged in high pressure aluminum or steel cylinders (Cavasin et al., 2008). ECO$_2$FUME application manual mentions that pressurized carbon dioxide serves as a propellant for delivering the product and enhances the fumigant's effectiveness by helping to quickly disperse phosphine into the space to be fumigated. Carbon dioxide retards flammability. Phosphine and carbon dioxide are both gases that, under sufficient pressure, can exist in a liquid state. It is this "liquefied gas" that is stored in the cylinder. The product is withdrawn from the cylinder as a liquid, but dispensed as a gas. In expanding from a liquid to a gas, it increases in volume by hundreds of times. As a new product in Indonesia, the effectiveness of ECO$_2$FUME 2 LG needs to be confirmed through efficacy trial on commodity.

The objective of this field efficacy trial was to evaluate the effectiveness of ECO$_2$FUME 2 LG (2% phosphine and 98% CO$_2$ by weight) against stored-product pests in milled rice, i.e *Tribolium castaneum* Herbts (Coleoptera: Tenebrionidae)*, Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae) and *Oryzaephilus surinamensis* L. (Coleoptera : Silvanidae).

MATERIALS AND METHODS

1. Time and location
The field efficacy trial was conducted from June to July 2011 at SEAMEO BIOTROP's Postharvest Research Warehouse for fumigation and at the Entomology Laboratory for the preparation of insect test, observation of insect mortality and number of insect spawns. SEAMEO BIOTROP is located in Bogor, Indonesia.

The temperature and relative humidity inside the warehouse during the fumigation period were in the range of 24.5 to 29.0^{o}C and 69 to 86%, respectively.

2. Materials and equipments
Materials and equipments used in this trial were: milled rice in polypropylene bag, test insects (*T. castaneum, S. zeamais*, and *O. surinamensis*), PVC plastic fumigation sheets, wooden pallets, sand snakes, full face masks, filter canisters for phosphine gas, standard application equipments for ECO$_2$FUME application, phosphine monitor, phosphine leak detector, thermohygro meter, grain moisture meter and other appropriate equipments.

3. Methods
3.1. Experimental design
Efficacy trial of ECO$_2$FUME 2 LG was performed using Randomized Complete Design. Four dosage levels of ECO$_2$FUME 2 LG tested were: 17.5 g/c.m. (= 250 ppm), 35 g/c.m. (= 500 ppm), 52.5 g/c.m. (= 750 ppm), and 70 g/c.m. (= 1000 ppm) and control (0 g/c.m. (= 0 ppm). Each treatment consisted of 5 replications.

Twenty five staples of milled rice were used as trial units. The volume of each unit was 1.15 c.m. All trial units were laid out randomly at Postharvest Research Warehouse.

3.2. Test insects

The test insects used for each experimental unit in this trial were 200 adults of *T. castaneum, S. zeamais*, and *O. surinamensis* which were collected from insects reared at BIOTROP's Entomology Laboratory. These insects were split into 5 groups and put in small plastic bottles with milled rice as a culture media. Each bottle contains 40 insects and covered with gauze to allow the fumigant gas to penetrate into the plastic bottles through it. Adult of test insects have been incubated in the milled rice media for one week before fumigation treatment. It was expected that the eggs of each adult test insects could be found in the media. The eggs that were laid by adults also were used for test insects.

3.3. Efficacy trial procedures

Plastic hose was mounted on each staple to measure the phosphine concentration during fumigation. Bottles containing test insects were put randomly inside each experimental staple, including control. PVC plastic cover sheets (thickness 0.15 mm = 150 μm) were used to cover each treatment which was infested by the test insects for fumigation enclosures. To avoid gas leaking, the edge of plastic sheets was pressed with sand snakes and also taped to the concrete floor. Procedure for applying ECO$_2$FUME followed the application manual issued by Cytec. ECO$_2$FUME cylinder was placed on top of a digital weighing scale with the capacity of 100 kg.

The ECO$_2$FUME liquefied gas, then injected into the enclosure using high pressure dispensing hose. Fumigation was conducted for 36 hours under PVC plastic cover sheets. Fumigation was terminated by aerating the enclosure until the phosphine concentration reached the threshold limit value (TLV), i.e. below 0.3 ppm then plastic cover sheets were removed.

Parameters observed were mortality of test insects and the number of larvae that emerged from the eggs two weeks after fumigation terminated. Milled rice moisture contents were checked before and after fumigation and were analized with digital grain moisture meter. Phosphine consentrations were checked at 2, 8, 20, 26 and 32 hours after fumigation started.

After fumigation was terminated, the adult test insects were removed from culture media in the plastic bottles for mortality counting, then plastic bottles containing milled rice for test insects food were incubated for 14 days until the eggs hatched. Adult test insects were re-collected and counted to find out how many were alive and dead. Mortality data was then used to calculate the effectiveness of fumigant according to Abbott formula, then analyzed by analysis of variance (ANOVA) with 5% difference level. Fourteen days after fumigation, the presence of individual larval offspring of test insects was observed. This revealed that the eggs were not killed by fumigant applications, so they can still continue their life until they hatch into larvae. The presence of the individual generation of test insects after fumigation was analyzed descriptively.

Treatments of tested fumigant is considered effective based on the following criteria: 1) test insect mortality is significantly more effective compared to the mortality in the control, 2) there is no development of egg into larva after incubation for 14 days on fumigation treatment.

RESULTS AND DISCUSSION

1. Efficacy of ECO$_2$FUME 2 LG

The effectiveness level of tested fumigant on test insects *T. castaneum, S. zeamais,* and *O. surinamensis* can be divided into 1) the effectiveness of the adult stadia and 2) the effectiveness of the eggs stadia.

1.1. Efficacy of ECO$_2$FUME 2 LG against adult stadia

Efficacy of ECO$_2$FUME 2 LG against the adults of *T. castaneum, S. zeamais,* and *O. surinamensis* based on corrected mortality at all doses tested : 70 g/c.m. (= 1000 ppm), 52.5 g/c.m. (= 750 ppm), 35 g/c.m. (= 500 ppm) and 17.5 g/c.m. (= 250 ppm) was up to 100%. The efficacy of the control (no treatment of fumigant) is 0% meaning there were no dead test insects found. Result of analysis of variance at 5% difference level on the efficacy of fumigant showed a significant difference between control and all doses of fumigant treatment i.e., 70 g/c.m. (= 1000 ppm), 52.5 g/c.m. (= 750 ppm), 35 g/c.m. (= 500 ppm) and 17.5 g/c.m. (= 250 ppm). There was no significant difference (α = 5%) between each dose treatment. It could be concluded that all dose treatments of fumigant are effective in controlling *T. castaneum, R. dominica,* dan *S. zeamais* compared to control (without fumigant).

ECO$_2$FUME application manual mentions that fumigation success depends on the use of appropriate doses, sufficient exposure period, correct application procedures and proper sealed enclosure. The recommended dose by Cytec for ECO$_2$FUME applications at temperatures above 26°C is 200 to 1000 ppm with a 36 hour exposure period or 500 to1000 ppm with a 24 hour exposure period. To maximize control, extreme care must be observed in sealing, higher dosages must be used, exposure periods must be lengthened, proper application procedures must be followed, temperature and humidity must be favorable.

1.2. Efficacy of ECO2FUME 2 LG against eggs stadia

Determination on the effectivity of the fumigant on eggs differed from determination on the effectivity of the fumigant on adults which is based on adults mortality of the test insects. The effectivity of the fumigant on eggs of the test insects was not counted from egg mortality because in this efficacy test did not have the initial count of the eggs. One week before fumigation, the milled-rice to be used for food of test insects was filled with adults of the test insects to allow the adults to lay eggs. Hence it was assumed that in the milled-rice of the test insects, eggs were already available. The number of eggs laid by the adult of the test insects was not counted.

To determine the effectivity of the fumigant on egg stadia, the presence of hatched larva was observed after the used milled-rice test media was incubated for 14 days after fumigation and after the removal of the test adults right after the fumigation finished i.e. at the time of counting of mortality of the test insects.

The number of larvae did not determine the effectiveness of the fumigant to eggs. Effectiveness of fumigant treatment depends on the presence or absence of larvae. The fumigant treatment is considered effective if no live larvae is found in a single treatment, and not effective when live larvae still exist.

The incubation results showed that in all doses of fumigant treatments i.e., either 70 g/c.m. (= 1000 ppm), 52.5 g/c.m. (= 750 ppm), 35 g/c.m. (= 500 ppm) and 17.5 g/c.m. (= 250 ppm), did not indicate any development of eggs into larvae, while the milled rice of untreated fumigant (control) contained larvae. At control, the larvae that emerged from 200 adults averaged 184 larvae of *T. castaneum*, 324 larvae of *S. zeamais* and 266 larvae of *O. surinamensis*. This shows that all test doses of ECO$_2$FUME 2 LG were effective to control eggs.

2. Milled rice moisture content

The high moisture content of commodity can affect its quality during storage. Therefore, in testing the efficacy of this fumigant, moisture content analysis was conducted both before and

after fumigation to determine the fumigant's influence in increasing the moisture content of milled rice. Based on samples analyzed, the moisture content of milled rice before and after fumigation were 15.9% and 15.7%, respectively. This fact proves that ECO_2FUME 2 LG does not cause an increase in moisture content that can result in decreased quality of stored milled rice.

3. Phosphine gas concentration

Phosphine concentrations were checked to ensure the target concentration was reached. During fumigation, if due to the leaks, phosphine concentration dropped down below the target concentration, topping up should be done. Topping up is done when concentration levels drop more than 10% from the target concentration. The ability to top up the gas is one of the advantages in using liquefied cylinderized phosphine like ECO_2FUME compared to tablets or plates to ensure the success of fumigation. Refumigation after the failure of a fumigation is costly and renders the insects to develop resistance.

The formulation of ECO_2FUME 2 LG can be applied with shorter time exposure than that of tablet, pellet, or plate because there is no need to react with oxygen to produce phosphine gas. Decrease in exposure time will help storage manager to distribute their product faster.

The benefits in using ECO_2FUME are: 1) more effective to control insects due to its ability to maintain minimum target concentration by top up any time during fumigation and reduced risk of target insects experiencing narcosis (hibernation due to localized exposure to very high phosphine concentration), 2) more efficient to utilize the gas with greatly reduced amount of phosphine required to maintain the minimum target concentration or concentration-time (CT) product of 100% efficacy, 3) more friendly to environment due to no waste deactivation and disposal of residue, 4) needs shorter time due to quick establishment of target concentration thereby reducing total effective fumigation time.

Based on this efficacy result, the Ministry of Agriculture Republic of Indonesia has issued a minister decree No.: 4198/Kpts/SR.140/10/2011 permitting the distribution of ECO2FUME 2 LG in Indonesia.

ACKNOWLEDGEMENT

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COMPARATIVE MORTALITY OF *LIPOSCELIS ENTOMOPHILA* (ENDERLEIN) EXPOSED TO ETHYL FORMATE IN EMPTY BINS

Wang   Dianxuan*[1], Li Hui[2]

[1]Henan University of Technology, Zhengzhou，Henan, P. R China
[2]Yunan Grain and Oil Science Institute,Kunming,Yunnan,P.R.China
*Corresponding author's e-mail: *wangdianxuan62@126.com*

ABSTRACT

Fumigation was carried out in a PVC bin that served as fumigation chamber, of 300 mm in diameter and 7500 mm in height. The bin was located in an empty horizontal warehouse. *Liposcelis entomophila* (Enderlein) served as test insect exposed to the fumigant in cages placed at heights of 7.50, 5.61, 3.74, 1.87 and 0.1 m from the bottom of the bin. Ethyl formate (EtF) was applied at a dosage of 15.1 μL/L, from different heights of 7.5, 3.74 and 0.1 m. When EtF was applied from 0.1 m height complete mortality was achieved at the height of 0.1 and 1.87 m.. While the mortality of the insects at the heights of 3.74, 5.61 and 7.5 m were near 94, 27 and 21%, respectively. When EtF was applied from 3.74 m height, 100% mortality was observed in the cages located at 0.1, 1.87 and 3.74 m high. While the mortality of the insect at height of 5.61 and 7.5 m was about 90 and 8%, respectively. When EtF was applied from 7.5 m height, complete mortality was achieved at all five heights. Results indicate that EtF can effectively control insects near the application point or when applied from top layers of the bin. Since EtF is heavier than air, it rapidly sinks after it is volatized. Therefore, for space fumigation, especially in large scale warehouses, it is necessary to assist the fumigant distribution by aid of mixing techniques.

Key words: ethyl formate, insect mortality, *Liposcelis entomophila,* space fumigation, empty warehouse fumigation, fumigant application method

INTRODUCTION

The stated owned grain in P. R. China is usually stored in large scale warehouses of 21 m to 30 m wide, more than 50 m long and 11 m high. The height of grain mass in these bulks is 6 m. Loading of grain should be carried out after the control of all insects in the empty bin. DDVP (dicholovos) is the fumigant of choice due to its quick effect in killing insects. But after dicholovos spray, there is a possibility that some survival insect population remain at top

of empty warehouse. Ethyl formate (EtF) is a liquid at normal ambient temperatures, it boils at 55°C, and vaporises readily at normal grain temperatures, its vapour was shown to be toxic to stored product insects (Muthu et al.,1984). It has the potential to serve as alternative to methyl bromide, it is faster in action than phosphine on insects and may contribute in relieving the selection pressure for resistance to phosphine. To some degree, EtF is an alternative to dichlorvos in large scale warehouses. Cao et al (2003) has reported its potential application in empty warehouses. However, the effectiveness of EtF fumigation at different heights of empty warehouses remained unclear. Vapormate™ is fumigant formulated by BOC Australia, a member of the Linde Group, and contains 16.7 wt% EtF in liquid carbon dioxide (Finkelman et al, 2010). Both EtF and Vapormate™ have not been registered and used as fumigant in China Therefore, the present work aims at investigating the differences in insect survival at different heights of a fumigated warehouse using EtF.

MATERIALS AND METHODS

1.1 Fumigation chamber
The fumigation chamber was made of PVC (to simulate a bin) of 300 mm in diameter and 7,500 mm in height and sealed at both ends by PVC material. The bin was located in an empty horizontal warehouse of 27 m wide, 60 m long and 11 m high. There was a small hatch on the 10 cm height and on the top of the PVC bin for the application of the fumigant and inserting the cages containing the test insects. The half life of pressure decay time from 500 Pa to 250 Pa was 43 seconds. The temperature was monitored by electronic sensors that were installed in the bin (Fig. 1).

1.2 Bioassays
Glass tubes of 70 mm long and 10 mm in diameter were covered at both ends by flax fabric to permit ventilation, served as insect cages. Thirty adults of *Liposcelis entomophila* (Enderlein) and some broken wheat were placed in each cage. For each height of the PVC bin, there were three replications in one group. Each group of cages was hung up with string to the heights of 7.5, 5.61, 3.74, 1.87and 0.1 m in the PVC bin that served as fumigation chamber. Another group of insect cages served as control outside the bin but located in the warehouse.

1.3 Ethyl formate and application
The fumigant EtF was analytical reagent of 98% purity produced by Bodi Chemical Plant, Tianjin, P R China. EtF was injected through a plastic pipe into an open Petri dish installed at heights of 7.50, 3.73 and 0.10 m from the bottom. The applied EtF dose of15 µL/L volatilized immediately after injection into the Petri dish. The temperature was in the range of 18° to 22°C during the fumigation. The mortality of insects was checked 24 h after the end of exposure to the fumigant and aeration using a small fan.

Mortality results of was analyzed by Duncan's new multiple-range test Processed with Date Processing System, edition V 7.05, developed by Hangzhou Ruifeng Information Technology Ltd., China.
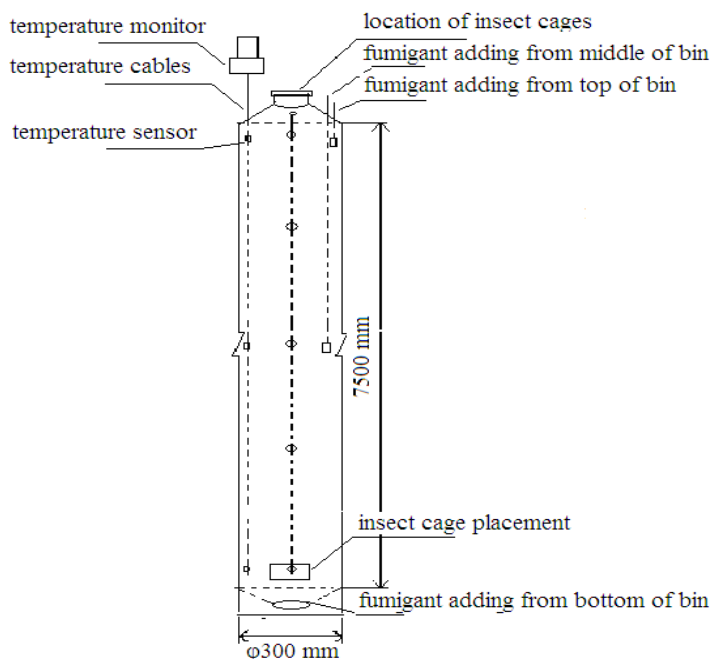
Fig. 1- Plan of PVC fumigation chamber and position of insect and fumigant introduction points.

RESULTS AND DISCUSSION

Mortality of *L. entomophila* adults exposed to EtF at different heights of the PVC chamber is shown in Fig. 2. In this fumigation, EtF was applied from the 0.10 m port. Fig. 2 indicated that the Insect mortality at 0.10 m and 1.87 m height was 100%. Whereas the mortalities at heights of 3.74, 5.61 and 7.5 m from bottom were near 94, 27 and 21%, respectively.

When the fumigant was applied from the point located at 3.74 m high, 100% mortality was observed in the cages located at 0.1, 1.87 and 3.74 m high. However, mortalities of the insects at 5.61 and 7.5 m height were about 90 and 8%, respectively.

When the fumigant was applied from the point located at 7.5 m high, complete mortality was achieved at all five tested heights.

The results indicate that it was not possible to achieve complete mortality for the height more than 2 m above the application point. As the height of fumigant application point was higher, the mortality was higher. This means that in space fumigation, EtF is more effective when applied from top layers. The natural reason is that vapor density of EtF is 2.55 times heaver than that of air (=1. The molecules would sink by gravity after application in the space.

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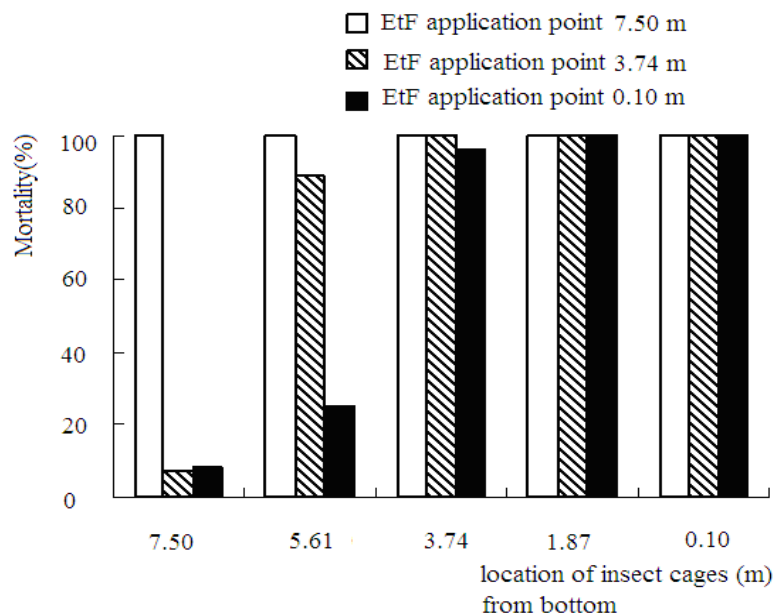
Fig. 2 - Mortality of *Liposcelis entomophila* adults exposed at different heights in the fumigation chamber and three application points of EtF. Fumigation temperature was in the range of 18° to 22°C.

EtF has been used successfully in disinfestation of food plant and machinery. Recently small-scale field trials have been carried out on dried fruits, wheat, barley, canola, and oats. All trials resulted satisfactorily without problems related to the application method, operator safety, insect control, commodity damage, ventilation, or residues at out loading (Annis, 2002). EtF fumigation has been reported using recirculation, or in combination with CO_2, or applied in a sealed system and allowed to mix freely by natural or forced air movement (Annis, 2002; Simpson et al., 2007). Vapormate™ is now fully registered for use in grain and horticultural products in Australia, in New Zealand for use in grain and for quarantine treatment of bananas and in Israel for dates and stored grains (Finkelman et al., 2010). EtF or Vapormate™ may be used as fumigation in future in China. The current results conclude further that for space fumigation in large scale warehouse, the recirculation or forced air is necessary for the even distribution of the fumigant to achieve complete insect control, especially at the top of warehouses.

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ETHANDINITIRILE (C$_2$N$_2$): TIMBER AND LOG FUMIGATION UPDATE

Chung-Gyoo Park[1], Jin-Hoon Cho [1], Max Yoo[2], Bong-Su Kim[4], Byung-Ho Lee[3*], YongLin Ren[4]

[1] Division of Applied Life Science, Gyeongsang National University, South Korea
[2] The Linde group, South Korea
[3] Dongbu ARI, Dongbu Hannong Co. Ltd, South Korea
[4] School of Biological Science and Biotechnology, Mordoch University, Australia
[*]Corresponding author's e-mail: *byungholee@dongbu.com*

ABSTRACT

Ethanedinitrile (C$_2$N$_2$) is a potential fumigant to replace methyl bromide (MB) for timber and log treatment. It is highly toxic to wood destroying pests and timber related insect pests such as *Anaplophora glabripennis* (Asian Long-horned Beetle)*, Monochamus alternatus* (Japanese Pine Sawyer), *Rhyzopertha dominica* (Lesser Grain Borer), *Reticulitermes speratus* (Japanese Termite), *Tomicus piniperda* (Pine Bark Beetle), *Hypanria cunea* (Fall Webworm) and *Bursaphlenchus xylophilus* (Pine Wilt Nematode). Several field trials have been reported at low temperatures in Korea. The trial reported here demonstrates control of natural infestations of PWN and of artificial inoculation of JPS larvae in pine logs at high moisture content. The L(Ct)$_{99}$ of C$_2$N$_2$ to larvae of JPS was 329.4 g h m^{-3} at low temperature (5±0.5°C).

Key words: Fumigant, Fumigation, Ethanedinitrile, Methyl bromide alternatives, Timber and log, Wood destroying pest

INTRODUCTION

In Korea, pine wilt disease (PWD) is the most serious cause of damage to pine forest systems (Kwon et al., 2005). It well known that the main vector of the Pine wilt nematode(PWN) is the Japanese pine sawyer (JPS). Under the Clean Air Act and Montreal protocol, the use of MB is not an option in Korea. Fumigation with metam sodium (MS) is the only one of registered practical option to prevent the spreading disease in Korea.

Since ethanedinitrile (C$_2$N$_2$) was developed in 1995, it has been demonstrated to be highly toxic to wood destroying pests and timber related insect pests such as *Anaplophora glabripennis* (Asian Long-horned Beetle)*, Monochamus alternatus* (Japanes Pine Saywer), *Rhyzopertha dominica* (Lesser Grain Borer), *Reticulitermes speratus* (Japanese Termite), *Tomicus piniperda* (Pine Bark Beetle), *Hypanria cunea* (Fall Webworm) and *Bursaphlenchus xylophilus* (Pine Wilt Nematode) (Ren and Lee 2008). The penetration of C$_2$N$_2$ into timber blocks occurred with and across the grain of the timber (Desmarchelier and Ren, 1996). Among the MB alternatives, C$_2$N$_2$ has excellent penetration and is the fastest to achieve even concentration throughout the timber (Ren et al., 2011). Efficacy of C$_2$N$_2$ to JPS, PWN, JT and YMB(Yellow minute bark beetle, *Crypahlus fulvus*) in natural infestations at low temperature

have been shown (Ren et al., 2005, Ren et al., 2006, Park et al., 2007, Ren and Lee 2008, Cho et al., 2011).

We report here trials of C_2N_2 against artificial inoculation of JPS larvae in logs infested with PWN.

MATERIALS AND METHODS

Artificial Inoculation of JPS Larvae into Logs

Pine wood was sawn from Korean red pine (*Pinus koraiensis*) that had been naturally infested with PWD. The Korean pine tree with 15-20 cm in diameter cut approximately 40-50 cm in length. Moisture content of pine trees averaged 18.8%. To investigate artificial inoculation, larvae of JFS were placed in pre-drilled holes in pine logs (Figure 1) and left for 1 day before fumigant was added. Larvae of JPS in these experiments were purchased in Kinsect Co. in Korea. Mortality of untreated pests was to be obtained same experimental conditions for 30 days.

| Drilling hole in the pine logs infested PWN and then JPS larvae was put inside holes | Covering with sawdust gently | Covered aluminum foil wrap and incubated at 5℃ |
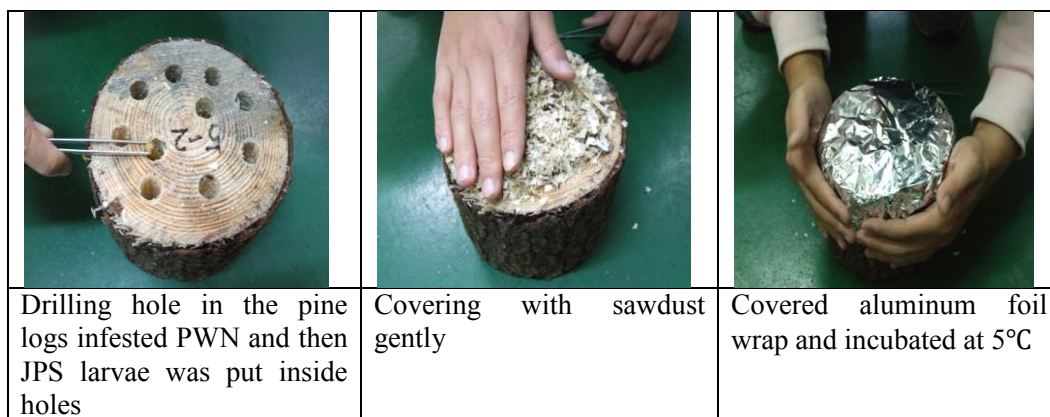
Fig. 1- Artificial inoculation process of Japanese pine sawyer larvae into the pine logs which infested with Pine wilt nematode

Fumigation trials under LDPE film

Field fumigation trials were conducted at the Kyungnam Province, Korea. Fumigation tents (100 × 100 × 100 cm) were constructed of wooden frame and covered with LDPE. Each tents had a load of similar sized dimensions of prepared woods. Wood loads per tent were approximately equal. Calculated amounts of EDN or MB were injected into the tents to give nominal concentrations (g m^{-3}). The amount of fumigant required was calculated from the ideal gas laws as outlined by Ren et al., 2011.

The air temperature in each chamber was recorded using Thermo Recorder (TR-71U). After 24hr fumigation, the fumigated tents were aerated for 24 hours with natural condition.

Measurement of EDN and MB concentration

Ethanedinitrile and MB were determined on a Agilent GC(7890A), equipped with a flame ionization detector (FID) after separation on a HP-5 Column (J &W Sci, 19091J-413). The oven temperature was 150°C. Injector and detector temperature were 200°C. During

fumigation, gas samples in the fumigation tents were pumped into tedlar bags and analyzed by gas chromatography. The concentrations of gas were calculated from the relative response of fumigation samples to gas standards.

Bioassays of pine wilt nematode and pine sawyer larvae
PWN mortality was counted after 7 days fumigation. Five randomly sawn timber blocks (approximately 2 cm thicknesses from the 30 cm bottom of logs with diameter size over 13 cm) were taken for bioassay. Nematodes were then extracted using the modified Baermann funnel procedure (Southey, 1985). In the assay of JPR larvae, each fumigated piece of wood was carefully removed from sawdust. All larvae were kept under natural conditions for 72 hours and then counted. Non-fumigated logs with artificial inoculation of JPS larvae were used for control mortality.

Determination of concentration × time products (Ct)
Concentrations of fumigant were monitored at timed intervals over the exposure periods (24 hours) and were used to calculate the product Ct = Concentration × time. The Ct products were calculated from Eq. 1.

$$Ct = \sum (C_i + C_{i+1})(t_i - t_{i-1})/2 \qquad \textbf{Eq. 1.}$$

Where: **C** is fumigant concentration (g m^{-1})
t is time of exposure (hours)
i is the order of measurement
Ct is concentration × time products (g h m^{-1})

RESULTS

Survival rate of JPS larvae at different conditions in artificial inoculation assay
Survival rate of JPS larvae at different temperatures in artificial inoculation assay without fumigant treatment are shown in Table 1. Mortality was very low for 3 d exposure at low temperature, such as 5°C or 7.3°C (Table 1). However, there were no survived JPS larvae founded at 25°C. For fumigant bioassays, the conditions chosen to minimize mortality were assessed for 3 days inoculation at 5°C.

Table 1. Number of alive Japanese pine sawyer larvae at different temperatures and periods after 3, 15 and 30 days inoculation in artificial inoculation assay without fumigant treatment

Temperature (°C)	No. inoculated insect	No. Alive insects		
		3 days	15 days	30 days
25±1	30	10	0	0
5±0.5	30	29	22	10
7.2±1.3	30	30	30	28

Toxicity of EDN to pine wilt nematode and Japanese pine sawyer larvae

Ethanedinitrile gave 100% control of JPS larvae and PWN when the Ct product was 329.3g h m^{-3} (Tables 2 and 3). This product was obtained for an initial application of 34 g m^{-3} for 24 hours exposure at 5.0±0.5$^{°}$C. The L(Ct)$_{99.0}$ of ethanedinitrile was estimated at 329.4 g h m^{-3} at 5.0±0.5$^{°}$C. Also, MB gave good control of JPS larvae and PWN when applied 80 g m^{-3} for 24 hours exposure at 5.0±0.5$^{°}$C; the Ct products of MB was 495.4 g h m^{-3}. This was lower than that reported by Barak et al (2005) who reported the Ct products was 1000 g h m^{-3} to *A. glabripennis* which applied in well sealed chambers.

Table 2. Efficacy of EDN and MB to larvae stage of Japanese pine sawyer larvae with artificial inoculation assay

Fumigant	Ct (g h m^{-3})	Time (hours)	Total insects	Alive insects	Dead insects	Mortality (%)	Temperature (°C)
Untreated	-	-	30	30	0	0.0	
EDN	152.5	24	30	28	2	5.0	
EDN	231.7	24	30	6	24	80.0	5.0±0.5
EDN	329.3	24	30	0	30	100.0	
MB	495.4	24	30	0	30	100.0	

Our trials demonstrated that EDN and MB can control PMN and JPS on cold and wet logs. As fumigation procedures were not ideal, it is possible that the dose can be further reduced. The novel bioassay procedure of artificial inoculation will provide a new tool for timber research. In summary, EDN has great potential for the treatment of pine wood logs to control insect pests and nematodes, particularly at low temperatures.

Table 3. Efficacy of EDN and MB to Pine wilt nematode

Fumigant	Ct (g h m^{-3})	Time (hours)	Mean No. of nematodes per 100g wood chips	Mortality (%)	Temperature (°C)
Untreated			8738	-	
EDN	152.5	24	4	99.99	
EDN	231.7	24	0	100	5.0±0.5
EDN	329.3	24	0	100	
MB	495.4	24	0	100	

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SESSION 5

MA and hermetic storage application technologies

Chairpersons:
Bhadriraju Subramanyam, USA
Cornel Adler, Germany
Mevlut Emekci, Turkey

GLOBAL CHALLENGES FOR THE SUCCESSFUL APPLICATION OF MA AND HERMETIC STORAGE

Shlomo Navarro*

Food Technology International Consultancy Ltd., P.O. Box 3300, Beit Yehoshua, 40591, Israel.
*Corresponding author's e-mail: snavarro@ftic.info

ABSTRACT

Storage insects are aerobic organisms requiring oxygen for their survival. Therefore, they respond to altered atmospheric gas compositions containing low oxygen (O_2) or high carbon dioxide (CO_2). To have an insecticidal toxic effect, a "high-CO_2 atmosphere" must contain a substantial proportion of CO_2, often more than 60%. Insect response depends on the species, developmental stage and age, the physical conditions in the environment, exposure time, and the type of the atmospheric composition used as treatment. Lowering the r.h. increases the effectiveness of MAs. Desiccation plays a large role in the mortality of stored-product insects exposed to some MAs. To obtain good control, the temperature should be above 21°C during the application of MA. The influence of temperature over the range of 38–42°C on the effects of hypoxia and hypercarbia on insects was demonstrated. The main cause of deterioration of dry grain is insects. While the main cause of deterioration of moist grain is microflora. Therefore, hermetic storage may be addressed to dry grain or moist grain storage. Hermetic storage takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the MA by reducing O_2 and increasing CO_2 concentrations through respiratory metabolism. An ingress rate of 0.05%O_2/day is sufficient to arrest the theoretical weight loss, caused by insects or microflora, at a level of 0.018% over one year storage period. For dry grain storage, this level is critical since even at short storage periods of 3 to 6 months at this ingress rate, the possibility of a residual surviving insect population is eliminated at an economical threshold. This low O_2 ingress level, is difficult to obtain in rigid structures, but is achievable in practice using flexible liners. It could serve as a guideline for the sealing specifications of structures appropriate to the hermetic storage method.

Key words: modified atmospheres, hermetic storage, carbon dioxide, low oxygen, stored-product insects, stored-product microflora, desiccation, respiratory metabolism

INTRODUCTION

The beneficial effects of modified atmosphere (MA) treatment as an alternative that is safe and environmentally benign, to the use of conventional residue-producing chemical fumigants for controlling insect pests attacking stored grain, oilseeds, processed commodities and packaged foods have been well documented (Navarro, 2006). Serious interest in using the technique in a practical, routine manner was not pursued until the 1970s and 80s, probably due to the success of conventional fumigants and grain protectants in controlling

stored-product pests. During this period, a realization began to develop that the chemicals, if used improperly, left objectionable residues, were hazardous to apply, and that there was a potential for the development of insect resistance to them. Research was initiated during this time in Australia, in the U.S. and several other countries on the use of modified atmospheres (Ripp et al., 1984). MA and controlled atmosphere (CA) treatments for the disinfestation of dry stored products have received increasing scientific attention during the last 32 years. Although CA has become well established for control of storage pests, its commercial use is still limited to a few countries. The widespread scientific activities on this subject resulted in several international conferences, such as the International Conferences on Controlled Atmospheres and Fumigation in Stored Products with the report of the last meeting by Daolin et al. (2008), and the International Working Conferences on Stored-Product Protection with the report of the last meeting by Carvalho et al. (2010).

Reviewing the reports on MA and hermetic storage carried out over the last 32 years reveals that more field trials were carried out on MA, CA and fumigation than on hermetic storage using flexible containers. Only in the last several years has hermetic storage emerged as a significant alternative method of post harvest storage, particularly in tropical climate countries using several hermetic storage methods (Villers et al., 2010) in South America using the silobags (Bartosik, 2010), the use of hermetic SuperGrainbags^{TM} for small farmers for rice seed since 2004 as reported by the International Rice and Research Institute (IRRI) (Rickman and Aquino, 2011) and in Africa the Purdue Improved Cowpea Storage (PICS) hermetic bags (Murdock et al., 1997; Murdock et al., 2003; Baributsa et al., 2010; Anon., 2012).

In spite of the numerous advantages of MA and hermetic storage have, these technologies still need additional field data and practical know-how. The present paper aims at describing the existing global challenges for the successful application of MA and hermetic storage.

GASTIGHTNESS OF THE STRUCTURES

Rigid structures
Structural requirements:
A fundamental requirement for the successful application of gaseous treatments to control stored-product insects is a well-sealed structure. Fumigants have been used for many years with limited requirements for structural tightness, and covering the grain bulk or the storage with plastic sheets was usually considered satisfactory. Lack of gastightness has for years been a problem for the application of fumigants in storage. The consequences of poorly sealed storages under fumigation are now more considered in view of the development of insect resistance to phosphine in poorly sealed structures (Casada and Noyes, 2001). The requirement for gastight storages for application of CAs and MAs appears to be more critical than for application of fumigants (Navarro, 1999). Therefore, before MA application, careful examination should be made of sealing requirements to obtain a standard acceptable for maintaining the gas composition over the designed exposure period.

Although practical guides for requirements for silo gastightness exist (Banks and Annis, 1977), they are very seldom implemented by the grain industry. Their specifications correspond to the pressure-decay times needed to maintain the atmospheric composition in the silos. These tests were designed to estimate the permissible limits for effectively maintaining the gas composition in the storages during the treatment (Navarro and Zettler, 2001). Comparisons of variable-pressure tests are scarce. A table was prepared to provide provisional guidelines based on the best estimates available in the literature (Navarro, 1999).

Accordingly, for example, for MA storage, with large structures of up to 500 tonnes capacity, a decay time of 5 min from 250 to 1250 Pa was regarded as satisfactory. To ensure successful application of MA in rigid structures, the grain industry should adopt the concept of sealing the structures adequately and run a suitable pressure test before MA treatment.

Cost of sealing:

A major challenge in the application of MA is to convert an existing structure into sufficiently gastight for the treatment (Burton, 1998). Although sufficient expertise has been gathered in countries like Australia (Newman, 2006), such expertise is lacking in many other countries that renders the initial cost sufficiently expensive to create commercial reluctance in the application of the technology. In practice, storage structures designed specifically for the application of MAs are practically nonexistent, apart from those in Australia (Ripp et al., 1984). Newman (1990) noted an increasing trend in Australia toward the use of sealed storage for dry grain, accompanied by the conversion of existing structures to sealed storage rather than construction of new installations.

In a recent study (Navarro et al., 2012a) cost of sealing of 2,400 tonnes capacity bin was 15,700 € or 6.54 €/tonne (AU$ 8.28/tonne) of grain. According to Newman (2006) "The costs of sealing a horizontal 21,800 tonne storage in 1982 was nearly AU$ 3/tonne, therefore the full cost of AU$ 64,400 amortised over 10 yr is AU$ 0.30/tonne. In 1999 the costs of sealing a storage ranged from AU$ 3.50 - 4.50 per tonne depending on the structure. Now in 2006 the costs are closer to AU$ 5 per tonne equating to AU$ 0.50/tonne over 10 yr using the previous example". This exemplifies the significant differences of sealing works carried out in a country like Australia with existing technological infrastructure and in a country that strives to initiate MA technology like Cyprus. The 2006 sealing cost in Australia was AU$ 5/tonne which may not be comparable to 2012 cost in Cyprus at AU$ 8.28/tonne. Although the costs of sealing any storage will depend entirely on the complexity of the task, the above figures may provide a perspective for the sealing challenge before MA treatment is initiated.

Flexible structures

Flexible structures can be used for MA/CA treatments and for the application of hermetic storage technology. However, currently, there are more flexible structures used for hermetic storage than for MA/CA storage in rigid structures (Navarro, 2006; Navarro et al., 2012b). It is assumed that flexible structures are easier to seal than rigid structures. However, gas loss through the structural membrane during gaseous treatments is an important phenomenon. Membranes of plastic permit gas permeation and gas exchange. Pressure tests, are not capable of measuring the degree of permeability losses. Since it is difficult to maintain complete gas tightness without any O_2 ingress into the large commercial structures, some tolerances that would permit quality preservation of the grain during hermetic storage should be established.

Parameters for testing gas tightness for hermetic storage of grain:

The following parameters were set for hermetic storage of cereals. Since this technology is relatively the newest and the terminology used is less elaborated, it creates much confusion of what is meant by hermetic storage of grain. This type of storage has been referred to a type of MA that can be applied for the protection of grain also termed as "sealed storage" or "air-tight storage" or "sacrificial sealed storage". This method takes advantage of sufficiently sealed structures that enable insects and other aerobic organisms in the commodity or the commodity itself to generate the MA by reducing oxygen (O_2) and increasing carbon dioxide (CO_2) concentrations through respiratory metabolism (Navarro et al., 1994; Navarro, 2012).

Respiration of the living organisms in storage (insects, fungi, and grain) consume oxygen (O_2), reducing it from near 21% in air to 1 to 2% while production of carbon dioxide (CO_2) rises from an ambient 0.035% to near 20% or higher according to the level of moisture content. This environment kills insect and mite pests and prevents aerobic fungi from growing. Elevated CO_2 and depleted O_2 levels will generally maintain stored grain quality for long periods. Grain with excessive moisture may be invaded by lactate-forming bacteria and yeasts. The key to successful hermetic storage is air tightness and control of condensation. In modern times, storage size has increased from small family storages to large bulks representing many producers or a portion of a country's total production.

The main cause of deterioration of dry grain is insects. While the main cause of deterioration of moist grain is microflora. The grain responds differently in the ecosystem of storage when it is at intermediate moisture but close to the critical level where fungi is the dominant microflora (Navarro and Donahaye, 2005). While at higher moisture levels, the dominant microflora are; mostly yeasts and bacteria (Elepano and Navarro, 2008; Weinberg et al., 2008). Therefore, hermetic storage may be used for storing dry or moist grain (Navarro and Donahaye, 2005).

For the application of hermetic storage to dry grain an ingress rate of $0.05\%O_2$/day is sufficient to arrest the theoretical weight loss, caused by insects or microflora, at a level of 0.018% over one year storage period (Navarro et al., 1994). For dry grain storage, this level is critical since even at short storage periods of 3 to 6 months at this ingress rate, the possibility of a residual surviving insect population is eliminated at an economical threshold. For higher O_2 ingress rates, the weight loss continues to rise in proportion to the O_2 ingress rate and insect damage might be very significant and cannot be arrested. Ingress rates of up to $0.15\%O_2$/day can be tolerated. However, for moist grain, at higher O_2 ingress rates than 0.15%/day, permits grain deterioration that might lead to development of mycotoxins (Weinberg et al., 2008).

This low O_2 ingress level, is difficult to obtain in rigid structures, but is achievable in practice using flexible liners. It could serve as a guideline for the sealing specifications of structures appropriate to the hermetic storage method. Flexible structures with higher O_2 ingress rates than $0.15\%O_2$/day, may be used to protect the grain from rain or increase of moisture provided the grain is dry and without any infestation. The question is whether these structures should be considered under the term of "hermetic storage" or just simply "sealed storage" without the expectations that they will develop a biogenerated atmosphere to protect the grain and use fumigation to control the insects.

Size of the flexible structures
Enclosures that are mostly destined for indoor hermetic storage of bagged commodities are now available in the market (PICS or Purdue Improved Cowpea Storage) (Anon., 2012; Baributsa et al., 2010; Baoua et al., 2012). The dimensions of the structure are dictated by the manageability of the stack. Unit containers in the range of 80 L to 120 L capacity named SuperGrainbags™ (SGB) exists (Villers et al., 2008; Rickman and Aquino, 2011). The SGB is a 7-layer coextruded plastic with thickness of 0.078 mm, 2.14 mL/(m^2 24h) permeability levels for oxygen and for water vapour of 4.28 g/(m^2 24h). These features of SGB maintain commodity quality, even with long transport times and in humid environments. Using the same material, the SuperGrainbag-HC™ has become available for use with mechanized loading, which handles up to a 1-tonne capacity for bags or bulk storage.

For outdoors hermetic storage of grain larger structures have been reported by Villers et al. (2008). The most widely used form of hermetic storage is the Cocoon™. It is

manufactured in capacities of up to 300 tonnes. Cocoons, used for storing grain commodities, are made from specially formulated flexible 0.83 mm thick PVC with permeability to oxygen varying from 87 to 400 mL/(m^2 24h) and to water vapour of 8 g/(m^2 24h). They are sealed with an airtight zipper. A newer type of Cocoon called the MegaCocoon™ has more recently been introduced for larger scale storage of up to 1050 tonnes.

Silo Bags of 200 tonnes capacity for on-farm grain storage are used directly in the field and, with the available handling equipment, is quite simple to load and unload. This technique was originally used for silage; it involves storing dry grain in sealed plastic bags. This sealed storage method adopted in South America is used for temporary storage of dry grain and oilseeds (Bartosik 2010).

The size factor in hermetic storages:
Experience shows that hermetic storage works best for large structures. This is obvious from the lower surface area/volume ratio in large bulks compared with small bulks. The factor of O_2 ingress rate, in practice is a goal difficult to achieve. Therefore, depending on the commercially available membrane permeability, engineers should aim at designing hermetic structures of sufficiently large dimensions. To emphasize the importance of the size of the structure in hermetic storage, calculations were made assuming a permeability level of 200 mLO$_2$/(m^2 24h) for structures of different dimensions ranging from 1 to 1,000 m^3 (Navarro et al., 1994). The calculations demonstrate that a tenfold increase in the volume of the bulk causes an approximate twofold decrease in the initial O_2 ingress rate. This indicates the importance that low-permeability liners must be preferred for hermetic storage at farm-levels in developing countries.

Gas permeation through the membrane:
Although insect respiration causes depletion in the O_2 level of the hermetic storage, to arrest insect development, a sufficiently low ingress rate O_2 is critical to control the insect population or to eliminate the possibility of a residual surviving insect population. Such critical residual O_2 level remaining in the hermetic storage structure is exemplified in Fig. 1, where insect respiration (4 insects/kg grain, each 157 μL/insect/day), the O_2 ingress rate, and its difference as the volume of residual O_2 remaining in the hermetic storage was plotted on the same graph. From Fig. 1 it is clear that the residual O_2 concentration would reach to about 5% in about 13.5 weeks.

This low O_2 ingress level is achievable in practice using flexible liners. It could serve as a guideline for O_2 permeability specifications of flexible liners appropriate to the hermetic storage method. For small volumes, such as bag size hermetic storage structures, a low permeability to O_2 is essential and for large volumes higher permeability levels can be tolerated. To exemplify such tolerances Fig. 2 was prepared that clearly shows the importance of selecting extremely low O_2 permeability liners when using small size (bag) hermetic storage units. According to Fig. 2, hermetic storage structures with capacities greater than 50 m^3 would require liners of a permeability level of 100 mLO$_2$/(m^2 day) for ingress rate of 0.05%O$_2$/day. For capacities of greater than 100 m^3, liners of permeability level of 400 mLO$_2$/(m^2 day) will be suitable for ingress rate of 0.15%O$_2$/day.
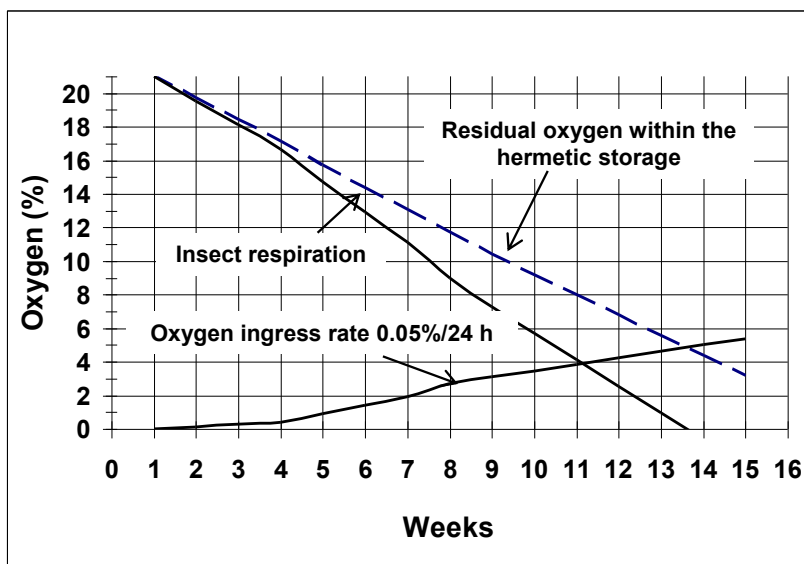
Fig. 1 - Insect respiration (4 insects/kg, each 157 μL/insect/day), O₂ ingress rate (0.05%/24 h), and its difference as the percentage of residual O₂ remaining in the hermetic storage to demonstrate the process of obtaining an O₂ depleted atmosphere in hermetic storage of dry grains.
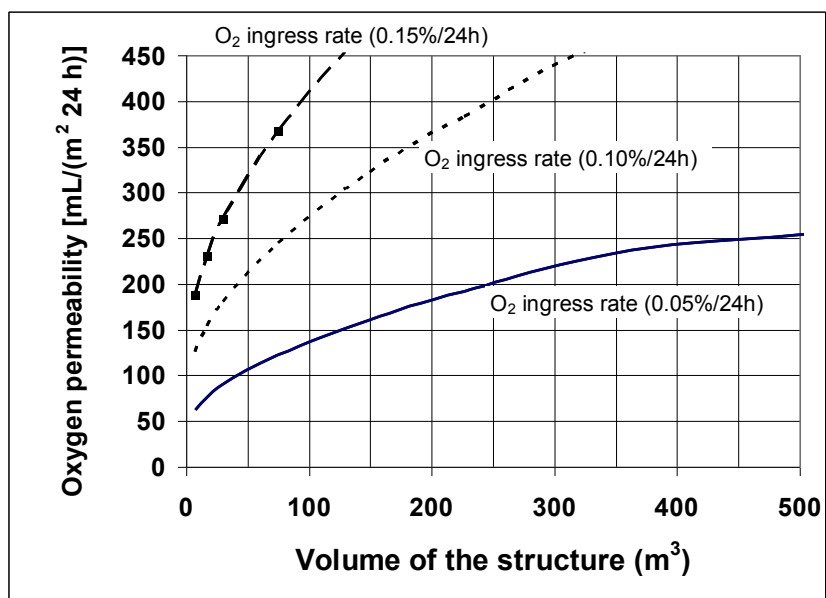

Fig. 2 – Oxygen permeability requirements [mL/(m² 24 h)] of liners in relation to various storage capacities (m³) and oxygen ingress rates (%/24 h) for successful application of hermetic storage of dry grain.

Liner durability and resistance to insects

Flexible packaging films vary in resistance to penetration by insects. A major drawback of flexible liners is that pests leading to infestation of foods can penetrate them. The degree of pest infestation of packaged foods depends upon the pest species involved, the time of exposure to invading pests, and the prevailing environmental conditions. There are two types of insects that attack packaged products: penetrators, insects that can bore holes through packaging materials, and invaders, insects that enter packages through existing holes, such as folds and seams and air vents. *Sitophilus* spp., *Rhyzopertha dominica* (F.), *Prostephanus truncatus* (Horn), *Plodia interpunctella* (Hübner), *Lasioderma serricorne* (F.), *Callosobruscus maculatus* (F.) and *Stegobium paniceum* (L.) are some of the stored product insects that are capable of penetrating the flexible liners destined for hermetic storage of grain or pulses.

With the increase use of hermetic storage technology in bags, farmers have quickly adopted the technology. The hermetic bags provide storage opportunity to farmers and consumers interested in organic and bio products. However, liner vulnerability to insect penetration places the technology at risk. A major challenge is therefore, to explore the possibilities of preventing insect penetration through the liner to eliminate the gastightness needed for successful application of the technology.

LETHAL ACTION OF MA ON INSECTS

Low oxygen and anoxia

Nitrogen (N_2) is commonly used to produce a low-oxygen atmosphere to cause anoxia on storage insects. Generally, the lower the oxygen level, the higher the mortality. For effective control, the O_2 level should be <3% and preferably <1% if a rapid kill is required. Although suppression of storage-insect development was observed at about 5% O_2, the exposure time required to kill the insects was very long. Experiments with *Tribolium castaneum* (Herbst) in N_2 showed significant differences in adult mortality between 0.1 and 1.0% O_2. Similar experiments with *T. confusum* in N_2 showed a critical oxygen level at 0.9%, and >1.4% O_2 was found to be ineffective. The adults are generally the most susceptible to the treatment and *S. oryzae* or *R. dominica* was demonstrated to be more tolerant than *Tribolium* spp. The lowest level of tolerance to lack of O_2 was attained around the 1% concentration level. There are more laboratory data for *S. oryzae* than for any other stored-product pest and, except for *Trogoderma* spp.

Effect of air relative humidity and MA

Lowering the r.h. increases the effectiveness of MAs. Results with adults of *T. confusum*, *T. castaneum*, and *Oryzaephilus surinamensis* (L.), have shown that, in atmospheres containing 99% N_2 (balance O_2), decreasing the r.h. from 68 to 9% increased the mortality from 3 to 98.5% in a 24-h exposure of the red flour beetle. These three species also exhibited a similar response to mixtures of CO_2 in air at lowered r.h.

Desiccation plays a large role in the mortality of stored-product insects exposed to some MAs. It was shown that when larvae, pupa, and adults of the red flour beetle were exposed to varying concentrations of CO_2 or O_2, weight loss was much higher in some of the atmospheres than in others or in air. A linear relationship of the combined effect of low O_2 or high CO_2 and r.h. in producing a lethal environment for *Ephestia cautella* pupae was shown (Navarro, 2012). In these trials the importance of the desiccation in relation to the ambient r.h.

as a result of opening the spiracles under the influence of low O_2 concentration was demonstrated (Navarro, 2006).

In contrast to these observations Murdock et al. (2012) attributed *C. maculatus* mortality to the dependence of the insect on carbohydrates for energy, carbohydrates must represent its main source of water. According to Murdock et al. (2012) the mode of action of hermetic storage, namely cessation of feeding, growth, development and reproduction and eventual death resulting from inadequate metabolic water due to lack of oxygen, may apply to a wide range of insect pests of stored products.

Effect of temperature and MA

At temperatures of 20–30°C, most species and developmental stages show >95% mortality in <10 d at both 0 and 1.0% O_2. *Trogoderma granarium* Everts larvae (12 d at 0% O_2), *S. oryzae* pupae (20 d at 0% O_2; >14 d at 1% O_2), and *Sitophilus granarius* adults (16 d at 1% O_2) are the only exceptions so far found. The influence of temperature on the length of time necessary to obtain good control with MAs is as important as with conventional fumigants. To obtain good control, the temperature of the grain should be above 21°C during the application of CO_2 (Navarro, 2006).

It was shown that, at 15.4°C, complete control of immature *R. dominica* was obtained after four weeks of exposure to 60% CO_2. Responses of larval, pupal, and adult stages of the nitidulid beetles *Carpophilus hemipterus* (L.) and *Urophorus humeralis* (F.) exposed to simulated burner-gas concentrations at three temperatures of 26, 30, and 35°C were reported. Comparison of exposure times showed that the effect of temperature on treatment efficacy was most pronounced at the 1% O_2 level, where, for the three stages of both species tested, values of LT_{50} at 26°C were about half those at 35°C. However, at 3% O_2 and 35°C, LT_{50} levels were only marginally reduced.

Eggs, larvae, pupae, and adults of *T. castaneum* to three low-oxygen concentrations at 26, 30, and 35°C were exposed. At all levels of O_2 (1, 2, and 3%), in typical respiration atmospheres under hermetic conditions (similar to burner-gas atmospheres), the LT_{99} values at 35°C were significantly lower than those at 26°C. Work on all four development stages of *E. cautella* showed the strong influence of temperature on mortality values when the insects were exposed to CO_2 concentrations varying from 60 to 90% in air.

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LONG TERM SEMI-UNDERGROUND HERMETIC STORAGE OF GRAIN

Jonathan Banks*

10 Beltana Rd, Pialligo, ACT 2609 Australia
*Corresponding author's e-mail: *apples3@bigpond.com*

ABSTRACT

Two trials, here called the Cunningar and Narrabri trials, were carried out to investigate the feasibility of long term (> 10 yr) storage of grain in underground and semi-underground structures. In the Cunningar trial 104 tonne of wheat, about 12.5% moisture content (w.b.) was held for 10 yr in a specially constructed, semi-underground, sealed concrete storage. The design was modelled on the semi-underground Argentinian stores constructed in the 1940s. In the Narrabri trial 160 tonne of wheat, about 9.3% m.c., were held in a polyethylene-lined, earth-covered semi-underground bunker for 34 yr. No infestation was found at outturn in either trial, but insects were added in one trial (Cunningar) to assist reducing the interstitial oxygen content rapidly to insecticidal levels and in the other (Narrabri) the grain was initially treated with malathion and later with phosphine.

Oxygen, carbon dioxide, temperature and relative humidity were recorded during the storage and at final outturn. In the Cunningar trial, oxygen levels fell to <2% after addition of infested grain as an oxygen absorber. Carbon dioxide levels remained low (<3%), presumably because the new concrete in the bins absorbed the metabolic CO_2. In the Narrabri trial, oxygen levels fluctuated from about 8 to 18% over most of the trial, but were low (<2%) at outturn. The low oxygen level was associated with water ingress late in the trial. This also resulted in some grain loss (30 tonne contaminated out of 160 tonne load).

Acceptable loaves could still be baked from the wheat after 10 yr of storage, but not after 35 yr. Falling number values were very high (>900 s) and the grain was not viable.

Key words: postharvest systems, long term grain storage, non-chemical alternatives, hermetic storage, modified atmospheres.

INTRODUCTION

Two long term grain storage trials are described here – the Cunningar and Narrabri trials. Both systems were intended to operate as hermetic stores. The first trial, the Cunningar trial, was initiated by S.W. Bailey. He was concerned over the use of chemical treatment of grain even at that time (the late 1950s) and sought to apply the basic knowledge of the mechanism of hermetic storage that he (Bailey, 1955, 1956, 1957, 1965) and Oxley and Wickenden (1963) had gained, to give a modern, functional and 'non-chemical' method of grain preservation. The second trial, the Narrabri trial, was carried out to determine the suitability of the then recently-developed, Australian earth-covered bunker system for long term storage of grain.

Neither trial provides a true example of pure, dry grain, hermetic storage, as in both cases there was intervention that altered the system. In the Cunningar trial, insects were intentionally added to accelerate the removal of oxygen. In the Narrabri trial, the wheat used was treated on inloading with malathion and the bunker was later fumigated with phosphine after mechanical damage to the seal was detected. Oxygen levels in this trial were not sufficiently low (i.e., <2%) to cause rapid elimination of insect pests. However, both trials provide data on oxygen loss and trends in quality changes during long term storage in systems that could probably be used for true hermetic storage. There is very little good contemporary information on this subject, apart from the studies of Burrell (1980) and Pixton (1980).

THE CUNNINGAR TRIAL

Background

Bailey's studies (1955, 1956, 1957, 1965) had shown that insect pests of stored grain could be killed by atmospheres containing less than 2% oxygen and, furthermore, that such atmospheres could be generated by holding infested grain in gastight systems under laboratory conditions. He also showed that it was the lack of oxygen and not, as then believed, the elevated CO_2 levels which eliminated the pests. He also noted that the quantity of product consumed and damage caused by the insects under fully sealed conditions was very small, with about 500 g of wheat starch per tonne of wheat stored metabolised to create the insecticidal conditions.

S. W. Bailey had constructed in 1960 an airtight silo approaching the size of commercial operations to investigate hermetic storage at a scale greater than under laboratory conditions. Storages incorporating the hermetic storage principle have been used in many parts of the world for centuries (e.g., Sigaut, 1980). Of particular interest at the time were the storages installed by the Ministry of Agriculture in Argentina (see Hyde et al., 1973), in which cereals had been stored successfully for periods in excess of 6 yr. These partly subterranean pits consisted of concrete walls up to ground level with an arched brick cover and were fairly easily rendered airtight. A design based on the Argentinian stores was chosen for construction of the store at Cunningar, NSW.

Design and gastightness of the storage

The storage contained two cells each of about 50 tonne wheat capacity, constructed of sprayed, reinforced concrete, and covered with an airtight seal, consisting of four layers of bituminised felt, each sealed to the next with hot bitumen, then a further coverage of sprayed concrete. The two cells had an ovoid cross section with an arched roof and had a common wall separating them (design shown in Fig. 1).

The effectiveness of the door seals and level of gastightness of the cells was checked using a tracer technique. About 3 m^3 medical oxygen was released into each empty cell, raising the oxygen level therein above atmospheric. Regression analyses on oxygen concentration with time showed leakage rates for the western (Near) and eastern (Far) bins of 1.9 and 4.2% d^{-1}, respectively, showing the Near bin to be well sealed, but the Far bin to be somewhat more leaky.

After modifications to reduce condensation problems on the metal inloading hatches, pressure tests for both the Near and Far bins for 500-250 Pa decay was 2 min 5 s.
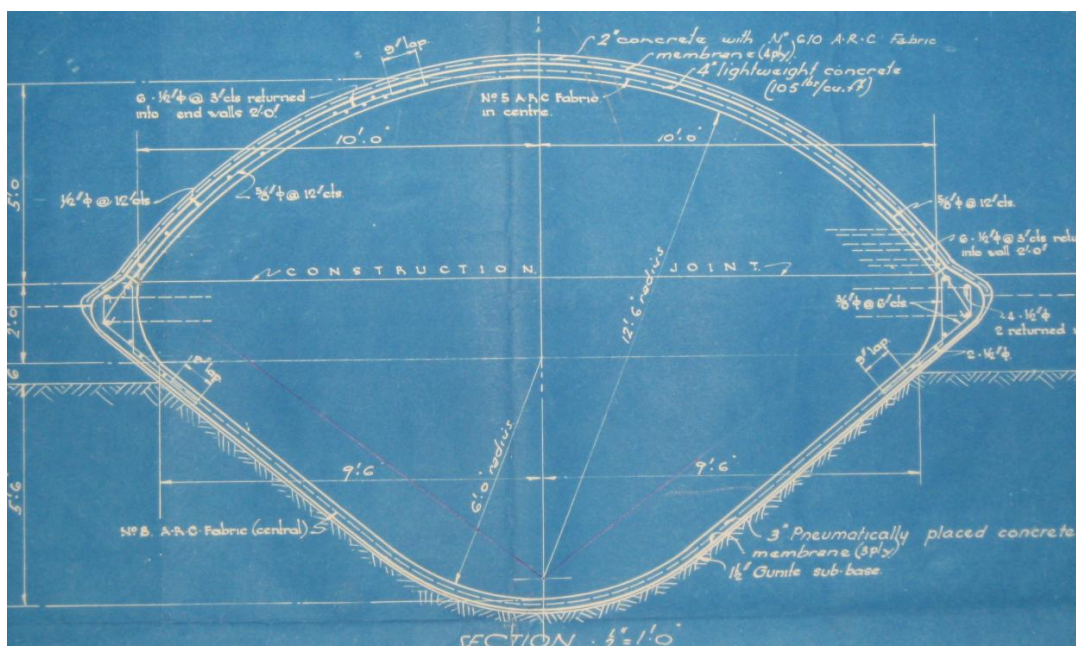
Fig. 1- Cross section of the Cunningar hermetic storage pit. From original 1960 construction blueprint.

The two cells were filled with 56 and 48 tonne FAQ wheat, probably at around 12% m.c, for the Near bin and Far bin, respectively. The two cells were sealed at 4:30 p.m. on 10 January 1962, soon after filling and conditions within the two cells were monitored during the subsequent 10 yr period of storage.

RESULTS

Oxygen concentration and infestation levels

The initial low rate of oxygen loss suggested that insects were either absent from the mass or were present in very low numbers. As the complete absence of insects could prolong the investigation unnecessarily and render the experiment unconvincing in terms of insect control, it was decided to open the bins and introduce a population of insects into one bin.

Approximately 100 kg of wheat in nylon gauze bags, heavily infested with all stages of several insect pests, primarily of *Sitophilus granarius* (L.) and to a lesser extent with *S. oryzae* (L.)*, Rhyzopertha dominica* F.*, Oryzaephilus surinamensis* (L.) and *Tribolium castaneum* (Herbst)*, were placed in the Near bin 251 days after the cell was originally sealed. The bags allowed the insects to escape but prevented the admixture of the heavily damaged culture grain.

The oxygen concentration in the Near bin was reduced much more rapidly after the addition of the insects, falling from atmospheric to 8.2% in the next 10 weeks, while during the same period the levels in the Far bin fell from 16.5% to 13.3%. The oxygen levels in the Near bin continued to fall over the succeeding weeks reaching a minimum of 1.8% on 30 January 1963 (4.5 months). The Far bin oxygen concentrations also continued to fall during this period, suggesting that either there was a leak between the two cells or there was a low infestation in the grain that did not develop substantially until the grain warmed up after winter

but then increased rapidly, depleting the oxygen to a similar level to that in the Near bin. The oxygen content in the Far bin reached a minimum of 2.7%. Oxygen concentrations in both cells then tended to even out and gradually increase to between 9 and 10% by the following autumn, peaking in October 1963 then dropping again during January and February 1964 to between 4 and 5%. This trend indicated that the contractors had been unable to achieve a sufficiently good seal to prevent ingress of some atmospheric oxygen. At no time since the sealing of the cells had the oxygen concentrations remained low enough for sufficiently long periods, i.e. <2% for several weeks, to ensure that all insects had been killed. To check this, the Near bin was again opened in June 1964 and samples taken. Visual examination showed the grain was still in a sound condition but 17 live *S. granarius* were found on the walls above the grain surface indicating a light general infestation. On further inspection a small patch consisting of 1-2 kg of damp grain was found at the lower edge of the hatch cover. This grain contained a few hundred adult *S. granarius*. The patch had become damp due to condensation on the inside of the steel hatch cover.

The Far bin was opened in September 1965 for inspection and modification. The grain was examined for insects and for condition. Samples were taken for culturing to check for presence of live immature stages and for milling, baking and germination tests. On the surface of the bulk fair numbers of dead *S. granarius* and a few dead *O. surinamensis* were found. No live or dead insects were recovered from the samples drawn from depth. Examination of the bin walls revealed one moribund *S. granarius* which died a few hours later. A small patch of mouldy grain was found at the lower edge of the loading hatch, resulting from condensation on the metal hatch cover. Examination of this grain in the laboratory revealed one live *S. granarius* adult.

The conditions inside the two cells continued to be monitored; the oxygen concentrations fluctuating in a cyclic manner generally reaching a yearly high around 5% in September/October and reducing to a minimum of between 1 and 2% in summer, around February/March.

Carbon dioxide levels

Only after 29 months of storage (June 1964) were appreciable (>0.1%) CO_2 concentrations observed. Presumably the alkaline freshly formed concrete structure of the cells was absorbing any CO_2 as it was produced. After 29 months, CO_2 levels ranged between 0.25 and 1%. Low concentrations were again detected in April 1966 reaching less than 0.5% in the Far bin and 2.5% in the Near bin. From November 1967 regular analyses were made. Concentrations in the Far bin remained fairly constant between 1 and 3.5% to the end of the experiment in May 1972. In the Near bin more CO_2 was found ranging between 3 and 7.2% with the peaks in CO_2 corresponding with the troughs in oxygen concentration.

Temperatures and relative humidities

There was an overall cooling of the grain from the time it was placed in the storage throughout the 10 yr of the experiment. The maximum average temperature of the grain in the Near bin was 26°C soon after filling, with yearly maxima falling progressively to 19.6°C after 10 yr storage. Minimum temperatures usually observed ranged from 13.2 to15.4°C in 1963 and 1964, but then fell steadily back to 13.4°C in 1971.

The average r.h. was 58% in the Near bin and 54% in the Far bin at the commencement of the storage period corresponding to moisture contents of about 12.7 and 12.0%, respectively. The r.h. also showed a tendency to fluctuate, reaching the highest each year around February and March and dropping around August. A gradual increase was

recorded over the 10 yr storage period to r.h. corresponding to final moisture contents of 14.6 and 12.6%, respectively.

As the construction of the cells incorporated a gas tight membrane, it is unlikely that moisture was gained from the surrounding earth or the atmosphere, so is probably associated with the increased metabolic activity. The Near bin had received an additional substantial quantity of living insect material, while the Far bin did not. Apart from this metabolic activity there was evidence of mycological activity.

Milling, baking and germination characteristics
Milling and baking tests on samples of the stored grain are provided in Table 1.
Germination of the grain after the 10 yr storage period was very low, the Near bin giving a figure of 1.25% of grains giving normal seedlings (5 grains in 400) and a further 0.5% (2 in 400) showing some germination activity. The Far bin fared worse, with 0.25% giving normal seedlings (1 in 400) and no other grains (0 in 400) showing activity. The operator conducting the germination tests noted an unusually heavy fungal infection developed on the grains during the tests.

THE NARRABRI TRIAL

Background
The design of the Cunningar trial storage was capital intensive and logistically inflexible. However, consideration of the fundamentals of its design and operation contributed substantially to the concept of the earth-covered bunker for emergency storage of grain. The first modern examples of this storage construction type were semi-underground and of a similar cross section shape to the Cunningar pit. A long pit was excavated and lined with polyethylene sheeting. After filling, the grain bulk was first covered with polyethylene and then some of the soil obtained from the excavation. The remainder of this soil was used to build the storage walls. Similar pits were recommended for storage of dry grain in South Australia in the 1930s (Spafford, 1939).

A prototype storage containing 1888 tonne of wheat was constructed at Narrabri, NSW in December 1975 (McCabe 1976). This prototype performed well, despite exceptional rainfall and flooding during the storage period, with a loss of about 31 tonne through water damage. Experience gained with the store indicated that earth-covered bunkers could be suitable for long term protection of dry grain.
The results of a trial to evaluate the system for long term preservation of grain are described below.

Storage construction
The storage for the trial was constructed at Narrabri, NSW essentially as described by Champ and McCabe (1983). The storage pit was excavated below ground level to a depth of 3 m. It was 2.4 m wide and 9 m long at floor level tapering out to 4.2 m x 12 m at ground level. The walls were built up to a further 0.6 m above ground level and the pit was lined with 150 μm black polyethylene sheeting. After filling, the grain bulk was covered with 150 μm polyethylene sheeting and then a further cover of double-coated 16 x 16 m woven polyethylene (Canvacon). These sheets were overlaid with 100 mm of river sand to facilitate the removal, at completion of the trial, of the nominal 900 mm of top cover of soil. A cross-section of the pit, showing construction details, is given in Fig. 2.
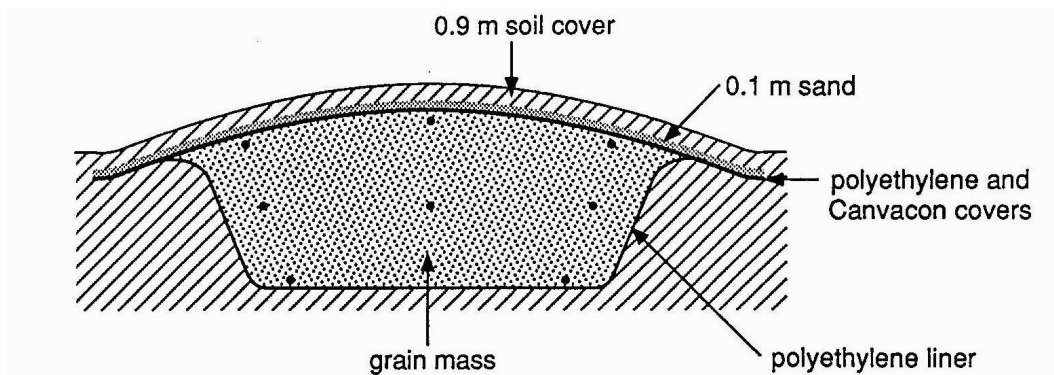
Fig. 2- Cross section of semi-underground Narrabri storage pit, showing construction detail.

Experimental design

The storage was filled in June 1976 with 160 tonne of Northern Hard 1 wheat. The wheat used had an initial moisture content of 9.0% and a temperature of 15°C. It had been treated with a grain protectant (malathion) at the nominal rate of 18 ppm one month prior to being placed in the storage and had a residual malathion content of 5.2 ppm at the time of sealing.

The storage was instrumented with thermocouples, humidity sensors (PCRC-11) and gas sample lines during filling. Relative humidity readings were converted to equivalent moisture contents using the wheat isotherm of Pixton and Warburton (1971).

Samples of grain were taken at the time of loading and also from time to time throughout the 34 yr storage for milling and baking tests.

RESULTS

Oxygen and CO_2 concentrations

The oxygen concentrations within the storage atmosphere fell steadily over the first 15 months to 14%, then tended to fluctuate between 9.6% and 17.7% before settling down around 14%. They dropped again between September 1985 and May 1987 after about 10 yr of storage to an average of 8.4% and in June 1991 were measured at 8.0%.

Carbon dioxide values fluctuated between 2.8% and 7% for three years of storage then tended to even out around 4%. An average value of 12% was observed after 15 years of storage (June 1991) after a three-year break in the series of readings.

The relative decrease in oxygen content and increase in CO_2 observed in 1991 would generally indicate some increase in metabolic activity in the bulk, but the five 10 kg samples of grain taken at the time disclosed no reason for the increase.

At outloading after 34 yr of storage, oxygen levels were <1%, with CO_2 levels at 20%.

Moisture content

The samples of grain taken in June 1991 had moisture contents (oven method) ranging from 9.1 to 9.8%, average 9.4%. These values compare with samples tested with a capacitance meter (Marconi model TF933A, St Albans, UK) at the time of loading which indicated an average of 9.0% and values deduced from readings from the r.h. sensors (PCRC-15) in the bulk which indicated a range of 8.8% to 9.4% during the first 15 yr storage.

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Temperatures

The grain was loaded into the bunker in winter and had an initial temperature of 15°C. Thermocouple readings at each visit to the site showed that grain temperatures near the surface fluctuated seasonally between 9°C and 34°C following winter and summer trends in temperature. Temperatures at the bottom of the store varied to a lesser degree, between 1 9°C and 25°C with a seasonal lag of about six months.

Grain quality

A comparison of milling and baking test results are shown in Table 2 for samples taken during the trial.

Table 1. Milling and baking test results for wheat samples from the Cunningar trial.

	Near bin			Far bin		
Test date	Nov 62	Oct 64	Aug 72	Nov 62	Nov 65	Aug 72
Storage period (yr)	0	1.9	9.8	0	3.0	9.8
Wheat						
Test weight (kg hL^{-1})	71.1	73.6	72.3	72.3	70.8	72.3
Moisture content (% w.b.)	13.2	14.8	14.3	12.2	14.6	14.1
Wheat protein (N x 5.7,%)	9.8	9.9	11.4	12.3	12.1	11.8
Acidity (mg KOH/100 g d.b.)	-	43	62	-	65	74
Flour						
Farinograph						
Water absorption (%)	55.0	53.0	57.4	60.5	58.4	59.2
Development time (min)	0.75	2.5	8.0	4.0	5.5	6.0
Diastatic activity (% maltose)	-	1.85	1.51	-	1.67	2.02
Extensograph (135 min)						
Length (cm)	19.1	18.5	18.3	22.9	19.8	15.6
Height at 5 cm (BU)	125	195	395	255	430	530
Maximum height (BU)	130	225	-	385	-	-
Area (cm^2)	39	62	45	123	166	144
Alveograph						
Stability	32	34	57	51	46	61
Strength	7	16	28	38	42	34
Extensibility (cm)	7.6	11.3	8.7	15.2	14.5	8.5
L/P	2.62	3.60	1.67	3.30	3.45	1.55
Baking test						
Score (%)	61	61	65	76	80	58
Volume (mL)	(Fair)	-	600	(Fair+)	770	550

After 34 yr of storage, most of the wheat, about 135 tonne of the initial 160 tonne, was recovered in good condition. It was free running, had no mouldy or other off-odours, was free of infestation and quite dry. A poor loaf could be baked from the grain and the grain

was sold for stockfeed. The main change observed in storage was a toughening of the gluten and an increase in Falling Number as expected from the low final α-amylase value.

Caked grain, in most cases containing a white mould, and also blackened grain was present on the base of the store and also particularly at the western edge of the store at ground level. In both cases it was clear that some water had been able to leak into the store. Two years prior to outloading, the top cover had suffered unauthorised damage, allowing rainwater to enter.

GENERAL CONCLUSION

Both the Cunningar and Narrabri trials, described above, demonstrate that long term storage of dry grain is feasible in some semi-underground or underground structures under conditions typical of the Australian wheat growing regions over 10-15 yr, but with some changes to wheat baking quality and also loss of germination. Insect infestation can be expected in freshly harvested grain as delivered to the central bulk handling system. This normally becomes easily detectable in untreated grain within 3 months of storage. In both trials, the grain at outturn after storage in excess of 10 yr was pest free. However, in neither case were insects eliminated by pure, unassisted hermetic storage. In one case insects were added to assist the process (Cunningar) and in the other (Narrabri) the grain was pesticide-treated before storage. The continued low oxygen content (<3%) maintained over a long period in the face of leakage of air into the system in the Cunningar trial indicates a significant level of metabolism. This may have resulted from the metabolism of damp grain but is more likely to be from the continued presence of a population of *Sitophilus* at a level below that normally detectable at outturn.

The grain in the Cunningar trial was initially at quite high moisture content, about 13%. This increased to over 14% during the 10 yr storage (Table 1).

Such an increase is to be expected as a result of the metabolic activity apparent from the continuing low oxygen levels and CO_2 production. Although a satisfactory loaf could be baked from the grain from this trial after storage there was a marked off-odour in the bread, possibly associated with the very high free fatty acid content.

In the Narrabri trial the grain was taken in very dry, with a moisture content of about 9%. Its quality was well maintained during the first 15 yr storage period. In contrast to the Cunningar trial, there was evidence that metabolic activity within the bunker was low, with oxygen levels generally being about 12%.

The maximum moisture limit for long term storage of wheat in systems such as used here is probably about 11.5% (see Annis and Banks, 1993, for discussion). This is low enough to have little metabolic activity and sufficient margin to allow for some moisture generation during storage without reaching a moisture content where rapid metabolism and mould activity can become a problem.

With correct control of moisture content, both systems described here appear promising for long term grain storage.

Underground bunkers similar to the Narrabri trial bunker were extensively used in Victoria during the late 1970s and were constructed in Iraq for long term storage of wheat. The design was the forerunner of the modern Australian plastic-covered bunker system. Use of the earth cover has been discontinued, though it may still be useful where very long term storage of grain is required, as it will provide the insulation needed to reduce moisture migration. Moisture migration can be a significant problem during long term storage in plastic-covered systems.

Table 2. Milling and baking test results for wheat samples from the Narrabri trial.

Test date	Jun 76	Oct 78	Aug 83	Jun 91	Sep 10
Storage period (yr)	0	2.3	7.2	15.0	34.3
Wheat					
Test weight (kg hL^{-1})	80.7	81.0	80.6	82.2	-
Protein (%)	13.3	13.5	13.5	13.5	12.9
Fat acidity (mg KOH/100 g d.b.)	10.4	17.5	27.2	-	-
Falling number (s)	527	553	810	1426	991
Milling yield (%)	77	75	76	74	78
Flour					
Protein (%)	12.8	12.3	12.5	12.5	12.3
Diastatic activity (mg)	225	182	195	-	-
Farinogram					
Water absorption (%)	67.1	62.0	66.5	67.5	64.3
Development time (min)	4.4	5.4	3.9	3.6	3.6
Extensogram (135 min)					
Extensibility (cm)	21.6	17.9	21.0	16.3	11.0
Maximum resistance (BU)	330	410	500	640	640
Viscogram					
Peak viscosity (AU)	520	750	783	-	-
Baking test					
Loaf score (%)	81	79	79	-	19
Loaf volume (mL)	860	840	775	693	438

Since these trials were initiated there have been many attempts to design and commercialise systems that truly work on hermetic storage principles. Some of these are described by Villers et al. (2008).

ACKNOWLEDGEMENTS

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WORLDWIDE USE OF HERMETIC STORAGE FOR THE PRESERVATION OF AGRICULTURAL PRODUCTS

T. De Bruin[1]*, S. Navarro[2], P. Villers[3], A. Wagh[4]

[1]President, GrainPro Philippines, Inc., Subic Bay Freeport Zone 2222, Philippines
[2]Food Technology International Consultancy Ltd., Beit Yehoshua 40501, Israel
[3]President, GrainPro, Inc., 200 Baker Avenue, Suite 309, Concord, MA 01742, USA
[4]Continent Manager, 463 Kamal Sagar RCF Colony, Bhandup, East Mumbai, India
*Corresponding author's email: *tdb@grainpro.com*

ABSTRACT

This paper discusses various forms of hermetic storage and transport, which preserve dried commodities such as grains (rice, maize, beans, wheat), silage, coffee, cocoa and seeds. Discussed are its limitations, and the expanding needs of 5 continents and 90 countries that use hermetic storage. Hermetic storage has proven especially effective in hot, humid climates to combat frequent, devastating post harvest losses and prevent the growth of mold related aflatoxins, a major public health hazard. With hermetic storage systems, post harvest losses are reduced to < 1% in hot, humid climates; this is in marked contrast to conventional storage systems requiring pesticides or refrigeration, which often reach 25% losses or more. Scientific information based on insect and commodity respiration, as well as the use of injected CO_2 (in some cases) to more rapidly decrease oxygen levels, is now a superior alternative to refrigeration for multi-month seed preservation. Finally, the paper addresses the cost effectiveness of hermetic storage versus alternative storage systems.

Key Words: Safe Hermetic Storage, Modified Atmosphere, Long Term Storage, Cocoon™, Insect Control, Flexible Storage Structure, Hermetic Bunker™, SuperGrainbag™, TranSafeliner™, Solar Dryer, SilBag™, GrainSafe™, Collapsible Dryer Case™, GrainKeep Center™

INTRODUCTION

The use of fully hermetic storage and the number of hermetic applications have grown rapidly since the last CAF conference in 2008 (Villers et al., 2008). Several scientific questions about the technology have been answered. The year 2012 saw the introduction of a number of new forms of hermetic storage such as the SilBag™, Collapsible Dryer Case II™ (CDC II), GrainSafe™, GrainKeep Center™ (GKC) and larger scale bulk storage. Semi-hermetic storages, such as the Purdue PICS (Purdue Improved Cowpea Storage) bag and the Argentine Silo Bag, have also been used primarily in Africa and Latin America for some applications.

The SilBag:
The SilBag™ simplifies the process of storing high quality silage for cattle and dairies, eliminating the need for extensive compression because of its airtight environment (Fig. 1).

Fig. 1- SilBag™ for cattle farms and dairies.

SuperGrainbag IV R™:
The SuperGrainbag IV R is made using a significantly tougher plastic than that of previous SuperGrainbags to provide a high level of insect resistance against larger grain borers and cowpea weevils, which have been known to penetrate thin plastic membrane walls.

GrainSafe XL™:
The new 10 to 50 tonne capacity GrainSafe XL is a small bulk storage device for larger farms.

IMPROVEMENTS FOR SUCCESSFUL APPLICATION OF HERMETIC STORAGE

Conditions for Implementation of Hermetic Storage:
Hermetic storage relies primarily on the respiration of insects, microorganisms, and the commodity itself (Villers et al., 2006a; Villers et al., 2006b). Any dry commodity that has been previously fumigated to control insects, may take weeks to reduce oxygen levels without the injection of supplementary carbon dioxide. Respiration rates of insects and the time required for oxygen levels to drop sufficiently is a strong function of initial infestation and ambient temperature. Further, the time it takes to achieve low oxygen levels in hermetic storage of dry commodities increases as the temperature drops significantly below 20°C; below this temperature, respiration rates begin to drop dramatically. On the other hand, respiration rates of wet commodities are dictated by the level of moisture content.

Supplemental Carbon Dioxide:
In certain applications, respiration alone is too slow in reducing oxygen levels. The injection of supplemental CO_2 speeds up the process of reducing the O_2 level to critical levels of <3% for insect survival and has been shown to be especially beneficial in the storage of peanuts, where the process may otherwise take 30 days or more (Navarro et al., 2012). It has also been used for organic fig storage (Ferizli and Emekci, 2000) and for the fumigation of flowers and books.

Oxygen Absorbers:
The insertion of an inexpensive oxygen absorber packet into small hermetic storage systems is more practical for field applications than the injection of CO_2.

Novel Scientific Data on Beneficial Use of Hermetic Storage:

The detrimental effects of high levels of aflatoxins on public health (i.e., HIV and cancer) have been studied for some time (Williams, 2011). In 2010, 10% of the Kenyan maize crop had to be rejected because of excessive aflatoxin levels. Many countries have restricted permissible aflatoxin levels for humans to five to 10 ppb. According to the 2011 World Bank report about grain losses in East Africa, "Due to the combined effect of aflatoxins and insect infestation, losses in a number of areas are 25% [for maize]" (World Bank Report, 2011).

Recent work on peanuts, summarized in Table 1 attempt to demonstrate that aflatoxin development was prevented at low O_2 atmospheres developed under hermetic conditions due to the respiration of peanuts only or at high CO_2 atmosphere generated by purge of the CO_2. The lack of the presence of the toxigenic strain of microflora did not produce sufficient evidence to determine whether aflatoxin growth was inhibited. But at the same time the tested hermetic conditions or high CO_2 levels did not promote aflatoxin development. On the other hand, the most significant data on peanuts was obtained on prevention of FFA growth. Table 1 shows that FFA increase can be prevented under hermetic storage with a low O_2 or a high CO_2 level atmosphere (Navarro et al., 2012). In commodities such as peanuts, decrease of O_2 through respiration alone (in the absence of sufficient insect population and at low moisture content) may take several weeks – too long to prevent significant aflatoxin growth and oxidation. The injection of CO_2 or the use of an oxygen absorber appears suitable means for generating the appropriate hermetic storage atmospheres.

Dr. Silverio Garcia-Lara at Technological University de Monterey, Mexico has recently successfully tested the use of a newer, more resistant plastic membrane (0.078 mm) (now known as the SGB IV R) to prevent penetration of hermetic storage bags by cowpea weevils (*Callosoburchus maculatus*(F.)) and larger grain borers (*Prostephanus truncatus* (Horn)), which have been known to penetrate earlier hermetic and semi-hermetic bag liners.

TECHNOLOGIES DEVELOPED FOR THE PROPER IMPLEMENTATION OF HERMETIC STORAGE

Collapsible Dryer Case (CDC):

Successful, long term hermetic storage requires proper drying to reduce moisture content below critical levels and prevent deterioration in storage. The availability of low cost, portable, rain protected solar dryers has greatly facilitated this drying of commodities prior to storage. During rainfall, the Collapsible Dryer Case II (CDC) with inflatable edges (Fig. 2) can be zippered shut, with one end pulled over the other.

Fig. 2- CDC III™ Collapsible Solar Dryer.

Table 1. Moisture Content (%), FFA (% oleic acid), Aflatoxin (µg/kg) values and CFU (Colony Forming Units) for molds at the beginning of the trials for the targeted 7 and 8 % moisture contents of peanuts after 90 days of storage at 30°C

Moisture Content (%)	Tested parameters	Initial	After 90 days				
			Hermetic sound peanuts	Hermetic with 3% broken peanuts	CO_2 with 3% broken peanuts	Control	Control with 3% broken peanuts
7	% Moisture Content	5.97±0.03	6.80±0.20	7.20±0.21	6.60±0.40	6.33±0.53	6.60±0.26
	FFA (% oleic acid)	0.36±0.01	0.63±0.53	0.70±0.17	0.43±0.07	0.57±0.03	1.50±0.12
	Aflatoxin (µg/kg)	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
	CFU molds	$3*10^2$	$1.8*10^3 \pm 1.2*10^3$	$1.7*10^3 \pm 7*10^2$	$9.7*10^1 \pm 28$	$1.3*10^4 \pm 9*10^3$	$4*10^4 \pm 3*10^3$
8	% Moisture Content	7.53±0.07	6.87±0.15	6.37±0.2	7.10±0.32	6.63±0.19	7.30±0.17
	FFA (% oleic acid)	0.42±0.09	0.67±0.17	2.13±0.07	0.77±0.03	2.57±0.47	4.00±0.42
	Aflatoxin (µg/kg)	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
	CFU molds	$3.1*10^2$	$8.4*10^3 \pm 5*10^3$	$6.3*10^1 \pm 18$	$1.2*10^1 \pm 4$	$7.6*10^5 \pm 5*10^5$	$7.5*10^5 \pm 2*10^5$

(Navarro et al., 2012)

Grainkeep Center (GKC):

Using the "spoke and hub" concept, the GKC "brings the market to the farmer" to within a 25 km radius (Fig. 3). The first two GrainKeep Centers are public/private partnerships owned and operated by a local entrepreneur (KPMC) in Kenya. Farmers can bring bags of grain to a hermetic storage unit within their own village and when the local storage unit is full, the bags are transferred to the Center. The Center can measure aflatoxin levels and provide access to simple, modern equipment such as threshers and dryers. Because the GKC provides safe hermetic storage for large volumes, it attracts large buyers such as the World Food Program (P4P). Typically, one GrainKeep Center can service about 2,200 farmers with a capacity of 2,000 tonnes. This significantly improves family income for approximately 11,000 family members.

Fig. 3- GrainKeep Center™ - Bringing the market to the farmer.

Guatemala:

The World Food Program (P4P) has widely distributed more than three thousand GrainSafes™ (Fig. 4) to farmers in Guatemala in response to the demand for medium sized hermetic containers for bulk grain with continuous "in" and "out" capabilities. Also, after testing metal and rigid plastic silos that require the use of fumigants, the Guatemalan farmers found that they preferred pesticide free hermetic storage (GrainSafes™).

Fig. 4- GrainSafe™, World Food Program, Guatemala, 2011.

Ghana:

The Cocoa Board of Ghana uses several hundred large Cocoons™ with a capacity of up to 320 tonnes to store cocoa beans (which are highly susceptible to rancidity) prior to exporting them. More recently, they started using TranSafeliners™ in shipping containers for protection during transport for organic cocoa.

Nepal:

In Nepal, a large number of small farmers have started using man portable SuperGrainbags™. Fig. 5 shows a farmer storing her corn in a Coop in Mulpani village near Kathmandu, Nepal.

Fig. 5- Maize storage in Nepal.

Afghanistan:
The largest scale application of SuperGrainbags for small farmers was funded by USAID in Helmand Province, Afghanistan in 2010 and 2011, along with large quantities of CDC rain protected solar dryers.

Brazil, Peru, and the Philippines:
Large scale storage of maize and rice seeds in Cocoons is also current in Brazil, Peru and the Philippines. Studies on rice seed by the International Rice Research Institute (IRRI) and PhilRice in the Philippines show that adequately hermetic storage can preserve rice seeds for up to a year with almost the same results as in cold storage, without the energy requirements. (Villers and Gummert, 2009; Sabio et al., 2006) Elimination of insect damage, reduction of moisture fluctuation, and low oxygen/high carbon dioxide atmospheres create ideal conditions for preserving germination and vigor.

<center>SAFE STORAGE FOR 5 MAJOR CROPS</center>

Seeds:
PhilRice and BPHRE in the Philippines have studied long term storage of hybrid rice seed extensively. They compared four methods: hermetic, cold room, air conditioning and unprotected PP bags. Table 2 shows that of the various storage technologies observed, hermetic storage was the most effective in controlling insect infestation and reducing weight loss (Sabio et al., 2009). In addition, the benefits of hermetic storage of rice seeds, and paddy over the alternative storage methods are further described by Villers and Gummert (2009).

Table 2. Mean percent germination rate of Mestizo 1 (PSB Rc72H) hybrid paddy seeds stored under different storage technologies and durations

Storage method	Storage duration (months)			
	0	3	6	9
Hermetic storage	96.16 abA*	96.47 a A	93.30 a A	86.15 b B
Cold room	96.80 a A	97.57 a A	92.95 a B	89.60 a C
Air conditioned	94.30 ab A	94.75 ab A	88.13 b B	85.82 b B
Control	92.87 b A	92.92 b A	76.38 c B	74.70 c B

*Means in a column followed by a common small letter are not significantly different at 5 % level of significance.
Means in a row having a common capital letter are not significantly different at 5 % level of significance.
(Sabio et al., 2006)

Coffee:
Farmers, traders, importers and roasters now use hermetic storage for coffee in some 20 countries (Aronson, 2005). *Coffee and Cocoa International* writes, "As coffee growers, logistics companies and roasters search for a way to protect high quality coffee during storage and transportation, one particular technique seems to be coming to the fore. Hermetic products are being used around the world to preserve the quality of coffee prior to roasting, and prevent the development of FFAs and OTA in cocoa" (Coffee and Cocoa International,

2011). Green coffee or cocoa can be economically stored and transported intercontinentally when hermetically protected with TranSafeliners™ (Table 3) (Villers et al., 2010).

Rice:
IRRI recommends hermetic storage for all rice growing regions because of reduced losses, improved germination and milling recovery (head rice) (Villers and Gummert, 2009).

Maize:
Dry maize stored hermetically in hot, humid climates, as described in *African Farming and Food Processing*, has storage losses of less than 1% and arrests increases in aflatoxin levels (Anon. 2011).

Cocoa:
Cocoa is especially susceptible to rancidity (free fatty acids) as well as to insect damage due to its high fat content. As mentioned earlier, the Ghanaian Cocoa Board successfully uses large Cocoons and SuperGrainbags to store cocoa for export (Fig. 6) and, more recently, TranSafeliners™ for protecting cocoa during intercontinental shipments (Jonfia-Essien et al., 2008; 2008b; 2010).

Fig. 6- Cocoon™ for storing 320 tonnes of cocoa at COCOBOD, Ghana, 2012.

ECONOMIC ANALYSIS

A simple method of comparing the addition of hermetic storage as an alternative to "traditional," non-hermetic containers are shown in Table 2 (Sabio et al., 2006).

Cost effectiveness of hermetic technology is an important consideration for all users. A GrainPro document (#LT2263PV1111-1, unpublished data) provides an interactive calculation of return on investment (ROI) and payback in years.

The costs of various methods used to store rice seed was investigated in the Philippines, an examination which showed hermetic storage to be the lowest total cost alternative (Sabio et al., 2006).

For protection during intercontinental transport of cocoa, Fig. 7, courtesy of Dorman (VolCafe, Kenya), shows a cost comparison of various ways of protecting coffee during shipment.
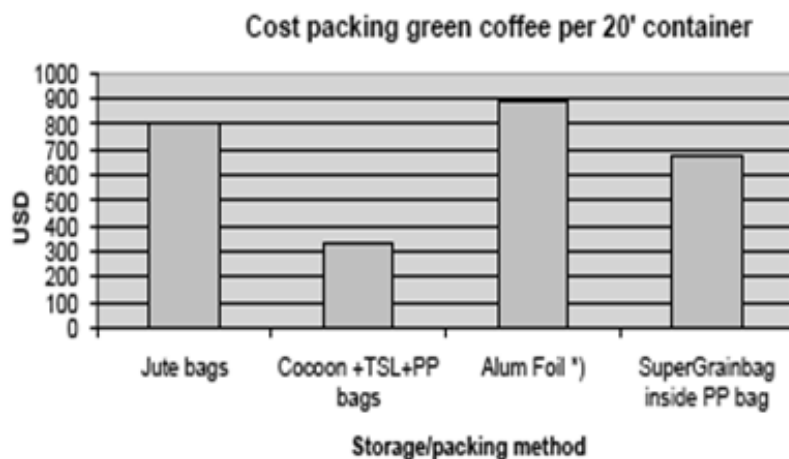
Fig. 7- Dorman (VolCafe, Kenya) shows a cost comparison of various ways of protecting coffee for intercontinental shipments.

CONCLUSIONS

Hermetic storage, when sufficiently airtight, is a modern, transportable, sustainable, chemical free, user friendly, "green" and cost effective solution to five previously difficult storage problems:

1) Protecting crops from insect infestation;
2) Preventing aflatoxin growth;
3) Preventing rancidity in commodities;
4) Safe, long term storage for quality preservation;
5) Suitable and economic for preservation of seed germination during storage;
6) Eliminating the need for pesticides, fumigants or refrigeration in storage.

Conventional storage in hot, humid climates has failed to protect stored commodities (Villers et al., 2006). Almost 25 years after the introduction of the first hermetic storage systems, countries can now better meet their food security requirements, reduce costs, and increase the incomes of their local farmers.

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STATUS AND PROSPECTS OF HERMETIC STORAGE OF RICE SEEDS IN THE PHILIPPINES

Caesar Joventino M. Tado[1]* and Martin Gummert[2]

[1]Philippine Rice Research Institute, Maligaya, Science City of Muñoz, Nueva Ecija, Philippines,
[2]International Rice Research Institute, DAPO Box 7777, Metro Manila, Philippines
*Corresponding author's e-mail: *cjmtado@yahoo.com*

ABSTRACT

Hermetic storage is a type of modified atmosphere storage that uses air-tight storage containers or structures that reduces oxygen (O_2) and increases carbon dioxide (CO_2) concentration through respiration of the rice seeds, insects and other aerobic organisms inside the sealed container. It provides for a sustainable, pesticide-free safe storage of seeds that prevents insect pest infestation particularly in hot, humid climates. In the past, the application of hermetic storage in the Philippines consisted of household storage of seeds of cereal grains and vegetables by individual farmers using "air-tight" containers. In the 1990s, the National Food Authority employed hermetic storage of milled rice using the Volcani Cube. In 2002, the Philippine government launched the Hybrid Rice Commercialization Program (HRCP) which promotes the utilization of hybrid rice seeds to boost rice production in the country. Since the recommended locally-developed variety, the PSB Rc72H, also popularly known as "Mestizo 1" is more vulnerable to insect pest infestation and fungi than inbreds, the seed producers resorted to use hermetic storage to preserve their costly seeds. By the time the HRCP program ended in 2005, several types of hermetic storage containers such as Volcani Cubes, GrainPro Cocoons™, Super Grain Bags, IRRI Superbags, and PhilRice-developed SACLOB have already been introduced to the market. Interest on hermetic storage of rice seeds waned during the post-HRCP years. Cognizant of its potential in the local rice seed industry, IRRI and PhilRice, with funding support from the Asian Development Bank, started advocating the hermetic storage technology to the seed growers and other stakeholders in the country. This paper presents the status of hermetic storage of rice seeds in the Philippines, constraints in its adoption, as well as future prospects of the technology.

Key words: rice seed, grain storage, modified atmospheres, pesticide-free alternatives, hermetic storage, quality preservation.

INTRODUCTION

The very high relative humidity and temperature in many tropical countries results in a faster respiration rate of stored paddy, increased insect activity and hastens the development of moulds. These factors, coupled with poor drying techniques, contribute to the deterioration of quality of the grains, which ultimately affects its germination and seedling vigor. In the Philippines, the minimum standard for germination of high quality seeds is 85% (Orge and

Abon, 2007). A drop in germination of the rice seeds below the standard would have a significant effect on the income of the seed producers, as this would mean around 50% reduction in the price of seeds.

Fumigants are still widely used to control storage pests due to their relative ease of application. Methyl bromide is a broad spectrum pesticide that has been used to control storage insect pests, pathogens and rodents. However, its use is being phased out in accordance with the Montreal protocol due to its effect on the earth's stratospheric ozone layer (EPA, 2008). In contrast, phosphine has continued to be the most widely-used fumigant for the control of stored-product insects, particularly in developing countries because of its low cost, ease of use, and absence of residues (Zeng et al., 2007; Abdullahi, 2010). However, the sustained viability of this fumigant has been challenged by the observed development of resistance of some insects toward phosphine in Asia, Australia, and Brazil (Nayak et al., 2003; Athie and Mills, 2005; Pimentel et al., 2006; Ahmedani et al., 2007; Pimentel et al., 2008).

Due to the problems associated with the use of pesticide-based pest control in storage products, non- chemical and environment-friendly methods of pest control in the postharvest sector are becoming increasingly important (Villers et al., 2007a; Silva et al., 2012). Storage problems often occur in high relative humidity and temperature, and in the presence of adequate oxygen. These problems are eliminated through a low O_2 and high CO_2 atmosphere produced through respiration processes of biological agents (Villers et al., 2010).

Hermetic storage is a type of modified atmosphere storage that uses air-tight storage containers or structures that reduces the O_2 and increases the CO_2 concentration through respiration of the rice seeds, insects and other aerobic organisms inside the sealed container. It provides for a sustainable, pesticide-free safe storage of seeds that prevents insect pest infestation particularly in hot, humid climates. It also prevents the development of cancer causing mycotoxins such as aflatoxins and ochratoxin A (OTA). The low permeability of the hermetic structure also maintains safe constant moisture levels in the stored product regardless of ambient exterior humidity (Villers et al., 2007b).

APPLICATION OF HERMETIC STORAGE IN THE PHILIPPINES

In the Philippines, paddy rice are commonly bagged and stored in warehouses at ambient conditions, which range from 29-33°C and 65-75% r.h. The seeds are exposed to ambient air, insects, rodents and birds. Losses in both quantity and quality are often experienced due to the consumption by insects, rodents and birds.

Moisture migration is the phenomenon that discourages the use of airtight storage in the tropics. With the advances in the development of sealed flexible plastic containers that reduce the intensity of moisture migration, the use of hermetic storage for outdoor, alternative or temporary storage facilities for use by farmers' organizations, cooperatives and seed growers became feasible in the Philippines and other tropical countries (Navarro et al., 1999).

In the past, the application of hermetic storage in the Philippines consisted of household storage of seeds of cereal grains and vegetables by individual farmers using "air-tight" containers. The attempts by the government to adopt this technology for storing grains started in the 1990's through joint research and development programs that were carried out by the Agricultural Research Organization (ARO) of Israel and the Philippine Center for Postharvest Development and Mechanization (PhilMech - formerly NAPHIRE and BPRE) of the Philippines to study outdoor storage of paddy and maize using hermetically sealed plastic liners called Volcani Cubes. Results of the experiments showed that under Philippine climatic

conditions, the gastight storage prevented moisture migration during 4 months of storage and provided acceptable protection by maintaining the number of live insects below the threshold of economic damage without the need for pesticides (Alvindia et al., 1994; Navarro et al., 1997).

Laboratory and field trials using sealed flexible structures of 10 tonne capacity also showed that paddy can be stored hermetically at 18% moisture content (w.b.) for up to 1 month, without any perceptible deterioration (Donahaye et al., 2001).

The investigation on the suitability of hermetic storage to store milled rice and its by-products was conducted by the National Food Authority. Laboratory experiments indicated that the quality of rice bran was preserved during 6 months storage (De Dios et al., 2007). Field results showed that after 3, 6 and 11 months of continuous storage, the oxygen concentrations in the Volcani Cubes dropped to 11.4%, 5.4% and 2.7%, respectively. The modified atmosphere also suppressed insect development, and the quality of milled rice remained high throughout the storage period. In contrast, the untreated control stacks stored under ambient conditions were heavily infested by insects after 3 months of storage (De Dios et al., 2001).

Promotion of the hermetic storage technology for paddy and maize to the Philippine countryside was conducted by PhilMech in the late 1990s (Estigoy, 2001), although the focus was for food and feed, respectively. The use of this technology for storage of rice seeds took a big leap during the implementation of the Hybrid Rice Commercialization Program (HRCP) from 2002 to 2005. With the launching of HRCP in 2002, commercialization of the hybrid rice technology became the Philippine agriculture's banner program in attaining self-sufficiency and increasing productivity and profitability in rice, and generating rural employment (Redoña et al., 2005). Hybrid rice is known to have a yield advantage of 15% over inbred varieties under the same input levels (Tu et al., 2000).

Since the recommended locally-developed variety, the PSB Rc72H, also popularly known as "Mestizo 1" was more vulnerable to insect pest infestation and fungi than inbreds, storing these seeds for up to 6 months, in time for the next cropping season became a big problem for HRCP implementers. Consequently, researchers investigated the technical feasibility and cost effectiveness of hermetic storage using Volcani Cubes or GrainPro Cocoons[TM] to store Mestizo 1 seeds, in comparison with low temperature storage technologies (Sabio et al., 2006). With its technical and economic feasibility ascertained, hybrid rice seed producers resorted to use hermetic storage to preserve their costly seeds.

The Philippine Rice Research Institute (PhilRice), being the procurer and distributor of hybrid rice seeds during the initial years of HRCP implementation adopted hermetic storage technology to store the large quantities of seeds that could no longer be accommodated in its cold storage facilities. During this period, PhilRice was able to develop its own hermetic container called "SACLOB", using locally-available and inexpensive material, with a simple but durable zipping mechanism (Orge et al., 2008). The material used was 0.8mm thick PVC tarpaulin sheet, with Velcro strip as zipping mechanism (Fig. 1).

Its performance (Fig. 2) was found to be comparable to the imported ones (Estoy et al., 2008; Gergon et al., 2011). Collaborative work of IRRI with GrainPro, Inc. on hermetic storage systems also led to the development of a 50 kg Superbag that fits inside the traditional storage bags that farmers can easily use to hermetically store small amounts of seeds (Rickman and Aquino, 2007; Villers and Gummert, 2009). By the time the HRCP program ended in 2005, several types of hermetic storage containers such as Volcani Cubes, GrainPro Cocoons[TM], Grainsafe[TM], IRRI Superbags, and PhilRice-developed SACLOB have already

been introduced to the market. However, interest on hermetic storage of rice seeds waned during the post-HRCP years.
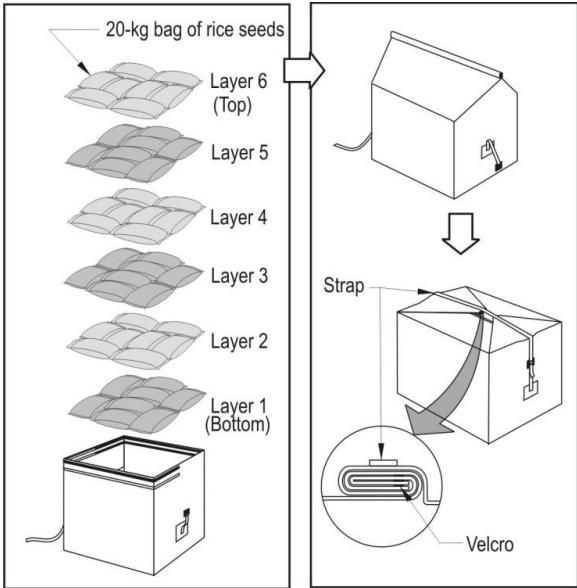
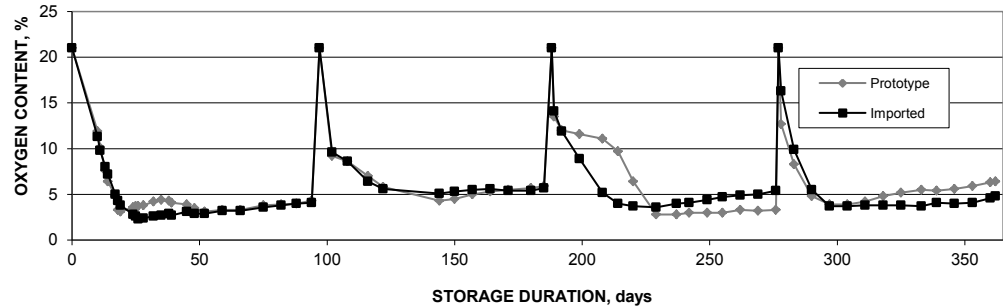Fig. 1- The SACLOB hermetic storage container developed by PhilRice

Fig. 2- Comparison between the oxygen level inside the SACLOB and its imported counterpart.

CURRENT EFFORTS TO PROMOTE HERMETIC STORAGE IN THE PHILIPPINES

Cognizant of its potential in the local rice seed industry, IRRI and PhilRice, with funding support from the Asian Development Bank, are currently advocating the hermetic storage technology to the seed growers and other stakeholders in the country. Information dissemination to seed growers and intermediaries are being done through the postharvest learning alliance, a multi-stakeholder platform where members freely share their experiences

and technologies to others. Adaptive research on the technology are also being conducted by farmers' groups in three pilot provinces in the country with the support from the private sector in terms of technology (GrainPro, Inc.) and funding (Catholic Relief Services). Constraints on the availability of Superbags and Cocoons[TM] are now being sorted out through partnership between the supplier and a large distributor of agricultural and veterinary products and supplies in the country. The project has also come up with information materials such as posters and video clips in the major local dialects. Finally, training on the technology has been integrated in the capacity enhancement programs of the government for its agricultural extension workers throughout the country under the Philippine Food Staples Sufficiency Program of the country's Department of Agriculture.

FUTURE PROSPECTS

With the increasing emphasis on chemical-free and environment-friendly methods of controlling pests in the postharvest sector, hermetic storage technology is seen to play a vital role in the Philippine rice seed industry in the near future. With the government's adoption of hybrid rice technology as one of the main interventions to attain its goal of self-sufficiency in food staples, many seed growers and other stakeholders will be using hermetic technology to store their precious seeds. The success of Bayer CropScience, one of the world's largest seed companies, in shifting from traditional warehouse storage to hermetic storage of its hybrid rice seeds (Villers and Gummert, 2009), points to the commercial viability of the technology on the large scale. On the other hand, the Superbag will also allow small farmers to use relatively cheap hermetic storage containers for their seeds. Finally, experience gained by farmers on the suitability of using this technology to store other products such as mungbean and cacao will further boost its application in the country.

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CONTROLLED ATMOSPHERE: LOW-OXYGEN DISINFESTATION OF POST HARVEST COMMODITIES IN CHAMBERS AND SILOS IN GREECE

Sotiroudas, V[1]

[1] AgroSpeCom Ltd., Food Safety and Hygiene, 3, N. Kountourioti street, 54625, Thessaloniki, Greece, Phone: +30 2310 543 125, Fax: +30 2310 543 147, Website: www.agrospecom.gr, *Corresponding author's e-mail: *v.sotiroudas@agrospecom.gr*

ABSTRACT

With the use of Controlled Atmosphere treatment (CA) based on low-oxygen and increased temperature, we have constructed gastight chambers in 7 locations in Greece and treated various products for commercial purposes since 2008. Additionaly we have used low oxygene atmosphere to control insects in post harvest commodities stored in silos.

Treatments in Greece are carried out by applying the controlled conditions in climate controlled rooms and normal silos using fixed or mobile installations of the EcO_2 technology. CA has shown to be effective under commercial use for controlling eggs, larvae, pupae and adult insects found present in many different commodities.

CA treatments have advantages over traditional fumigants, for commercial applications including worker safety, zero pest resistance and residue-free products. Products treated in Greece with CA are: sesame, nuts, rice, beans, flour, herbs etc.

Key words: Controlled atmosphere, low oxygen, disinfestations, stored product pest control, fumigation, quality preservation, insects.

INTRODUCTION

The use of Controlled Atmosphere (CA) to control insects in final products or raw materials was introduced in Greece in 2008 with the construction of a 6-chambers-complex for a sesame factory. CA was introduced to replace phosphine, to improve worker safety and logistic handling. The treatment of the organic line of products was succesfully accomplished as well. The CA technology and system of the Dutch company EcO_2 has provided the commercial side of the CA principles. These principles have been known for years but were difficult to apply succesfully and economically in a commercial way in the past. Nowadays the system is recognized and used in 17 countries world-wide by different industries.

All oxygen dependent insect species including their pupae, larvae or eggs lose their capability to live and develop vital functions when exposed during a period of time to total deprivation of oxygen as an integrated element of the ambient air mixture. In the ambient air, oxygen (O_2) is present in levels of approx. 21% and the element Nitrogen (N) is present with about approx. 79%.

Each insect species, their pupae, larvae and eggs requires a certain amount of oxygen to secure the capability to live and to use its full vital functions necessary to survive and develop naturally. The amount of oxygen required "both in volume and during a specific time" depends on the actual size, the life stage and the activity of the specie. This required amount of oxygen needs to be freely accessible and available under normal atmospheric pressure and in an ambient atmosphere (^{o}C, RH) to secure the vitality and full capability to live and develop naturally.

Oxygen deprivation as a tool to eliminate alive species and/or to handicap the vitality and stop irrevocably the capability to live and develop, implies that technically the availability and free access to oxygen for the species is diminished to an as near to zero level as technically possible during a predefined period of time. Whereby the maintenance of an ambient temperature, which is closely related to most viable ambient temperature for the species, is an additional factor to optimize the vitality drive of the species, their larvae and eggs.

The efficacy of oxygen deprivation as a tool to eliminate insects is dependent on physical factors such as temperature, O_2 concentration and duration, and on biological factors such as insect species, strain and development stage.

The maintenance of such low oxygen level and optimum ambient temperatures requires positioning the species in an enclosed environment/area in which continuously the actual presence of oxygen in the atmosphere and the ambient temperature can be fully controlled and maintained during a predefined period of time.

The CA principles that are used by the EcO$_2$ technology are based on the establishment of a low-oxygen environment able to kill insects of all stages. The principles of CA are established by means of an oxygen burner system or a nitrogen generator and applied to gastight treatment chambers or gastight constructed environments. In the case of gas-tight-chambers the low-oxygen atmosphere is applied in airtight environments with a volume from 40 to 100 m^3. In the case of silos, the conditions are met with the overflown of the silo with on-side produced Nitrogen. Commerical silos treated with CA under the EcO$_2$ systme had sizes of 100 to 2,000 tones. Some of the treated silos had no sealing at all while others were sealed with the use of special coating. Insects of all stages, present in the products treated, are eliminated (99.9 % lt) due to suffocation by the lack of oxygen and dehydration. This paper will describe the commercial application of CA in gas-tight chambers and silos in Greece as from 2008.

CA APPLIED IN GAS-TIGHT CHAMBERS

The CA system supported by EcO$_2$ was first chosen as the insect control treatment method by the largest sesame production factory in Greece. Since then more companies changed to CA treatments. See table 1 for an overview of CA applications in chambers.

With an experience of 4,5 years in commercial applications in Greece we can underline the following:

Main advantages of the method:
The duration of the treatments is comparable to phosphine. The precision of the method is much higher than in any commercial phosphine fumigation due to the monitoring and control software equipment that is installed in each terminal. The safety of the workers is guaranteed with the use of CA. Phosphine resistant strains are killed with CA.

Table 1. Overview of CA applications in chambers in Greece

Location	Product	No. of rooms	Yearly capacity
Thessalonica	Sesame seeds	7	35.000 tonnes
Thessalonica	Diverse commodities	1	1.200 tonnes
Thessalonica	Rice	1	2.400 tonnes
Lamia	Rice	4	9.216 tonnes
Athens	Diverse commodities	4	30.000 tonnes
Volos	Flour	2	7.200 tonnes

Main challenge:

Greece is a warm country and insects are very active for 6-8 months. After the CA treatment the treated products shall be stored in a clean room to prevent re-infestation. This is a model not so commonly seen in Greece. 5 out of 7 EcO_2 locations in Greece are equipped with a clean room.

The cost:
Example of using CA:

The rental of 1 average size chamber for 1 year is 20,000 euros. This chamber can take 60 pallets (tones) and will make 2 treatments per week in the 6 warm months and 1 treatment per week in the 6 cold months. This gives a total of 78 treatments per year x60 = 4,680 tones.
So the cost of using CA through the EcO2 system is 4,2 euros per tone for a small and relatively expensive system. The CA installations range from small to big sizes, which can handle more tones of products per year and which will lead to lower cost per ton of product.

Example of using Phosphine:

The fumigation of a 20" container with phosphine costs around 100 euros. Such a container takes around 20 tones.
So the cost of using phosphine is 5 euros per tone. Fumigation of larger volumes with phosphine will lead to lower cost per ton of product.

CA APPLIED IN SILOS

The principle of treating cereals in a silo with the use of CA is simple: overflow the silo with on-site produced nitrogen. The temperature of the grain in large silos cannot be artificially changed in a commercial way so the CA treatment in silos is applied only during the warm months of the year (in Greece April to November). Table 2 gives an overview of the CA systems for the treatment of silos.

Table 2. Overview of CA applications in silos in Greece

Location	Product	No. of silos	Yearly capacity
Thessalonica	Rice	5	12.500 tonnes
Volos	Grains	6	24.000 tonnes

Main advantages of the method:

The precision of the method is again higher than in any commercial phosphine fumigation. The safety of the workers is better supported with the use of CA. Phosphine resistant strains are killed with CA.

Main challenge:
The duration of the treatments is longer than phosphine. With a grain temperature of around 20°C phosphine needs 4-5 days while CA needs around 12 days depending on the insect.

CONTROL OF INSECTS WITH COMMERCIALLY USED CA CHAMBERS

CA is effective against a wide range of insects and their pre-adult stages: eggs, larvae and pupae. The insects are killed by a combination of temperature, atmospheric composition and exposure time.

During each treatment, the following parameters are controlled and monitored 24/7 to ensure an adequate treatment:
- The <u>temperature</u> within the treatment environment
- The <u>level of oxygen</u> within the treatment environment.
- The <u>duration</u> of the treatment.

Each insect species in the various life stages has its own optimum conditions to live and consequently its own parameters to be successfully eliminated.

During each CA treatment, the relative humidity of the product can be controlled to prevent change in product quality.

It is of basic importance that each industry will identify the insect they want to target in their CA chamber in each single treatment. For example in a freshly produced wheat flour one would expect to find *Tribolium* eggs while in a flour bag that has stayed for a long time in a warehouse more insects can be found including *Sitophilus* species. These two species (*Sitophilus* and *Tribolium*) have a significant difference on CA treatment duration with *Sitophilus* needing 40-50% extra time. This means that an important challenge for a company operating a CA chamber is to identify the target specie.

As previously mentioned another important parameter is to exclude re-infestation of the treated products. This means that a clean room makes a significant difference and still the clean room must remain free of insects. This is another challenge in commercial warehouses.

As part of the precision treatment each CA treatment must receive a close look to guarantee that the set parameters were met. This needs a detailed system, strict procedures and usually a third party overview. The EcO$_2$ system guarantees a specialist overview of the procedure for each treatment over the Internet. This overview has kept the good name around this company in Greece for more than 4 years.

CONCLUSIONS

The CA treatment makes no use of fumigants, chemicals or any other active ingredients. This guarantees; safety for employees, customers and environment, no residues on treated commodities and no development of insect resistance within treated insect population(s). Note that traditional fumigants can cause deadly accidents (Profume, MB and PH$_3$), cause ozone layer depletion (MB and Profume), and fumigant resistance issues are spread widely (PH$_3$).

The use of the toxic fumigant phosphine phase more and more the problem with increasing resistance of the insects and ultimately the increasing need for higher dosage of the gas, leading to higher expenses per treated ton of product. The CA technology requires investment in hardware (gas-tight chambers, CA generator machine) and energy costs when operating. Treating higher volumes of products makes CA competitive with toxic fumigation.

The CA treatment is an effective and safe treatment method suitable for treatment of conventional as well as organic commodities and effectively applied in Greece since 2008. The industries using this technology have all implemented this as part of their integrated pest management in their facilities.

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NITROGEN APPLICATION OFFERS FOR BOTH CONTROL OF INSECT AND GRAIN QUALITY

YongLin Ren[1,2,3], Cao Yang[4], James Newman[1,2], Manjree Agarwal[1,2], Ern Kostas[5], Hui Cheng[1,2]

[1] Cooperative Research Centre for National Plant Biosecurity, Australia
[2] Stored Grain Research Laboratory, Murdoch University, Murdoch, WA 6150, Australia
[3] Department of Agriculture and Food, Western Australia, 3 Baron Hay Court South Perth WA 6151, Australia
[4] Academy of State Administration of Grain, Beijing, P.R. China
[5] CBH group, Metro Grain Centre, 700 Alernethy Road, Forrestfield, WA 6058, Australia
*Corresponding author's e-mail: *y.ren@murdoch.edu.au*

ABSTRACT

Nitrogen (N_2) based atmosphere has been re-evaluated as cheaper, reliable and high efficient nitrogen generators (PSA – Pressure Swing Absorption and MNS – Membrane Nitrogen Separator) are available for grain industry. The systematic laboratory bioassay were conducted on four species of mixed-age cultures of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Trogoderma variabile* (Ballion) in five representative grains (wheat, barley, oats, Lupine and canola), and adult stages of Ladybird and Bronzed field beetle (*Adelium brevicorne*) in canola at 95-99% N_2 and 25-35ºC for 1 day to 4 weeks exposure. The techniques were tested on farmer storage using farm bins - Lake Grace (33°06′00″S 118°27′40″E) and Cooperative Bulk Handling Albany export terminal (35°1'50.90"S 117°53'10.54"E). This research successfully developed clean high nitrogen technology at a commercial scale for offering an immediate answer to the growing problem of insect resistance to phosphine and satisfies growing market demand for grain free of pest and chemical residues.

Key wards: grain storage, controlled atmosphere, nitrogen, PSA nitrogen generator, low oxygen, insect control, grain quality

INTRODUCTION

The Australian grain industry relies heavily on the internationally accepted treatment of fumigation with phosphine (PH_3) for grain treatment to maintain its harvest, free from insect infestation - an important criterion for market access. Currently, phosphine is the only fumigant available to treat bulk grains and oil seeds (more than 85% grains were treated / re-treated with phosphine) in each of the linkages from on-farm storage to the grain terminal. However, three factors are challenging the fate of using phosphine for treatment of grain; 1) OH&S and environmental issues, 2) a restrictive Codex Maximum Residue Limit - MRLs (marketing requirement and consumer safety) of 0.1 mg/kg, with some countries reducing the MRL from 0.1 to 0.05 or 0 mg/kg and 3) increasing resistance of insects. In the past 20 years,

however, resistance to phosphine in target insect pests has developed to such an extent that it now threatens effective control and, as a consequence, jeopardises market access.

Although controlled atmosphere storage has been in use for decades, but these did not lead to the adoption of the technique, as liquid nitrogen source and on site nitrogen generator is very costly. However, cheaper, reliable and high efficient nitrogen generators (PSA – Pressure Swing Absorption and MNS – Membrane Nitrogen Separator) are available, it is appropriate that N_2-based atmosphere be re-evaluated. Nitrogen based CA has several operational advantages over fumigation, particularly management of phosphine resistance and chemical residues issue.

- ➢ N_2 constitutes 78% of air, free source of nitrogen
- ➢ N_2 is not toxic, greatly reduced OHS&E risks
- ➢ Provides "organic" and truly residue-free grain
- ➢ No resistance problems
- ➢ No reaction with construction materials
- ➢ No need for ventilation before grain can be marketed
- ➢ No need for product registration

Our aim of this project is to develop cost-effective, viable non-chemical options to the current stand alone system based on phosphine. Research focus on development of nitrogen application technology to the "ready for adoption" stage, which can function as a direct replacement for specific uses of phosphine or eliminate resistant outbreaks.

MATERIALS AND METHODS

Grain samples
Newly harvested five representative grains (wheat, barley, oats, Lupine and canola) were used at moisture content of 9.9, 10.0, 8.4, 11.2 and 4.7%, w/w respectively. The moisture content of the grains was determined by using a Graintec HE 50 electronic moisture meter. The results obtained were expressed as a percentage calculated from replicates.

Insects and bioassays
Four species of stored product insects were used for bioassays. They were mixed-age cultures of *Sitophilus oryzae* (L.), *Tribolium castaneum* (Herbst), *Rhyzopertha dominica* (F.) and *Trogoderma variabile* (Ballion) which were established by adding adults (400-500) to media (1 kg) at 25°C and 65% r.h. The adults were left on the media (sterilised wheat for *S. oryzae* and *R. dominica*, wheat flour+yeast for *T. castaneum* and sterilised crushed canola for *T. variabile*) for 4-5 weeks, by which time there were representative numbers from each stage - egg, larva, pupa, and adult – based on knowledge of development rates (Howe, 1952; Beckett et al., 1994). The insects were sourced from susceptible strains MUWTC 8, MUWSO8, MUWRD 7, MUTV 11 of *T. castaneum, S. oryzae* (L.), *R. dominica* and *Trogoderma variabile* (Ballion) respectively held at the Murdoch University Post harvest Plant Biosecurity Laboratory, Perth, Australia. The resistant strains of *T. castaneum, S. oryzae* (L.) and *R. dominica* were also used for bioassays. Culturing and general handling techniques followed those described in Winks (1982). Data from these strains can be compared with laboratory bioassays and field results on several species of insects.

Bioassays were conducted by placing muslin bag (150 mm × 60 mm) at a depth of 6 and 23 cm within the fumigation chamber. Each bag contained 20 g of mixed-age cultures, in

standard laboratory culture medium, of approximately 100-120 adults, and an unknown quantity of eggs, larvae and pupae. The control bags containing a high population of insects (400-500 adults) was placed in a bottle containing unfumigated grain. The bagged insects were initially kept in controlled conditions of 25°C and 60% r.h. for one day before being placed in the fumigation chamber. The bioassay samples were retrieved at the end of the fumigation period, the adult insects counted and removed, and the remaining mixed-age cultures incubated at 25°C and 55-60% r.h. Subsequent-emerging adult insects were counted weekly for a period of 5 weeks with live and dead adults being removed at each count.

Nitrogen gas and apparatus

Food grade nitrogen was sourced from BOC Gases Australia. The laboratory bioassays were conducted at Murdoch University Post Harvest Plant Biosecurity laboratory. A gas purging flow system (Fig 1) was used to treat the insects with constant concentrations of nitrogen and oxygen and maintain low carbon dioxide concentrations during the period of treatment. Concentrations of nitrogen, oxygen and carbon dioxide were monitored twice a day during the 1-4 weeks treatment period.

Fig 1- A gas purging flow system was used to treat the insects with constant concentrations of nitrogen and oxygen and maintained low carbon dioxide concentrations during the period of treatment. All 15 cylinders were filled with a known amount (1.8-2 kg) of grain (wheat, barley, oats, lupin and canola).

The range of nitrogen concentrations were 97-99% balanced with oxygen for treatment of all stages of *S. oryzae*, *T. castaneum*, *R. dominica* and *T. variabile* in wheat, barley, oats, lupine and canola, and adult stages of Ladybird and Bronzed field beetle (Adelium brevicorne) in canola at 20-30°C.

Oxygen and carbon dioxide were analysed with Witt OXYBABY® 6.0 (WIT-GasetechnikGmbH & Co KG T, Germany). Accuracy 0.1-100% O_2/0.01-100% carbon dioxide.

Before and after exposure to nitrogen, grain moisture content, protein, oil content, starch or seed colour were analysed using a FOSS Infratec, 1241 Grain Analyzer (FOSS Analytical, Denmark).

Lake Grace farm bin trials

Farm bin-scale trials were conducted on the property of Doug Clarke near Lake Grace (-33.117, 118.607), Western Australia (Figure 2).

Fig. 2- The farm bin-scale nitrogen application trials were conducted at Doug Clarke's farm near Lake Grace (33.117, 118.607), Western Australia. A pressure swing adsorption (PSA) nitrogen generator (capacity of 30 m^3 of 99.5% N_2/hour) was used for purging nitrogen to wheat and canola bines (capacity of 75 tonne).

A pressure swing adsorption (PSA) nitrogen generator with a capacity of 30 m^3 of 99.5% N_2/hour was used to protect the grain retained on farm for sale. Nitrogen was applied to wheat and canola held in 75 tonne gas-tight storages (P½ ≥180s). The trial was conducted on wheat at 20°C. The final in-store nitrogen concentration was 97-98% for 3 weeks exposure and for canola trial at 35C for 7 days. Caged mixed-age culture (40-50 g) containing 100-120 of *R. dominica, S. oryzae* and *T. castaneum* adults and *T. variabile* larvae were used for bioassays. The cages were at different locations within the silo.

CBH Albany grain export terminal trials

A 350 m^3 of 99.5% N_2/hour PSA nitrogen generator has been installed at CBH Albany grain export terminal (35°1'50.90"S 117°53'10.54"E). The generator is plumbed to a bank of 10 × 10,000 tonne concrete cells. The project has conducted and completed trials on 5 × 10.000 tonne concrete cells containing newly harvested canola and barley at 30-32ºC (Figure 3). The grain was naturally infested with *T. castaneum*, Ladybirds and Bronzed field beetles. The final in-store nitrogen concentration was 97-98% for 2 weeks.

Laboratory bioassays.

a) Laboratory bioassays show that adults of *T. variabile*, *T. castaneum. R. dominica* and *S. oryzae* in wheat, barley, oats, lupins and canola at 99, 98, and 97% N_2 complete control was achieved at 24±2°C for 1, 3 and 7 days, at 30±2°C, for 1, 2 and 5 days, and at 35±2°C for 1, 2 and 4 days exposure periods.

Fig. 3- A 350 m^3/hour PSA nitrogen generator has been installed at the CBH Albany grain export terminal (35°1'50.90"S 117°53'10.54"E). The generator is plumbed to a bank of 10 × 10,000 tonne concrete cells.

b) Laboratory bioassays show that all immature stages of *R. dominica, S. oryzae* and *T. castaneum* in wheat, barley, oats, lupins and canola at 99, 98 and 97% N_2 complete

control was achieved at 24±2°C for 2, 3 and 4 weeks, at 30±2°C for 10 days, 2 weeks and 3 weeks, and at 35±2°C for 1, 2 and 3 weeks exposure period. For *T. variabile* at 98-99% N_2 complete control was achieved at 25, 30 and 35°C for 4, 3 and 2 weeks exposure period.

c) Laboratory bioassays show that all adult and all immature stages of *R. dominica, S. oryzae, T. castaneum* and *T. variabile* were controlled using the nitrogen treatment and there was no significant difference on mortality between phosphine-resistant and susceptible strains of these insects.

d) Adult stages of Ladybirds and Bronzed field beetles in canola were completely controlled at 95, 97 and 99% N_2 25°C for 6, 5 and 1 day exposure period, respectively.

e) The mortality of all stages of all insects tested increased with decreasing levels of oxygen, and increasing exposure time and temperature.

f) In comparison with wheat, barley, oats and lupin, high concentrations of nitrogen or low oxygen in canola kills all stages of all tested insects with higher efficacy.

Efficacy of high nitrogen (low oxygen) atmosphere on grain quality

Samples of wheat, barley, oats, lupins and canola were analysed both before and after exposure to 97, 98 and 99% N_2 for period of 1-5 weeks at 25, 30 and 35°C. There was no change in moisture content, protein, oil content, starch or seed colour.

Lake Grace farm bin trials

The trials on wheat at 20°C and 97-98% N_2, all adults of *R. dominica, S. oryzae* and *T. castaneum* were killed after one week and complete extinction of all life stages occurred after 3 weeks exposure, but 6-10% of *T. variabile* larvae survived. The trials in canola at 35°C shows that with 7 days exposure to nitrogen at 97% all Bronzed field beetles and Ladybirds were eliminated, and after 2 weeks exposure all stages of *R. dominica, S. oryzae, T. castaneum* and *T. variabile* were killed. Canola seed colour, oil content and levels of free fatty acid did not change during the 2 month storage period. This storage process of canola significantly contributed to maintaining quality by inhibiting the respiration process that can lead to rapid localised heating and prevented the oxidation that leads to seed deterioration at this high temperature.

Various atmospheric purging methods were evaluated during the trials. The most efficient method was to pump nitrogen into the base of the bin with the top lid closed and air purging from the silo through a pipe connected to headspace, exiting at ground level. The purge continued until the exhaust air contained 98% N_2. After one day, 1-1.5% O_2 desorbed from grain, requiring the storage to be topped up until the exhaust air again contained 98% N_2.

CBH Albany grain export terminal trials

After 2-3 weeks treatment with 98% N_2, all barley and canola was inspected for export with no live insect pests found. The bioassay with mixed age cultures show that all stages of tested *T. castaneum*, *S. oryzae* and *R. dominica* were killed after 2-3 weeks exposure. The treated barley and canola had no change in moisture content, protein, starch, oil content and level of free fatty acid and seed colour.

CBH Albany grain export terminal now incorporate the use of nitrogen as a management tool for grain coming in from up country that has been treated with phosphine. This means that effectively all grain exported from Albany will only be treated with phosphine once, or not at all, with the use of nitrogen only at port. The introduction of

nitrogen at CBH Albany grain export terminal has offered solutions for management of phosphine resistance an alternative to phosphine treatment and a grain quality control method.

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CONTROL OF STORED GRAIN INSECTS BY USING NITROGEN IN LARGE CONCRETE SILOS IN CYPRUS

Navarro[1] S, C. Athanassiou[2], A Varnava[3]*, N Vroom[4], D Yilasoumis[5], I Leandrou[5] and S Hadjioannou[5]

[1]Food Technology International Consultancy Ltd., P.O. Box 3300, Beit Yehoshua, 40591, Israel
[2]Laboratory of Entomology and Agricultural Zoology, Dept. of Agriculture, Crop Production and Rural Environment, University of Thessaly, Phytokou str. 38443, N. Ionia Magnesia, Greece
[3]Consultant, 36 Elia Papakyriakou str. fl.16, Strovolos 2003, Nicosia, Cyprus
[4]EcO2, P.O.Box 7488/3280 AG Numansdorp, The Netherlands,
[5]Cyprus Grain Commission, P.O.Box 21777, Kypranoros str. 15, Nicosia, 1061, Cyprus,
*Corresponding author's e-mail: *a.varnava@cytanet.com.cy*

ABSTRACT

Nitrogen (N_2) was applied in three concrete silos each containing 2,400 tonnes of grain for controlling stored grain insects. Structural sealing was carried out and air tight valves were installed to improve the air tightness of the bins. Pressure decay tests (250-125 Pa) carried out in full bins showed decay time from 120 s up to 290 s. Using a PSA N_2 generator the oxygen (O_2) concentration was reduced in 44-56 h below 0.9% and after that it was continuously maintained between 0.2-0.9% up to 23.8 days. Treatment of 18.7 and 23.8 days on grain temperature of 26°C and 22°C, respectively, was effective for the control of the adults of important stored grain insects *Oryzaephilus surinamensis* (L.)*, Tribolium confusum* J. du Val and *Rhyzopertha dominica* (F.) placed above and inside grain mass. In separate bioassays, which were placed in different zones right above the grain mass complete (100%) mortality of *T. confusum* (all life stages), *O. surinamensis* (larvae and adults), *Sitophilus granarius* (L.)(adults) and *R. dominica* (adults) was achieved in 18.7 and 23.8 days treatment as well. When applied to large quantities of grain, the N_2 modified atmosphere (MA) technology by using a N_2 generator proved to be cost competitive with the analyzed other treatment methods. Under trial conditions the cost of annual treatment of 40,000 tonnes using the PSA of EcO_2 system is about 1.6 €/tonnes in comparison to 0.5-0.8 €/tonnes by using Siroflo/ECO_2FUME, 0.4-0.8 €/tonnes by using contact insecticides and 0.40 €/tonnes for aeration. By increasing the annually treated quantity to 130,000 tonnes the treatment cost with N_2 MA EcO2 system is estimated 0.80 €/tonnes. The N_2 MA provided a full control of studied stored grain insects, free-residues products, no environment chemical contamination, low occupational hazard, no-dependence on insect resistance, no need for registration and full control and automation of the treatment operation. On the basis of these trials the N_2 MA technology is a successful alternative to phosphine Siroflo/ECO_2FUME and contact insecticides for the control of stored grain insects in large concrete silos.

Key words: Nitrogen, modified atmospheres, grain protection, grain storage, stored-grain insects, silo sealing, post harvest systems**,** non-chemical alternatives, oxygen, phosphine, contact insecticides, eco-friendly methods

INTRODUCTION

Phosphine and contact insecticides are still the main means used around the world for protection and disinfestations of grains stored in silos. On the other hand, there is an increasing need in new grain protection methods which should be friendlier to the environment, safer to products and employees and more effective for insect control on an acceptable cost basis. Modified atmosphere (MA) by using nitrogen (N_2) is one of the most promising alternative methods providing effective and residue-free insect control in sealed storage structures with reduced hazard to employees, no need for registration and no contamination of environment. The effects of low oxygen concentrations by using N_2 to control stored grain insects was reported in many works (Navarro, 1978; 2006; Jay, 1984; Banks and Annis, 1990). The method requires sufficiently sealed storage structures. The cost and difficulties of sealing large grain bins and the cost of N_2 supply, in combination with the widespread use of phosphine and liquid insecticides, have delayed the implementation of N_2 in large grain silo bins. During the last years there is an increasing interest in introduction of N_2 Modified Atmosphere technology for protection of stored grains in silos (Cassells et al., 1994; Banks and Annis, 1997; Timlick et al., 2002; Navarro, 2006). Liquid nitrogen from tank has been commercially and routinely used for grain treatment in 1800-tonnes sealed concrete silos of at least 5 min half life pressure decay time at Newcastle export terminal in Australia; the combination of IPM and nitrogen has been reported to be very effective (Clamp and Moore, 2000; Clamp and Banks, 2000).

The Siroflo/ECO$_2$FUME fumigation technology by using phosphine from cylinders in unsealed silos is an alternative to solid phosphine and liquid insecticides (Winks, 1992; Winks and Russell, 1994; Varnava et al., 1998). The Siroflo/ECO$_2$FUME was introduced to Cyprus in 1996; it was installed and successfully used in unsealed vertical metal and concrete grain silos. On the other hand, the need in increased doses of phosphine by Siroflo system and the non re-registration of ECO$_2$FUME in EU created the necessity for alternative solution. This trial was conducted at Cyprus Grain Commission's port concrete silo where Siroflo/ECO$_2$FUME fumigation system is used and aimed to demonstrate the application of N_2 MA technology as alternative to Siroflo/ECO$_2$FUME and define the real requirements, effectiveness and cost of sealing and using this technology. The results of this study are presented in this article.

MATERIALS AND METHODS

N_2 was applied in three bins at Cyprus Grain Commission's Limassol port silo. Bins are made of concrete with conical base floor (bin diameter 10.5 m, height to eaves 33.4 m, depth of cone 5.4 m, total storage capacity 3,046 m^3). Each bin is connected to a Siroflo/ECO$_2$FUME flow-through fumigation system and to an aeration system via two aeration ducts entering bins from the bottom. The bins were not constructed to be used with modified atmosphere.

Sealing works were carried out to improve gas tightness of bins A, B and C. At the bottom of bin B aeration duct inlets were sealed by installing two gas tight valves; the grain outlet gate was also replaced by a gas tight knife-type valve; at the bottom of the other two bins polyethylene sheet, multipurpose aerosol adhesive spray, tape and silicone were used for improving sealing at these places.

After this preparation, bins were loaded with grain up to about 1 m below bin roof leaving about 150 m^3 head space above grain. In bin A 2200 tonnes of barley (m.c. 12.9% wet

basis) and in each of bins B and C 2400 tonnes of feed wheat (m.c. 11.8% wet basis) were stored.

Additional sealing works were carried out at the top of bins. Sealing of the cracks and crevices inside silo bins between the roof and the wall joints was carried out from inside bins by using expandable foam polyurethane, plaster, gastight coating, tissue and sealing silicone. Aeration duct outlets were sealed using polyethylene sheet and tape; manhole inlets were covered and sealed with a temporary board, tissue, gastight coating and silicone; on board an over/under pressure valve, oxygen analyzer tube and temperature sensor were installed for measurement at different levels inside each bin and above grain. The top of each silo bin is equipped with two loading ports; silicone was used to seal the loading ports; in the case of bins B and C the second loading port (x-type valve) was removed and the opening was closed with a board, tissue, gastight coating and silicone; the x-valve of the bin A was sealed from inside bin using polyethylene sheet, multipurpose aerosol adhesive spray and tape.

For studying insect mortality two separate trials were carried out. In the first trial, test insects were separated from infested grain taken from commercial storages in Cyprus. Adults of *O. surinamensis*, *T. confusum* and *R. dominica* were placed in tubes (1.5 cm in diameter, 10 cm in height) with metal mesh walls and about 5 g of feed (flour and whole wheat kernels). The tubes containing the insects were placed on grain surface, at 0.5 m and 3 m below and at 1 m above grain surface, at the bin's centre.

In the second trial, the mortality of *T. confusum* (all life stages), *O. surinamensis* (larvae and adults), *S. granarius* (adults) and *R. dominica* (adults) was studied. Test insects were taken from laboratory cultures of *T. confusum* reared on wheat flour, *O. surinamensis* on cracked oats and *R. dominica* with *S. granarius* on whole wheat kernels. All adults used in the bioassays were <1 month old, while all larvae <2 week old and eggs of *T. confusum* were <1 day old. Twenty individuals from each species/life stage were placed in small cylindrical plastic vials (2.5 cm in diameter, 8 cm in height). About 0.3 g of diet was added to each vial before they were closed, but equipped with small openings in the lid to allow sufficient aeration. For each bin, there were 9 vials for each species and life stage combination. Three of them were placed in the center of the bin, three at the median of the radius and three at the edge, close to the bin walls, on grain surface.

Adults of *T. confusum* and *R. dominica* were placed in the control bin. After the termination of the N_2 treatment, all tubes and vials with insects were transferred to the laboratory and examined for surviving individuals.

The temperature inside bins was monitored using thermocouple cables at different locations 1 m above, on grain surface and 0.5 m and 3 m below grain surface, at bin's centre, before starting treatment and after completing it. The oxygen (O_2) concentration was monitored by taking gas samples at 1 m above and 3 m below grain surface and analyzing them using a portable meter. Additional measurements of oxygen and temperature inside treated bins at grain surface, at bin's centre, were taken by the Eco2 system during treatment continuously every 10 min.

Before starting treatment with N_2 a pressure test of full bins was carried out using the "half life pressure decay time method 250-125 Pa" to determine the bins gas tightness level.

The EcO_2 system was used for producing N_2 from ambient air and purging it into bins from the bottom. The EcO_2 generator was connected to two different points of the silo bins: a) from the top of the silo bins via the gas sampling tube and temperature sensor; and b) from the bottom of the silo bin via 25 mm (internal diameter) flexible tube connected to an already prepared 25 mm steel connection. The tube served for purging the silo bin using N_2 from the EcO_2 generator.

The EcO$_2$ generator used was based on air-to-N$_2$ production using pressure swing adsorption (PSA) technology and the system was installed in a mobile 6 m container with necessary equipment and control devices.

After reaching O$_2$ concentration below 0.9% in bins A, B and C, this concentration was continuously maintained by the EcO$_2$ system below 0.9% for 516 h, 399 h and 273 h, respectively.

Cost analysis data was carried out to compare N$_2$ based MA application using the EcO$_2$ system and other stored grain protection methods used in Cyprus Grain Commission (CGC) grain silos (Siroflo/ECO$_2$FUME, contact insecticides, aeration).

RESULTS AND DISCUSSION

Cost effectiveness of sealing an existing silo is an important factor for making a decision on which grain protection method to use. The use of gas tight valves improved sealing. The cost of sealing bins including the use of gas tight valves, and the half life pressure decay time achieved after sealing is shown in Table 1.

Table 1. Cost for sealing three 2400-tonne capacity concrete silo bins and gas tight pressure tests results in full bins after sealing

Bin	Sealing details	Cost (€)	Pressure decay test (250-125 Pa) time
A	Structural sealing works only*	2,500	2 min 20 s
B	Structural sealing works *	2,500	4 min 50 s
	2 gas-tight valves at aeration inlet ducts +	2,000	
	1 gas-tight knife-type valve at grain out loading port	11,200	
	Total cost for bin B	15,700	
C	Structural sealing works only*	2,500	2 min

* structural sealing works include sealing of the cracks and crevices inside silo bins between the roof and the wall joints, sealing of aeration exhaust vents and grain inlet ducts and manholes and other works except the installation of three gas tight valves.

The use of gas tight valves at aeration inlet ducts and at grain unloading port improved considerably the half life pressure decay time, but the sealing cost also increased close to 15,700 € per bin. Although the half life pressure decay time was at the border line of the acceptable levels for using MA, even in the bin where three gas tight valves were installed (4 min 50 s), in all bins the O$_2$ dropped below 0.9%. The time, the volume of N$_2$ and the energy needed by the EcO$_2$ generator to produce the required volume of N$_2$ is shown in Table 2.

The O$_2$ concentration was maintained between 0.1-0.9% for a period of 523 h in bin A, 404 h in bin B and 261 h in bin C. These values are comparable with the reports from previous studies from other parts of the world (Cassells et al., 1994; Clamp and Moore, 2000; Timlick et al., 2002). The purge time, maintenance time, the volume of N$_2$ produced, the power consumed by the EcO$_2$ generator and energy cost under trial conditions are presented in Table 3.

Table 2. Purge time, nitrogen volume and energy needed to reduce the oxygen concentration to below 0.9 % in three full silo bins using the EcO_2 system

Bin	Time (h)	Volume of N_2 (m^3)	Energy** to produce Nitrogen (kWh)	Cost*** for energy (€)
A	49	3709	1780	356
B*	44	2859	1372	274
C	56	3571	1714	343

* sealing includes structural works and 3 gas tight valves.
** energy consumption by EcO_2 converter during trials up to 0.48 kWh/m^3 N_2
*** average electricity cost 0.20 €/kWh

Table 3. Treatment duration, volume of nitrogen, energy and cost for treatment of three silo bins using EcO_2 system ($O_2 < 0.9$%)

Bin	Grain quantity (tonnes)	Total duration of treatment including purge, h (d)	Total vol. of N_2 (m^3)	Total volume of N_2 (L t^{-1} d^{-1})	Total energy to produce N_2** (kWh)	Total cost for energy*** (€)	Total cost for energy*** (€/t)
A	2200	572 (23.8)	17957	342	8619	1724	0.78
B*	2400	448 (18.7)	13381	298	6423	1285	0.54
C	2400	317 (13.2)	11399	360	5472	1094	0.46

* sealing includes structural works and 3 gas tight valves
** energy consumption by EcO_2 generator during trials up to 0.48 kWh/m^3 N_2
*** average electricity cost 0.20 €/kWh

Regardless of differences in the gas tightness of the bins and that the half life pressure decay time was lower than 5 min, in all bins the O_2 concentration was continuously maintained below 0.9% for the specified exposure time. Therefore, the difference among the bins was in the level of gas tightness that was reflected mainly on the amount of N_2 used to achieve O_2 concentrations below 0.9% and the same level of insect mortality; the more gastight was the bin, the less gas was used. Thus, the level of gas tightness affected the cost of treatment to achieve the same mortality level. Insect mortality of bioassays are shown in Tables 4 and 5. The trials were carried out for various exposure times to N_2 treatment, from 13 to 24 days. Table 4 shows that complete mortality of *R. dominica* adults, could be achieved when the treatment was 23.8 days but some survivals were observed when treatment was 18.7 days. The EcO_2 system was effective in maintaining the O_2 below 0.9%, which controlled the tested life stages of *O. surinamensis, T. confusum, R. dominica* and *S. granarius* (Tables 4 and 5).

After completing the treatment, operating the aeration system of the bins for 1-2 h was enough to restore the treated bins to atmospheric O_2 level. The cost of treatment using EcO_2 system in sealed silos mainly depends on: a) the fixed cost for rental of EcO_2 system, including maintenance and using the EcO_2 system control software and central communication system, b) cost of electricity for generating N_2, c) expenses for sealing a bin and depreciation of sealing, d) transportation of grain from one bin to a sealed bin for treatment with N_2, e) other factors like quantity (tonnes) of treated grain per year, the number

of gas tight valves and the expected life of bin sealing, the duration of treatment and other minor technical and logistical costs.

Table 4. Influence of different exposure times to oxygen concentration below 0.9% on mortality of adult insects in three bins treated with nitrogen generated by EcO$_2$ system

Bin	Total duration of treatment incl. purge time** (d)	Grain*** temper. (°C)	Air*** temper. (°C)	Oxygen concentr. during treatment (%)	Insect species in tubes at central zone of bin at different locations****	Total insects in tubes and sample	Insect mortality % (± SD)
A	23.8	22	15	0.2-0.9	*O. surinamensis*	63	100 ± 0.0
					T. confusum	265	100 ± 0.0
					R. dominica	263	100 ± 0.0
B*	18.7	26	16	0.5-0.9	*O. surinamensis*	203	100 ± 0.0
					T. confusum	109	100 ± 0.0
					R. dominica	308	91.6 ± 6.8
C	13.2	19	17	0.3-0.9	*O. surinamensis*	72	100 ± 0.0

* sealing of this bin includes structural works + 3 gas tight valves
** 44-56 h were required to fill bins with N2 and reach O2<0.9%
*** Air and grain temperature is the average of measurements taken before and after completing treatment, at bin's centre, at 1 m above and on grain surface and at 0.5 m and 3 m below grain surface respectively; in bin C it is the average of measurements taken before starting and after completing treatment, at bin's centre, at 1 m above and 0.5 m below grain surface, respectively.
**** in bins A and B tubes with metal mesh walls containing insects and feed were placed 1 m above and on grain surface and 0.5 m and 3 m below grain, at bin's centre; in bin C on grain surface.

Estimated cost for 18-day treatment of 2,400 tonnes grain in sealed concrete bins using the EcO$_2$ system under the Cyprus Grain Commission conditions at Limassol port silo is shown in Table 6. Estimated cost of about 1.6 €/tonnes is based on current data. Cost for rental, maintenance and use of EcO$_2$ system represents about 60% of the total cost of treatment with N$_2$. The electricity expenses for the generation of N$_2$ represent about 30% of the total cost. The rest of the cost that includes expenses for bin sealing and depreciation structural works, two gas tight valves at aeration inlet ducts and one gas tight valve at grain out loading port was about 10% of total cost.

A cost comparison of four different stored grain protection methods is shown in table 7. Cost estimates were based on 40,000 tonnes of grain treated annually. Since the EcO$_2$ system rental cost is fixed (about 38,000 €/year), the more grain is treated, the less the cost of the treatment is. For 40,000 tonnes of grain, the cost per treated tonne with N$_2$ MA using the PSA EcO$_2$ system including expenses for the rental of N$_2$ converter, electricity and sealing depreciation is about 1.6 €/tonne, in comparison to 0.5-0.8 €/tonne by using phosphine by Siroflo/ECO$_2$FUME, 0.4-0.8 €/tonne by using contact insecticides, and 0.40 €/tonne by cooling using aeration. By increasing the annually treated quantity to 130,000 tonnes the treatment cost with N$_2$ MA technology is reduced to 0.80 €/tonne making it cost competitive with any other available treatment method (Table 7).

Table 5. Insect mortality under different durations of oxygen concentration below 0.9% in two bins treated with nitrogen generated by EcO$_2$ system and in a non treated bin

Bin	Total treatment duration incl. purge time** (d)	Grain*** temper. (°C)	Air*** temper. (°C)	Insect species in vials at different locations on grain surface in bin****	Insect life stage	Central zone, insect mortality % (± SD)	Median zone, insect mortality % (± SD)	Peripheral zone, insect mortality % (± SD)
B* and A	18.7 (B*) and 23,8 (A)	26 (B) and 22 (A)	16 (B) and 15 (A)	*T. confusum*	eggs	100 ± 0.0	100 ± 0.0	100 ± 0.0
				T. confusum	pupae	100 ± 0.0	100 ± 0.0	100 ± 0.0
				T. confusum	larvae	100 ± 0.0	100 ± 0.0	100 ± 0.0
				T. confusum	adults	100 ± 0.0	100 ± 0.0	100 ± 0.0
				O.surinamensis	larvae	100 ± 0.0	100 ± 0.0	100 ± 0.0
				O.surinamensis	adults	100 ± 0.0	100 ± 0.0	100 ± 0.0
				S. granarius	adults	100 ± 0.0	100 ± 0.0	100 ± 0.0
				R. dominica	adults	100 ± 0.0	100 ± 0.0	100 ± 0.0
Control (non treated bin)	24 d, O$_2$=20.5%	17	15	*T. confusum*	eggs	43.4 ± 1.8	40.1 ± 8.9	51.0 ± 1.8
				T. confusum	pupae	9.4 ± 4.4	14.2 ± 4.9	11.9 ± 6.5
				T. confusum	larvae	17.4 ± 6.7	13.8 ± 7.4	9.8 ± 4.5
				T. confusum	adults	5.9 ± 3.1	2.9 ± 1.3	6.8 ± 2.1
				O.surinamensis	larvae	24.2 ± 7.8	24.8 ± 10.1	13.2 ± 6.5
				O.surinamensis	adults	19.3 ± 8.3	23.4 ± 4.3	15.4 ± 4.4
				S. granarius	adults	7.9 ± 5.8	8.9 ± 4.5	13.3 ± 4.7
				R. dominica	adults	5.7 ± 3.2	7.3 ± 4.3	3.4 ± 1.6

* sealing of this bin includes structural works + 3 gas tight valves
** 44-56 h were required to fill bins with N$_2$ to reach O$_2$<0.9%
*** Air and grain temperature is the average of measurements taken before and after completing treatment, at bin's centre, at 1 m above and on grain surface and at 0.5 m and 3 m below grain surface respectively; in the control (non treated bin) it is the average of measurements at 1 m above and 0.5 m below grain surface at bin's centre, taken before placing and after removing vials.
**** vials containing insects and feed were placed on grain surface

Table 6. Estimated expenses for 18-d treatment of 2,400 tonnes grain in sealed concrete bins using the EcO$_2$ system based on trial results, €/tonne

Cost parameter	Cost (€/t)	Share of the cost (%)
Rental, maintenance and operation of EcO$_2$ Nitrogen generator and system	0.95	60
Electricity for the production of used Nitrogen by EcO$_2$ generator and system	0.425	27
Depreciation of expenses for sealing a bin	0.16	10
Transfer of grain from one bin to a sealed bin for treatment with Nitrogen	0.06	3
Total expenses per treated tonne	1.6	100

Conditions/Assumptions: 18 d treatment including 2 d for purging with N$_2$; O$_2$<0.9%; electricity consumption by N$_2$ EcO$_2$ generator 0.35 kWh/ m^3 N$_2$; a group of four sealed bins with N$_2$ MA system; sealing includes structural works and three gas tight valves; expected life of sealing 10 years; rental, cost for maintenance and use of EcO$_2$ generator and system 38,000 €/year; average electricity cost 0.22 €/kWh; 0.3 m^3 N$_2$/tonne/day; 40,000 tonnes of grain treated using N$_2$ per year of which 20,000 tonnes will have to be transferred from another bin at a cost of 0.12 €/tonne.

Since the total annual sales of CGC is around 300,000 tonnes, the treatment of only 40,000 tonnes will contribute to prevent spread of infestation with a minor increase of 0.08% of total grain selling price by 0.21 €/tonne.

Another possibility is to use a smaller N_2 generator at a reduced rental cost. Although it might not be feasible to base all the treatments on a single technology, it is clear that increasing the amount of grain to be treated or reducing the rental cost, places the N_2 MA EcO_2 system in cost competition with all other analyzed treatment methods.

The main conclusions of this study are comparable with the conclusions of trials with liquid nitrogen MA in Newcastle grain terminal in Australia (Clamp and Moore, 2000). The N_2 MA technology proved to be cost competitive when applied to large quantities of grain and provided a full control of the tested various stages of stored grain insects, providing residue-free products, without environment chemical contamination, with contribution to improve occupational hazard, without the risk of insect resistance and full control and automation of the treatment operation. On the basis of these trials the N_2 MA technology was evaluated as a successful alternative to phosphine Siroflo/ECO$_2$FUME and contact insecticides for the control of stored grain insects in large concrete silos. On this basis the Cyprus Grain Commission is planning to replace the Siroflo/ECO$_2$FUME fumigation system by implementing the N_2 MA technology in four concrete silo sealed bins.

Table 7. Cost comparison of four stored grain protection methods, €/tonne

Calculations based on quantity of annually treated grain	Nitrogen MA using a PSA EcO_2 system in concrete sealed silos*	Siroflo/ Eco2fume**	Contact insecticides***	Aeration****
40,000 tonnes/year	1.60	0.50-0.80	0.40-0.76-0.80	0.40
130,000 tonnes/year	0.80	0.50-0.80	0.40-0.76-0.80	0.40

* Cost includes 18 days treatment with O$_2$<0.9% using a rental EcO2 nitrogen converter and operation system, four bins sealing cost depreciation (structural works and 3 gas tight valves), electricity for producing N_2 and partial transfer of grain.
** Dose 65-100 ppm PH$_3$ for 15 days. No grain transfer is needed.
*** The lower cost for 5 ppm Actellic EC 50; the middle cost for 0.5 ppm Spinosad Tracer 48 EC; the higher cost for 0.25 ppm K-Obiol ULV 6. Cost for transfer of grain from one bin to another 0.12 €/tonne is included.
**** 100 h aeration, 30 kW fan. No grain transfer is needed.

CONCLUSIONS

Each grain protection technology has its own strong and weak points. In comparison to Siroflo/ECO$_2$FUME, contact insecticides and aeration, the N_2 MA technology using a rental N_2 converter, when applied to limited quantity of grain, has the disadvantage being the most costly. By increasing the amount of treated grain makes the technology cost competitive with other conventional methods. Use of phosphine and contact insecticides face the problem of worldwide increasing insect resistance and the need for higher doses leading to higher expenses per tonne. The MA technology by using N_2 is a very effective and residue-free protection method, friendly to environment, contributes to improve occupational safety, suitable to organic commodities and without the potential insect resistance. These are important advantages that comply with increasing demand of the market and global environment protection and meet the expectations of a modern society.

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TAKING THE TROPICS HOME: CONTROLLED ATMOSPHERES FOR THE CONTROL OF TOBACCO BEETLES IN LARGE-SCALE TESTING

Cornel Adler[1]* and Annette Murray[2]

[1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants,
Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection,
Königin-Luise-Straße 19, D 14195 Berlin, Germany
[2]Reemtsma Cigarettenfabriken GmbH
An Imperial Tobacco Group Company
Max-Born-Strasse 4, 22761 Hamburg, Germany
Corresponding author's e-mail: *Cornel.Adler@jki.bund.de*

ABSTRACT

For years phosphine fumigation has been used for pest control in tobacco. Problems arose with the development of resistant insect strains. Moreover, toxic fumigants are potentially hazardous to man. This is why a consortium of interested tobacco companies, the CORESTA subgroup Pest and Sanitation Management in Stored Tobacco, decided for a large-scale testing of controlled atmospheres (CAs) at practical conditions. In most tobacco producing countries warm product temperatures favour the efficacy of hypoxic atmospheres and allow for sufficiently short treatment times. To obtain similar treatment times in Central Europe it was decided to heat the incoming tobacco prior to CA application. Samples of all stages of tobacco beetles were added to tobacco bales in order to monitor the efficacy of the treatment. Data loggers for temperature and r.h. were added to the samples to record physical conditions during transportation and treatment. Untreated control samples accompanied the experimental samples. One of the treatment chambers was a gastight cube measuring 10m in each direction. Practical treatments proved difficulties to reach uniform high temperatures in such a chamber. In some cases cold winter temperatures on the outside of the chamber led to massive condensation of water from tobacco leaves. Eggs, various larval stages, pupae, and adult beetles could be controlled with a hypoxic CA containing some 0.5% oxygen at temperatures of 28°C and within 9 days of exposure time. Few survivors were recorded, when a similar CA was tested at 38°C and 2.5 days exposure time.

Key words: tobacco, storage, disinfestation, *Lasioderma serricorne*, controlled atmospheres.

INTRODUCTION

Fumigants have been used for many years as the primary measure for insect pest control in large and commercial storages of dry stored products. In many regions of the world phosphine has been one of the fumigants heavily relied upon. This is still true in most cases even though some difficulties have arisen over the last decades: Continuous fumigation with just phosphine at low dosages (e.g. siro flow, J-system), competition for low costs and fast

treatment times, and low-quality sealing gave rise to the development of phosphine-resistant insect strains (Collins et al. 2003, Nayak et al. 2010). Moreover, phosphine was also used in-transit by untrained seamen or in rural areas by not sufficiently educated laymen and has caused a number of casualties by faulty use or abuse. Metal phosphides that develop phosphine in the presence of humidity sometimes gave surprising results that caused hazards when too high moisture contents caused too high PH_3-doses and ignition or when too low moisture contents let to insufficiently low dosages for pest control and caused problems when not completely degassed product had to be discarded. Cases were reported where such products caused fire when collected in a drum and where uninformed firemen increased the calamity when they tried to extinguish the fire with water. These are a few of the reasons that motivated tobacco producing companies organized in the CORESTA subgroup on pest control to look for new and less toxic ways of pest control.

One of the alternatives studied in this context was the use of Controlled Atmospheres. The efficacy of various CAs has been proven with many stored product insects at different temperatures, moisture contents and pressures (Lindgren and Vincent 1970, Jay et al. 1971, 1984a, 1984b, Bailey and Banks 1975, Annis 1987, Banks and Annis 1990, Navarro, Ripp et al. 1990, Adler 1994, Adler et al. 2000). The purpose of practical trials carried out at various locations was to prove the practical application of CAs in large-scale trials. The advantage of tobacco in this context is the high value per ton and the few potential pest species, namely the tobacco beetle *Lasioderma serricorne* (Col., Anobiidae) and the tobacco moth *Ephestia elutella* (Lep., Pyralidae). This paper describes results obtained with the tobacco beetle in trials carried out from 2009 to 2012. The main questions to be answered were:

1. Are the chosen conditions suitable for complete control of all stages of the tobacco beetle?
2. What is the optimum treatment temperature to secure a fast but still economically feasible pest control?
3. Are the chosen conditions achievable under practical conditions in a large storage chamber with some 100 so-called C48 cases of tobacco made of cardboard (weight 200 kg each)?

MATERIALS AND METHODS

Insect culture
Insects were reared at 25±1°C and 65±5% r.h. on wheat bran and broken tobacco leaves. The insect strain used was a phosphine resistant strain (COR 49) received from the Food and Environment Research Agency (FERA) in Sand Hutton, UK, or a phosphine resistant strain cultivated from a wild strain collected in Uganda. In the first trials 200 young beetles were placed onto 150 ml wheat bran (with glucose, glycerine and brewer's yeast added) and approx. 75 ml of broken tobacco. After 7 d the beetles were removed from the substrate and another 350 ml of wheat bran was added to provide additional feed to the developing larvae. This method was repeated in a weekly rhythm to receive the various developmental stages. In latter trials, 200 young beetles were placed onto cocoa powder. After 3 d the beetles were removed from the powder and batches of 80 eggs were counted into film tube cages together with 25 ml of wheat bran as substrate. This procedure was repeated weekly to receive the various developmental stages and gave more reliable numbers of individuals allowing calculation of percent mortality in case of survivors.

Preparation of samples and shipment

Eight developmental stages were taken from weekly cultures of the tobacco beetle. 50 adults were given into film capsules (length: 50 mm, diameter: 30 mm) together with 25 ml of fresh uninfested substrate. To allow easy gas exchange with the surrounding atmosphere, bottom and lid of the film capsules had an opening (diameter 6-10 mm) that was covered with a fine wire mesh gauze (mesh width approx 100 μm). Of eggs, larval and pupal stages, aliquots of 25 ml were taken from the respective culture jars. Four linen bags were filled with the eight film capsules, a fifth set of samples was kept as untreated control in a glass jar under laboratory conditions at 25°C. Data loggers to determine temperature and r.h. were added to the bags to be sent to Antwerp. Each bag was closed with a Velcro-fastener. In addition it was locked tightly with a metal wire. The four bags were placed into a cardboard box and were sent to Antwerp by mail on November 9, 2009. One of the four bags was an untreated control to determine the conditions during shipment, three bags were placed into tobacco bales at different positions within the treatment chamber. After treatment all four bags were sent back, opened, and checked for insect survival and mortality.

Treatment

In the majority of cases the treatment chamber consisted of a gastight cube with each side 10 m in length that was located inside an unheated storage building in Antwerp. It had a Salco door with a gas-tight seal and six 3000 W heating elements, as well as three fans placed at the sealing with an angle of 45%. The chamber was equipped with a nitrogen generator (membrane system) emitting nitrogen with a residual oxygen content of 0.5 %.

Prior to treatment the bags containing tobacco beetle stages and data loggers were placed into the tobacco in the C48 cases (Fig. 1).

Fig. 1- Insect sample bag in tobacco box prior to treatment

Handling after treatment

After treatment, samples were received back by mail and checked for survivors for at least 12 weeks. This was done because sub-lethal damages caused by CA are known to considerably delay developmental time (Adler and Reichmuth 1988). Climate data from shipment and treatment were turned into graphs and compared to figures given by operators responsible for the treatment. The hatch in shipped untreated controls was compared with hatch in untreated controls that had remained under laboratory conditions to determine the influence of adverse conditions during shipment on the results.

A total of 16 treatments were carried out in four different countries to test anoxic CAs with 0.5% O_2 at various temperatures and exposure times.

RESULTS

Heating large amounts of tobacco always resulted in a marked increase in relative humidity (Fig. 2). In Antwerp harbour, this lead to massive condensation at cool chamber walls during winter conditions.
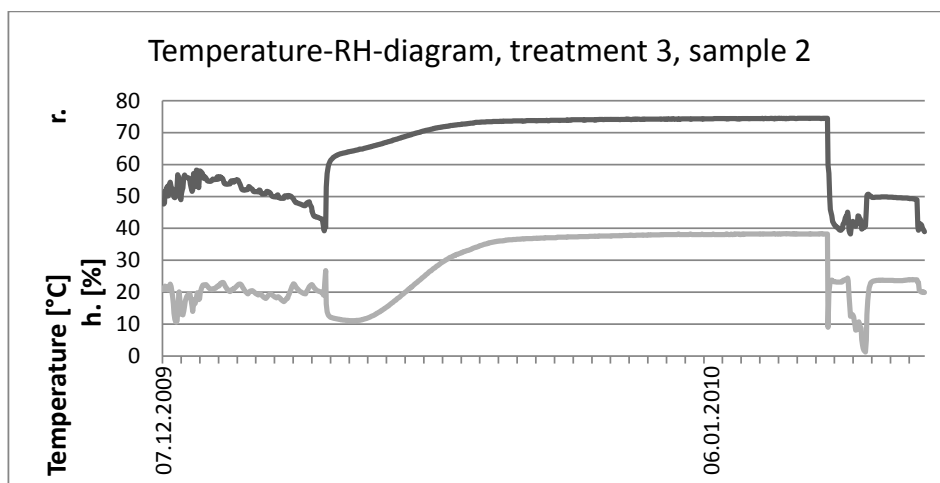
Fig. 2- Temperatures and r.h. over time in a treated sample during shipment and treatment. As may be noticed, humidity increased by approx. 20% during heating period and temperatures during shipment reached critically low levels on their way back to the laboratory.

During the first trials, temperature data of data loggers showed consistently much higher temperatures than those measured by the thermometers in the treatment chamber. Investigation showed that the temperature sensors in the chamber had not been calibrated.

During harsh frost periods in winter, shipment of samples could result in reduced numbers of survivors in the untreated control sent along with the samples compared to those of the sample remaining in the laboratory. From spring to early winter, however, hatch in both untreated samples was usually quite similar.

In a treatment in Indonesia were 38°C and a treatment time of 2.5 d at 0.5% O_2 had been chosen, few insects survived among old larvae of the tobacco beetles in our samples. At temperatures of 30°C or higher treatment times of 9 d were sufficient for complete control. Also at the same residual oxigen content, temperatures of 29°C, between 29 and 32°C and

higher temperatures for 9 d gave complete control. No survivors were found when 27°C were tested for 14 d, 25°C for 21 d, and 24°C for 28 d.

DISCUSSION

It could be seen that in heating stacked boxes or bales of tobacco an even distribution of temperatures is far from trivial. In many cases temperature recordings by data loggers differed by 2-5°C which could be attributed to their position in the stack. It is important to install strong transverse air currents directing hot air from the sealing down to the floor of a treatment chamber and to secure a good horizontal air flow, as well. The stacks of tobacco probably impede the air circulation and it could be useful to keep some free air space between the floor and the lowest tobacco box.

Fitness of a first strain utilized in this study seemed to be less than satisfactory when judged by numbers of offspring in untreated controls that had remained in the laboratory. Close investigation showed an infestation with microsporidia which motivated us to replace this strain by another phosphine resistant strain. Results obtained with the latter strain gave higher numbers of offspring but did not differ regarding the lethal effects caused by the CA treatment in combination with elevated temperatures.

The treatment at 38°C and 0.5% O_2 for 2.5 d seems to be a critical combination showing the first survivors in grown larvae close to pupation. From *Sitophilus granarius* it is known that this phase in juvenile development is most tolerant to CA treatments (Adler et al. 2000). The rice weevil *Sitophilus oryzae* could be controlled by CA at 38°C within 48 h (Jay 1987) and the granary weevil *S. granarius* at 40°C within 36 h (Adler 1997). However, the tobacco beetle was found to be comparatively tolerant to heat alone (Adler 2003). Obviously, the tobacco beetle is comparatively as tolerant as or even slightly more tolerant than the granary weevil to the combination of high temperatures and low levels of oxygen. A circumstance that may favour insect survival is the high relative humidity resulting from heating tobacco. It is known that part of the toxicity of anoxic atmospheres comes from dissiccation, and an increase in relative humidity may reduce the efficacy of this treatment.

It can be concluded that it is possible to achieve complete control of all stages of the tobacco beetle under the practical treatment conditions in a gastight chamber with a volume of approx. 1000 m^3. For the tobacco industry, a target residual oxygen content of 0.5%, temperatures of approx. 28°C, and a treatment time of some 9 d appear feasible also under economical aspects. Preheating cold tobacco may prolong total treatment times by several days. If the gastight chamber is in cold environment insulation could help to reduce the risk of condensation.

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CARBON DIOXIDE CONCENTRATION IN HERMETIC STORAGE OF SOYBEAN (*GLYCINE MAX*) IN SMALL GLASS JARS

Darío Ochandio, Ricardo Bartosik*, Alejandra Yommi, Leandro Cardoso

National Institute of Agricultural Technology (INTA), Ruta 226 km 73,5 (7620) Balcarce, Buenos Aires Province, Argentina
*Corresponding author's e-mail: *rbartosik@balcarce.inta.gov.ar*

ABSTRACT

This study aims to establish typical CO_2 concentrations in soybean stored in hermetic systems in the extremes of the common storage conditions (temperatures of 5 and 35°C and moisture content (m.c.) levels of 11, 13 and 17%). The experiment was carried out under laboratory conditions where soybeans samples were conditioned at the three different m.c. and incubated in hermetically sealed glass jars at different temperatures. The CO_2 concentration was measured by gas chromatography. When soybean was stored at 11% of m.c. and 5°C, almost no increase in CO_2 was observed after 1 year (less than 1%), but when it was stored at 35°C the CO_2 increased up to 5-7% after 230 days. At 13% m.c., the CO_2 concentration was also less than 1% after one year when stored at 5°C and was 12% when stored at 35°C for 230 days. For 17% m.c., the CO_2 concentration increased to 5.5-7% after 1 year, while for 35°C, CO_2 concentration was 20-23% after 100 days. Using the results of measured CO_2 concentrations under laboratory conditions, a guideline was developed for evaluating the storage condition of the grain in the silo-bags; however this evaluation has not yet been completed.

Key words: hermetic storage, carbon dioxide, soybean, biological activity, grain respiration.

INTRODUCTION

The silo-bag is a hermetic storage system adopted in many countries for storing dry grains. In Argentina, more than 16 million tonnes of soybean are stored in the silo-bags for a period of 6 months, either at the farm, at the elevator or at the industry level.

A technology was developed for evaluating the storage condition of the grain in the silo-bags based on the measurement of the CO_2 concentration in the bag (Bartosik et al., 2008). The CO_2 was measured in the bag and compared with a reference concentration to detect abnormal biological activity (i.e., grain spoilage, insect activity, etc). Thus, typical CO_2 values of the interstitial air are required as reference levels for this CO_2 monitoring system.

A comprehensive model was developed for simulating storage conditions in silo-bags. The model takes into account the heat and mass transfer according to the grain condition (temperature and m.c.), the ambient temperature and sun radiation. Also, the model predicts the change of O_2 and CO_2 concentrations taking into account grain respiration and permeability of O_2 and CO_2 through the plastic bag (Abalone et al., 2011a; 2011b).

However, in order to further refine the prediction of respiration (O_2 consumption and CO_2 generation) in a hermetic storage condition a suitable correlation that would take into account the effect of the oxygen depleting environment is required. Such a correlation is not available in the literature for soybean. This study aims to establish the typical CO_2 concentrations of soybean (*Glycine max*) stored in hermetic systems at common silo-bag storage conditions (temperatures of 5 and 35°C and moisture content levels of 11, 13 and 17%).

This study is a preliminary part of a more comprehensive study of soybean respiration under hermetic conditions. In the comprehensive study the CO_2 and O_2 concentration were measured for soybean stored at different m.c. and temperatures and the prediction equations were developed. Additionally, biota composition was characterized for different conditions of temperature, m.c. and gas concentration during storage. The germination test, commercial grade and volatiles (AGV and ethanol) were also correlated with storage conditions.

METHODOLOGY

The experiments were carried out at the Balcarce Integrated Unit (UIB in Spanish) of the research station of both the National Institute of Agricultural Technology (INTA) and the Agronomy College of Mar del Plata University, located near Balcarce city, Buenos Aires province, Argentina.

The soybean used in this experiment was collected during the 2011 harvest and consisted of a pool of different varieties grown in southern Argentina. All samples were graded according to the Argentine commercialization standard and also a germination test was performed to ensure that all of the samples were in good condition. Soybean with a condition that would grade them as "out of standard" (i.e., excess of mechanically damaged grains) or with low germination (i.e., due to high mold concentration) could affect the respiration rate and, hence, the experiment.

Soybean samples were conditioned to 11, 13, and 17% m.c. and placed in a chamber at 4°C until the experiment. The conditioning of the samples was done either by rewetting the soybean with distilled water, or drying the samples at laboratory conditions (temperature of 22-25°C and r.h. of 60-65%). The final m.c. was tested with a Dickey John GAC 2100 meter, and the results checked by the oven drying method according to ASAE S 352.2 standard (19 h at 130°C) (ASAE Standard, 2003).

Previous to the experiment, a test was implemented to check the gastightness of two different lids for the glass jars. Empty glass jars of 660 ml capacity were filled with a mix of gasses with 10.5% CO_2 and immediately closed with hermetic lids, one made of metal and other made of rubber. Both types of lids had at the center a perforation sealed with a rubber patch, from which a gas sample could be taken with a needle and syringe.

Gas samples were collected from the jars every 5 days approximately, and CO_2 concentration was measured with gas chromatography (Shimatdzu GC-17A, Kyoto, Japan) equipped with flame ionization red (FID) detector. Fig. 1 shows the results of the test, in which the jars sealed with the metal lid did not result with substantial change in the CO_2 concentration, while the jars sealed with the rubber lids showed a slow decrease in the CO_2 concentration. Thus, the metal lids were selected for a proper sealing of the experiment jars.
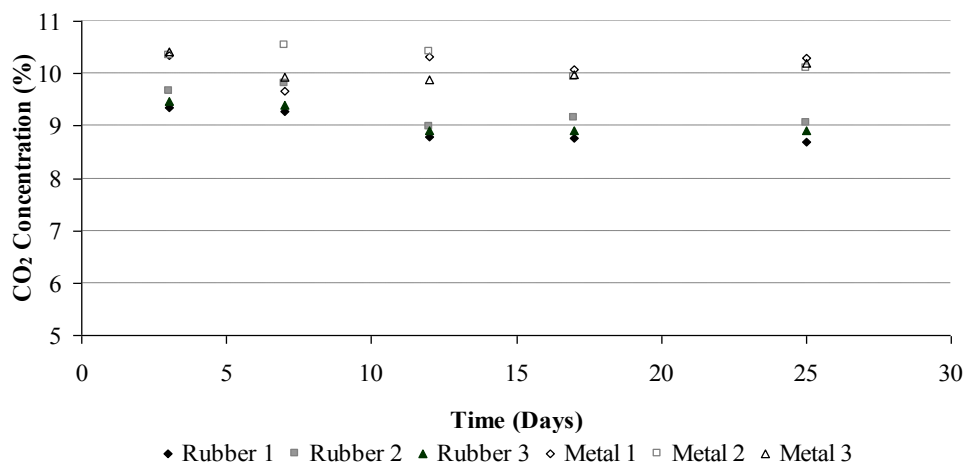
Fig. 1- CO_2 concentration (%) over time for two set of jars with different lids. The initial CO_2 concentration of the gas sample was 10.5%.

The glass jars were filled with 450 g of soybeans each at the three different m.c., occupied more than 90% of the volume of the jar. The empty space (voids) of the soybean filled jars was estimated by measuring the amount of distilled water required to completely fill the jar. The average volume of empty space was of 302.5 ml for 11%, 296.2 ml for 13% and 288.8 ml for 17% m.c. soybean

Two replicates for each m.c. were done at 35°C, while for the samples stored at 5°C three replicates were done. Air interstitial samples were taken with a needle and a 1 ml syringe and analyzed for CO_2 concentration.

RESULTS AND DISCUSSION

Soybean stored a 5°C had, in general, very low respiration. The CO_2 concentration after one year was between 5.5 and 7.5% for 17% m.c., while for 13 and 11% m.c. the CO_2 concentration did not reach 1% (Fig. 2). It could be possible that at 17% m.c. some microorganisms could be active, respiring and generating CO_2, while at 13 and 11% these organisms were not active.

Soybean stored at 35°C had a substantially greater respiration. After one month of storage, the CO_2 concentration in the 17% m.c. soybean reached 15%, tending to stabilize after 100 days to values between 20 and 23% (Fig. 3). The CO_2 concentration of the dryer samples had slower evolutions. At 13% m.c. the concentration reached 12% after 230 days, while for 11% m.c. the concentration reached only 5-7%.

It can be observed that in the experiments at 35°C and 13 and 17% m.c., there is an almost linear increase in the CO_2 concentration up to 10-12%, followed by a slow decay in the increasing rate. In a subsequent experiment (data not shown in this publication) it was observed that when the incubated sample reaches between 10-12% CO_2, the O_2 concentration drops below 1%. This would imply that at that point should be a change in the microbial activity and composition (from aerobic to anaerobic) and, hence, in the respiration rate.
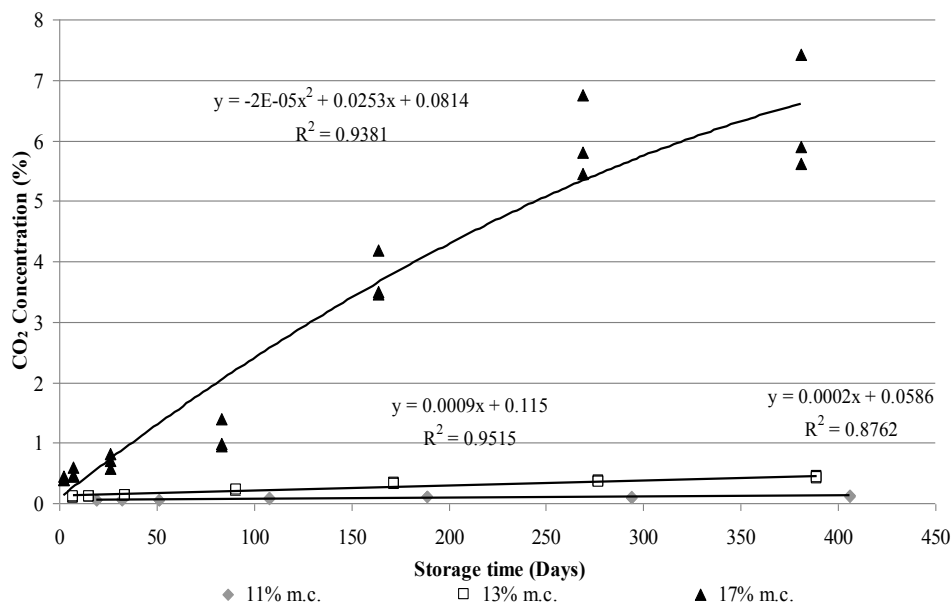
Fig. 2- CO_2 concentration (%) over storage time for soybean at different m.c. stored at 5°C.
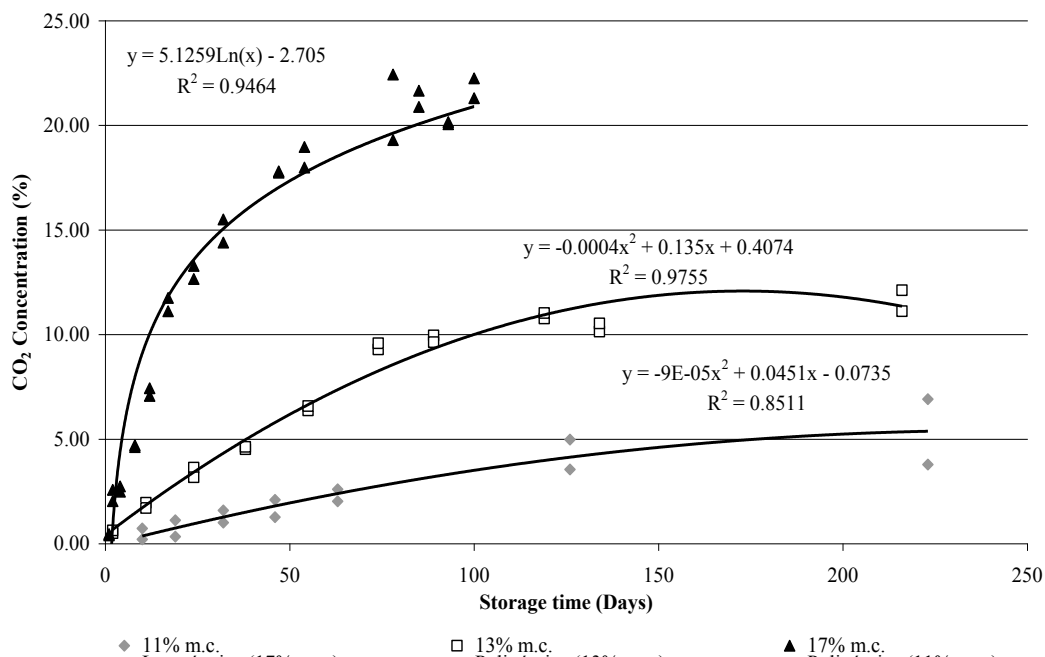
Fig. 3- CO_2 concentration (%) over storage time for soybean at different m.c. stored at 35°C.

Compared with corn, soybean has a lower respiration rate. In a similar study performed with corn at 14, 16, 18, 20, and 22% m.c. and incubated at 30°C, the CO_2 concentration over passed 21% for all the treatments, excepting 14% m.c. Most of the O_2 in the sealed containers with 14, 16, 18, 20 and 22% m.c. was consumed after 600 (25), 120 (5), 48 (2), 24 (1) and 12 (1/2) h (d), respectively. The CO_2 concentration measured after a plateau was reached was from 18% for 14% m.c. to 90% for 22% m.c. The time required to reach the plateau was from 1440 h for 14% m.c. to 480 h for 22% m.c. (Weinberg et al., 2008).

Cardoso et al. (2008) measured the CO_2 concentration in several soybean silobags at m.c. between 10 and 15% and found that, in general, the CO_2 concentration was lower than 1.5% for a storage time from 4 to 9 months in the field. On the other hand, a similar study Rodriguez et al. (2008) found that silobags with wheat from 13 to 14% m.c. had on average 5% of CO_2, showing a substantially higher respiration compared with soybean.

These observations are in agreement with the data presented in this paper. It is recommended in future works to address the evolution of O_2 in the hermetic environment of soybean and the respiration quotient.

CONCLUSIONS

The CO_2 concentration generated by soybean stored in hermetic glass jars was obtained for three different m.c. (11, 13 and 17%) and two different incubating temperatures (5 and 35°C).

Soybean at 11 and 13% m.c. incubated a 5°C almost did not show a CO_2 increase after one year, while soybean at 17% m.c. resulted with an increase of 5.5 to 7.5%.

Higher incubating temperature resulted in higher respiration and, hence, higher CO_2 concentration. Soybean incubated at 35°C during 230 d had a CO_2 concentration of 5-7% for 11% m.c., while when the m.c. was 13% the CO_2 reached 12%. When the m.c. was 17%, the CO_2 reached 20-23% after 100 d.

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EFFECTS OF CONTROLLED ATMOSPHERES FOR INSECT CONTROL AND QUALITY PRESERVATION OF MILLED RICE USING THREE WAYS TO REDUCE OXYGEN

Ling Zeng*，Chuanzhong Lao, Chao Guo, Qing Xian，Miao Zhen

Guangdong Institute for Cereal Science Research, P. R. China
*Corresponding author's e-mail: *zengling33@163.com*

ABSTRACT

Milled rice is a staple food for more than 60% of the world's population, especially in Asia. As a primary dietary source of human nutrition, milled rice plays an important role in meeting energy requirements and nutrient intakes. It is necessary for a country to store adequate milled rice to ensure the elementary living and the food safety. However milled rice is one of grain varieties which is the most difficult to storage and fresh-keeping. Aging, damage caused by pest and mould are main problems of milled rice during storage. Aiming at these main problems, the field experiment of milled rice under controlled atmospheres storage for stored grain insect control and quality preservation were undertaken in Guangdong Province, P. R. China. The purpose of this trial was to determine if three ways to reduce oxygen (O_2), including treatments of filling nitrogen (N_2), using deoxidizer and N_2+deoxidizer, could reduce and maintain the quantity of O_2 low enough and long enough to control stored grain insects and keep the quality of milled rice stored bag-stacks sealed in plastic enclosures. The results showed that all of three ways had reduced O_2 content inside the sheeted bag-stacks to lower than or about 2% since 1 week after treatment. Under controlled atmosphere for 3 months at the room temperature, tested stored grain insects, *Rhyzopertha dominica* (Fabricius), *Sitophilus oryzae* (Linnaeus)*, Tribolium castaneum* (Herbst)*,* were killed. The quality changes in senses indices, color changing, moisture content, acidity and tasting assessment value of milled rice treated were similar to those of untreated as a control, but milled rice treated made an increase to the cooking and eating qualities. It means the controlled atmospheres using three ways to reduce O_2 have effects on controlling stored grain insects and keeping rice quality. Among three ways, using deoxidizer is the lowest costs. The results will provide useful information for milled rice storage.

Key words: milled rice, controlled atmospheres, rice storage, quality preservation, *Rhyzopertha dominica* (Fabricius), *Sitophilus oryzae* (Linnaeus)*, Tribolium castaneum* (Herbst)

INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for more than 60% of the world's population, especially in Asia. As a primary dietary source of human nutrition, milled rice plays an important role in meeting energy requirements and nutrient intakes (Park et al, 2012). In recent years, as the natural disasters and other emergencies occur frequently, it is more and

more important for a country to store adequate milled rice to ensure the elementary living, emergency supplies of food and maintaining social stability. However, milled rice is one of grain varieties which is the most difficult to storage and fresh-keeping (Wang and Zhou, 2005). Due to having had its bran and hull layers removed by milling, and being directly exposed to the air, milled rice is more susceptible to insects and mould with changes in physicochemical and sensory-properties to speed up (Kim et al, 2000; Zhang et al, 2003). Aging, damage caused by pest and mould are important problems during storage of milled rice (Noomhorm et al, 1997). Over the years, grain reserves are mainly in raw grain, such as paddy and wheat, and there are relatively few studies on the theory, technology, equipment and facilities of milled rice storage. Therefore, safe storage of milled rice is now a challenge.

The conventional milled rice storage and fresh keeping technologies include storage at room temperature, storage at low temperature, controlled atmospheres storage and storage by chemical methods in and out of the country(Wu et al, 2008; Qu et al, 2009; Yang et al, 2010). With increasing concerns about health and environmental safety, consumers demand for chemical-free and insect contamination-free products is a general tendency. Controlled atmospheres storage used in milled rice is one of green grain storage technology devoting major efforts to developing (Gao and Xie, 2007; Zhu et al, 2010).This technology involves the alteration of the natural ratio of the atmospheric gases, oxygen (O_2), nitrogen (N_2) and carbon dioxide (CO_2), to render the atmosphere in stores unfavourable to pests (Annis, 1990; Banks and Fields, 1994; Adler et al, 2000; Conyers and Bell, 2007). It can inhibit the respiratory of insect pests and reduce the consumption of dry matter inside milled rice, thus effective control pests and to keep grain quality (Navarro, S.2009). The field experiments of milled rice under controlled atmospheres storage for stored grain insect control and quality preservation were undertaken in Guangdong Province, P. R. China. The purpose of this trial was to determine if three ways to reduce oxygen (O_2), including treatments of filling nitrogen (N_2), using deoxidizer and N_2+deoxidizer, could reduce and maintain the quantity of O_2 low enough and long enough to control stored grain insects and keep the quality of milled rice stored bag-stacks sealed in plastic enclosures.

MATERIALS AND METHODS

Bag-stacks preparation

The trials were undertaken at Xintang Grain Depot, Zengcheng City Grain Bureau, Guangdong Province, P. R. China in 2011. Bag-stacks stored milled rice was produced in location, belonging to long grain rice. The moisture content was 14%±1%.

Four bag-stacks of milled rice sealed in plastic enclosures were prepared for four kinds of treatment of controlled atmospheres, respectively for filling nitrogen (N_2), using deoxidizer, N_2+deoxidizer and blank-control. Each stack with 1 tone of 15kg bagged milled rice was sealed on six-sides with a new nylon composite film, which made from five-layers polyamide (PA) and polyethylene (PE) by co-extrusion. The covered film was 0.1mm thick. The size of each stack was about $1.4m^3$ (L1.05*W0.9*H1.5m). 3 plastic tubes with 6mm internal diameter were inserted into the stack at various locations for filling N_2 gas into stack, releasing air from the stack and monitoring the O_2 concentration respectively. Each plastic tube had a switch.

Test insects

Three tested insect species of laboratory cultures were rice weevil (*S. oryzae*), lesser grain borer (*R. dominica*), and red flour beetle (*T. castaneum*). All insects were adults with age mixed, and were placed in the insect cages made of PVC pipe sections covered with screens at

both ends. 30 adults of each specie with feed were placed in a insect cage as one replicate. There were 5 replicates for each insect species, with three species for each stack. The insect cages were inserted at various locations at a stack.

Gas-tightness

According to Van and Annis (1990) and the standard of "Technical regulations for controlled atmosphere storage of grain by purging carbon dioxide" (LS/T 1213-2008), set by The Chinese State Administration of Grain, application of vacuum revealed that a negative pressure was measured for gas-tightness. It requests that time to half pressure increase (negative 500 to negative 250 Pa) was more than 240s for controlled atmosphere storage.

Before treated, each stack sealing with a film sheeting was pressure tested using a micro-manometer model DP2000 made by Shanghai Guigu Instruments Co., Ltd. The film was checked for the leaks, and any leaks need to be heat sealed.

Controlled atmospheres application

N_2 was gaseous in a pressurized cylinder with purity (V/V)≥99.9%, manufactured by Guangzhou Yuegao gas industries Co., Ltd. The deoxidizer in food grade was manufactured by Guangzhou Pinnigao food additives Co., Ltd.

For filling N_2 stack, the plastic tube inserted into the bottom layer of the stack was connected with a N_2 cylinder, while the plastic tube on the top layer was connected with a vacuum air pump, and the monitoring plastic tube in the middle was connected with a gas analyzer model PA-Zr-O_2/CO_2-L made by Witt-gasetechnik GmbH & Co KG. First, to operate the vacuum air pump, let the pressure inside the stack to reach negative 500Pa. Then, gaseous N_2 from a cylinder installed a pressure reducing valves was introduced to the stack through the plastic tube at a constant rate, until O_2 concentration of 2% was reached and the pressure inside and outside the stack was balanceable.

For using deoxidizer stack, 1200g deoxidizer were place on the top of stack, the plastic tube inserted into the bottom of the stack was turned off the switch, the other two plastic tubes were connected with those as the same of filling N_2 stack. After operating the vacuum air pump and pumping gas to -500Pa, stop operating.

For using N_2+deoxidizer stack, 400g deoxidizer were place on the top of stack, three plastic tubes were connected with those of the same as filling N_2 stack. The operations were also like those of the filling N_2 stack, but the O_2 concentration just reached 5%.

For blank-control stack, no to fill N_2, no to use deoxidizer and no to pump gas, just to insert a monitoring plastic tube on the middle to measure the O_2 concentration.

Controlled atmospheres procedure

During the entire controlled atmospheres storage period, the temperatures of stored milled rice were recorded at a frequency of one hour with a data logger model RC-1+ made by Shanghai Jingchuang Electronics Co., Ltd, that can be inserted in the bag stacks in the central zone. The O_2 concentrations within the stacks were sampled daily and were monitored by a gas analyzer model PA-Zr-O2/CO2-L.

Post controlled atmospheres

At the end of 3 months of controlled atmospheres storage, milled rice quality and insect mortality were assessed for each stack. Samples of milled rice were collected from three different bag (top, middle and bottom) using a probe in each stack, and were placed in plastic bags with hermetic sealing and taken to the laboratory. The analyzed parameters were the

senses indices, color changing, moisture content (m.c.), acidity, tasting assessment value, cooking and eating qualities and so on, using the Chinese national standards methods according to GB5492-2008, GB5517-85 and GB/T15682- 2008. Insect cages were taken to the laboratory to observe. When insect mortality was recorded in the blank control stack, the adjusted mortality for the treated stacks was computed using the Abbott's formula.

RESULTS AND DISCUSSION

Gas-tightness
Four stacks were pressure tested. Time to half pressure increase (negative 500 to negative 250 Pa) was above 300s for all stacks. It means all treatment stacks had good gas-tightness.

Grain temperature
During the trials the temperatures of the milled rice were normal. The grain temperatures in the stacks were between 24.8-32.5 $^\circ$C, average temperature was 28.9 $^\circ$C.

O_2 concentration
The O_2 concentration curves during controlled atmospheres storage was shown in Fig.1. The results showed that all of three ways had reduced O_2 content inside the sheeted bag-stack to lower than or about 2% since 1 week after treatment. The O_2 concentrations in the stacks of filling N_2, using deoxidizer and N_2+deoxidizer, were respectively 2.35%, 1.84% and 1.52% at that time. Subsequently, the O_2 concentrations in treated stacks become calm. At the time of 7 weeks after treatment, the O_2 concentration was 0.90% for filling N_2 stack, 0.8% for using deoxidizer stack and 2.63% for using N_2+deoxidizer stack. Based on these results, the O_2 concentrations were maintained lower than or about 2% for at lease 7 weeks, and reached controlled atmosphere storage requirements.
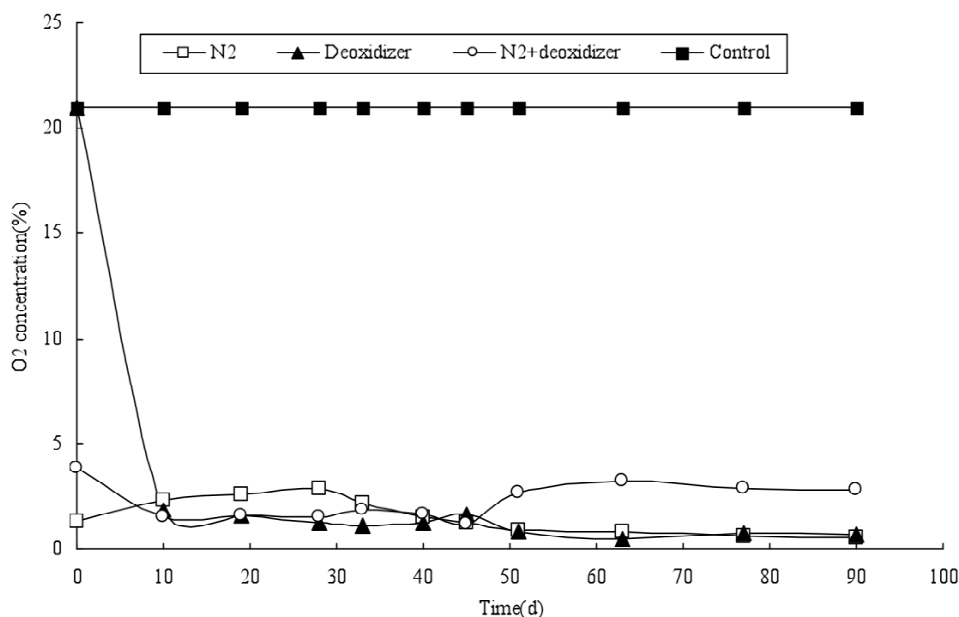
Fig.1- Concentrations of oxygen measured during controlled atmospheres of milled rice

Insect control

The population suppression results for three tested insect species were 100% (Table 1). Under three controlled atmosphere storage for 3 months at the room temperature, no live insects were found in any of the treated insect cages inspected in the laboratory. There were 86, 78 and 1373 live insects of *R. dominica*, *S. oryzae* and *T. castaneum* in insect cages of blank-control stack, respectively. The results showed that three ways to reduce O_2, including filling N_2, using deoxidizer and N_2+deoxidizer, could control stored grain insects well.

Table 1. Population suppression effect of controlled atmospheres using 3 ways to reduce O_2 on stored grain pests

Treatments	Number of live insects			The rate of suppression(%)		
	R. dominica	*S. oryzae*	*T. castaneum*	*R. dominica*	*S. oryzae*	*T. castaneum*
N_2	0±0 a	0±0 a	0±0 a	100	100	100
Deoxidizer	0±0 a	0±0 a	0±0 a	100	100	100
N_2+deoxidizer	0±0a	0±0a	0±0 a	100	100	100
Control	86±10 b	78±11b	1373±105			

* Means followed with same letters in the same insect species within the same column are not significantly different at 0.05 level by Duncan's multiple range test.

Quality of milled rice

Tables 2 and 3 showed the results of quality changes of milled rice under different controlled atmosphere storage for 3 months. In Table 2, the quality changes in appearance, yellow-colored rice, m.c., acidity and tasting assessment value of milled rice treated were similar to those of untreated as a control. In Table 3, milled rice treated made an increase total score to the cooking and eating qualities. It means the controlled atmospheres using three ways to reduce O_2 have effects on keeping rice quality under sheeted bag-stacks.

Table 2. Effects of controlled atmospheres for 3 months using 3 ways to reduce O_2 on milled rice quality

Treatments	Odour	Yellow-colored rice %	Moisture content %	Acidity ml/10g	Tasting assessment value
N_2	Normal	0.6	13.9	0.45	75
Deoxidizer	Normal	0.5	14.0	0.41	76
N_2+deoxidizer	Normal	0.4	13.7	0.41	76
Control	Normal	0.5	13.5	0.43	73

Cost-benefit analysis

Based on the results obtained, the controlled atmosphere using three ways to reduce oxygen had not significantly different effects on controlling stored grain insects and keeping rice quality, so the focus here were to compare their using costs. Costs should include labor cost, equipment cost, cost of chemicals (N_2 and deoxidizer) used and so on. Calculated according to amount used in the trails, cost of N_2 used to the filling N_2 stack was RMB 23.6 ¥/m^3, cost of deoxidizer used to the using deoxidizer stack was RMB 13.7 ¥/m^3 and cost of N_2 and deoxidizer used to the using N_2+deoxidizer stack was RMB 16.4 ¥/m^3. It means that among three ways, using deoxidizer is the lowest costs.

Table 3. Effects of controlled atmospheres for 3 months using 3 ways to reduce O_2 on cooking and eating quality of milled rice

Treatments	Odour	Appearance and structure			Palatability			Taste	Texture of cold cooked rice	Total score
		Color	Gloss	Rice integrity	Viscosity	Elasticity	Softness and hardness			
N_2	16.2	5.2	5.6	4	7	7.2	7.2	18.4	4	74.8
Deoxidizer	16	5.2	5.2	4	7.2	7.8	7.8	19.2	4	76.4
N_2+deoxidizer	15.8	5.4	5.4	4	7.4	7.6	7.6	19.2	4	76.4
Control	16	4.8	5.8	4.2	6.8	6.8	6.6	18	3.8	72.8
Score	20	7	8	5	10	10	10	25	5	100

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COMPARISON WITH PRESSURE SWING ADSORPTION AND MEMBRANE SEPARATION NITROGEN FOR REDUCE OXYGEN AT STORED GRAIN IN HORIZONTAL WAREHOUSE

Zhao Huiyi, Cao Yang*, Zhang Hongqin, Li Yanyu, Liu Jie, Li Jin,

Academy of State Administration of Grain, Beijing, 100037
№11 Baiwanzhuang Street, Beijng, P.R. China
Tel : 86-10-68623665   Fax : 86-10-58523599
* Corresponding author's e-mail: *cy@chinagrain.org*

ABSTRACT

In the 180t capacity of test horizontal warehouse，the research was made for comparison with pressure swing adsorption(PSA) and membrane separation(MS) nitrogen for reduce oxygen. During the research we had detected gas tightness of warehouse, full nitrogen flux and time, energy consumption, and oxygen decrease. The research results indicated that using facilities PSA reduced oxygen in grain mass with flux 45 Nm3/h and 49 Nm3/h, from 20.7% in normal atmosphere fall 15%, 10%, 5%和 2%，the average energy consumption were 55and 37.6 kWh，83 and 71 kWh，139 and 105kWh，189 and 160 kWh respectively. That were 61and 42 kWh,102 and 85 kWh，124 and 152kWh，193 and 195 kWh using facilities MS with flux 15.8 Nm3/h and 18.1 Nm3/h. And the average energy consumption of MS was higher than that of PSA. With higher flux it cost fewer energy but usage of nitrogen is lower. The paper had introduced the models and regulation for reducing oxygen in stored grain, also.

Key words: Stored Grain, Reduce oxygen, Pressure Swing Adsorption, Membrane Separation Nitrogen, Energy consumption.

INTRODUCTION

As the resistance of pests becomes stronger, reduced chemical application is the important task for grain storage (Benhalimaa et al. 2004, Bruce et ai. 1962). The research showed that under lower oxygen environment, the physiological activities of pests could be inhibited. Below 2% of oxygen (O_2) concentration for 20 days, the grain pests, such as *Sitophilus zeamais* Motschulsky and *Tribolium castaneum* Herbst, could be killed. Below 5%, the growth and development of pests could be inhibited. So low O_2 technology has been adapted to inhibit and kill grain pests without chemicals (Navarro 1978, Gilberg 1991).

For low O_2 process, high concentration carbon dioxide or nitrogen will be used to reduce the concentration of O_2 in barn (Valentin 1993). But comparing these two kinds of media, using

nitrogen should be more cheap and handy. In principle of making nitrogen, there are two kinds of equipment: pressure swing adsorption (PSA) and membrane separation (MS).

In this paper, PSA and MS were used for low O_2 process. And O_2 reduction time and consumption was used to compare these two kinds of equipment. At the same time the low O_2 model was also discussed.

MATERIALS AND METHODS

Test Horizontal Warehouse

The test horizontal warehouse is 180t capacity pilot-scale horizontal warehouse located in pilot-scale test base of Academy of State Administration of Grain. And its length is 9 meters, width 4.5 meters and grain loaded height 6 meters.

Nitrogen produced equipment

PSA equipment: max flux 50.35Nm^3/h and max O_2 concentration 99%(v/v%).
MS equipment: max flux 35Nm^3/h and max O_2 concentration 99%(v/v%).

Method of detection O_2 concentration

O_2 sensors were used to detect O_2 concentration. The O_2 sensors were distributed in three layers and six sensors in each layer. The distance form the first layer (bottom layer) to the bottom is 3m, the second layer (middle layer) 5m and third layer (top layer) is on the surface of the grain.

Method of power measurement

A power meter was installed on the input circuit of the making nitrogen equipment. And the power was record during the experiments.

Experiment method

Before the experiment, the nitrogen produced equipment was connected with the ventilation pipe using flexible pipe. The input nitrogen concentration was kept at 99% level. When nitrogen was input the barn, the power and the O_2 concentration for different layer was record. The equipment will be stopped until the average O_2 concentration of top layer is under 2%.

RESULTS AND DISCUSSION

Low O_2 process using PSA

Two different flux have been made to test the performance of PSA. The results showed in Fig. 1 (with flux 45Nm^3/h) and Fig.2 (with flux 49Nm^3/h).
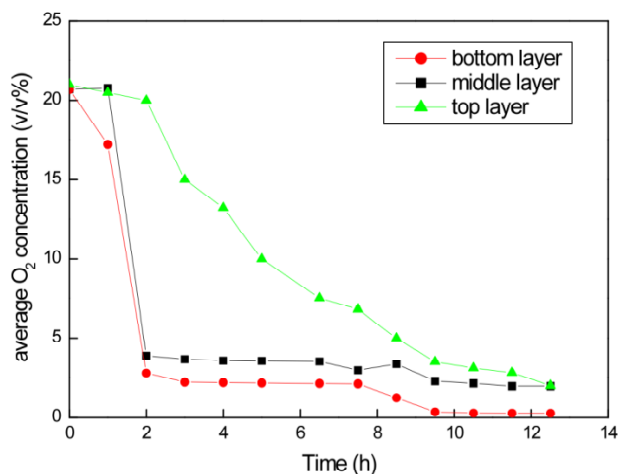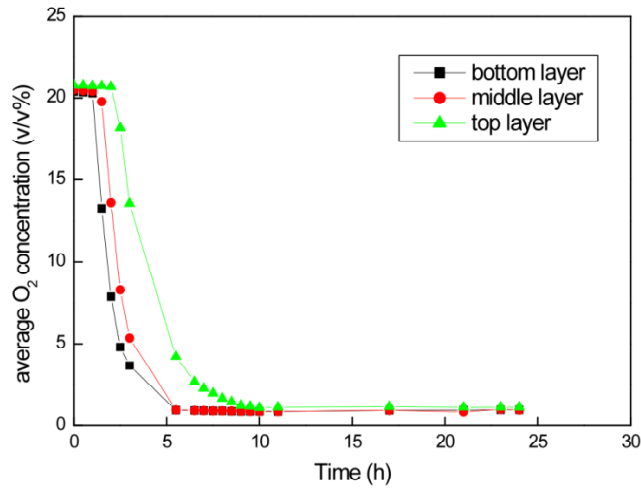
Fig. 1- The change curve of average O_2 concentration in different layer with time using PSA (flux $45Nm^3/h$).
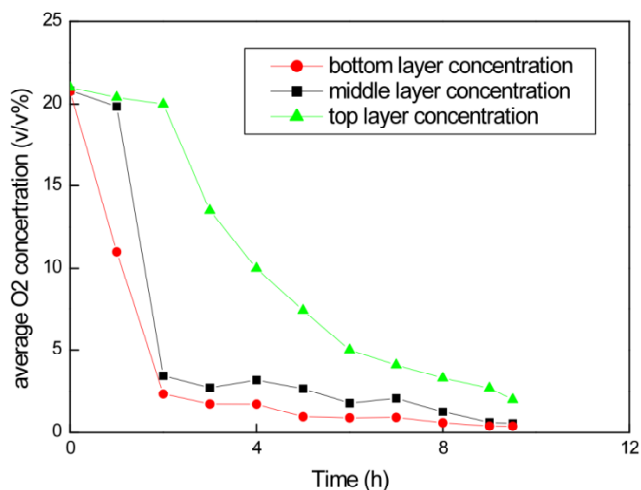
Fig. 2- The change curve of average O_2 concentration in different layer with time using PSA (flux $49Nm^3/h$).

As showed in Fig.1, Compared the different layers, the reduce rate of average O_2 concentration is depend on the distance of detection location. More close to the nitrogen inlet, it is faster for O_2 concentration to reach 2%. It spent 7.5 hours for O_2 to reduce to 2% for bottom layer and 13 hours for top layer. The same results are also showed in Fig.2 and the time is 3 hours and 9.5 hours. Compared Fig.1 with Fig. 2, the time to reach 2% is shorter with flux $49Nm^3/h$ than that with flux $45Nm^3/h$.

The power consumption with different flux is shown in Fig. 3 and Table 1. The total energy consumption is 189kWh for flux 45Nm^3/h and 160kWh for flux 49Nm^3/h. That means it consume less energy with higher flux. The energy consumption per hour and is higher with higher flux also, but energy consumption per concentration decreased is lower. The calculate data is shown in table1. As it discussed above, it is compatible to using PSA with higher flux.
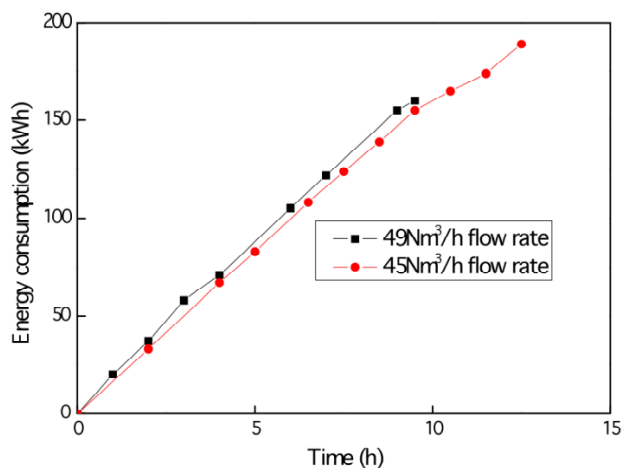
Fig. 3- The energy consumption curve using PSA with different flux.

Low O$_2$ process using MS

Two different flux have been made to test the performance of MS. The results showed in Fig. 4 (with flux 15.8Nm^3/h) and Fig. 5 (with flux 18.1Nm^3/h).

Table 1. The energy consumption using PSA with different flux

Flux (Nm^3/h)	O$_2$ average concentration of top layer (v/v%)	Time (h)	Energy consumption (kWh)	Energy consumption per hour(kWh/h)	Energy consumption per concentration decreased
	15	3	55	18.3	9.5
	10	5	83	16.6	7.7
45	5	8.5	139	16.3	8.8
	2	13	189	14.5	10.1
	15	2.2	37.6	18.0	6.5
	10	4	71	17.8	6.6
49	5	6	105	17.5	6.6
	2	9.5	160	16.8	8.5

Fig. 4- The change curve of average O_2 concentration in different layer with time using MS (flux 15.8Nm^3/h).
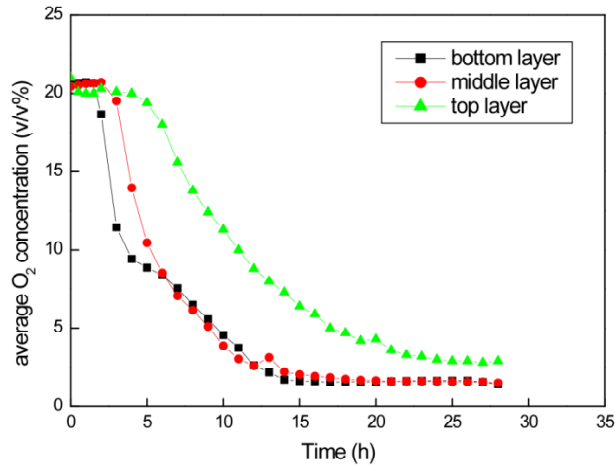
Fig. 5- The change curve of average O_2 concentration in different layer with time using MS (flux 18.1Nm^3/h).

As using PSA, the reduce rate of average O_2 concentration is depend on the distance of detection location when using MS. More close to the nitrogen inlet, it is faster for O2 concentration to reach 2%. It spent 5.5 hours for O_2 to reduce to 2% for bottom layer and 7.5 hours for top layer with flux 15.8Nm^3/h. The same results are also showed in fig.5 and the time is 4.2 hours and 14 hours. Different with using PSA, the time to reach 2% is shorter with lower flux when using MS. The reason for this difference may be due to the different flux. Using PSA,

the flux is three times of using MS. When using PSA, the nitrogen flux cannot be used to replace O_2 component efficiently and more nitrogen has been vent to the air. The power consumption with different flux is also shown in Fig. 6 and Table 2.
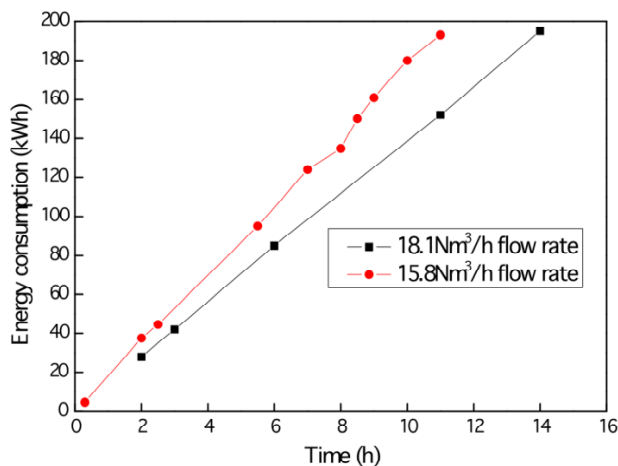
Fig. 6- The energy consumption curve using MS with different flux.

The total energy consumption is 193kW for flux 15.8Nm^3/h and 195kW for flux 18.1Nm^3/h. that means it consume almost the same energy with higher flux. The energy consumption per hour is lower with higher flux but energy consumption per concentration decreased is lower. The calculate data is shown in Table 2. As discussed above, it is compatible to using MS with higher flux also.

Table 2. The energy consumption using MS with different flux

Flux (Nm^3/h)	O_2 average concentration of top layer (v/v%)	Time (h)	energy consumption(kWh)	energy consumption per hour(kWh/h)	energy consumption per concentration decreased
15.8	15	3.2	61	19.1	10.5
	10	5.4	102	18. 9	9.4
	5	7	124	17.7	7.8
	2	7.5	193	25.7	10.2
18.1	15	3	42	14.0	7.2
	10	6	85	14.2	7.8
	5	11	152	13.8	9.6
	2	14	195	13.9	10.2

Compared with PSA, it cost more energy with MS. This is because the rate of work using MS is higher than using PSA.

According to the flux individually, the total nitrogen volume input into the bam is calculated in Table 3.

Table 3. Total nitrogen volume input into the bam with different flux

Flux(Nm^3/h)	Total nitrogen volume(m^3)
15.8	118.5
18.1	253.4
45	585.0
49	465.5

From Table 3, it is very clear that with high flux a large amount of nitrogen has been released to the air without used although it is quickly to reach the final. If considering the efficiency and usage of nitrogen, the low O_2 technology must be changed.

Low O_2 models

The low O_2 model is made to describe the average O_2 concentration changes with time. According to the three layers, total average O_2 concentration (C_w) has been made to be dependent variable as shown in expression 1.

$$C_w = \frac{\sum_1^n c_i}{n} \quad (1)$$

C_w: total average O_2 concentration (v/v%)
C_i: O_2 concentration of each detection sensor
i: the detection number
n: total detection number

The model function is adapted to simulated low O_2 process as shown in expression 2.

$$C_w = A_2 + (A_1 - A_2)/[1 + (t/t_0)^{A_3}] \quad (2)$$

C_w: total average O_2 concentration (v/v%)
t: time (h)
A_1, A_2, A_3, t_0: model coefficient

The model coefficients are shown in table 3.

From table 3, the correlation coefficient (R) and residual sum of squares (RSS) can be made to prove it is a suitable to describe the low oxygen process using the model as expression 2. And the comparison between calculated data and test data is shown in Fig. 7.
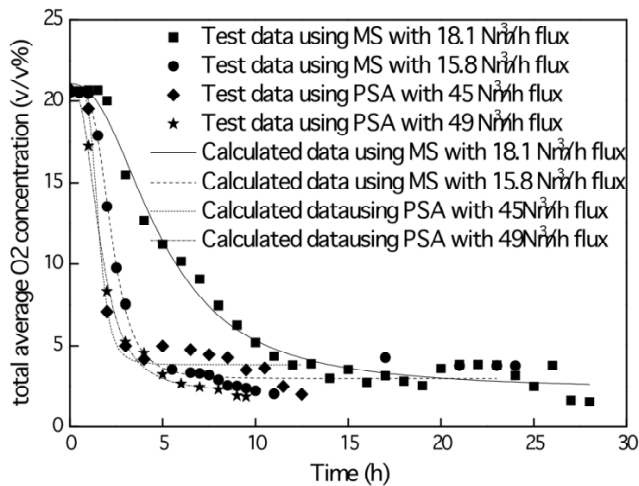
Fig.7- Comparison between calculated data and test data

CONCLUSION

- It is suitable using nitrogen produced equipment with high flux, it is cost fewer energy and energy consumption per concentration decreased.
- Using PSA has more advantage than using MS if the equipment running individually.
- Using single equipment, the usage of nitrogen is very lower. The low O_2 technology must be changed.
- The model can be used to describe the process of low O_2.

$$C_w = A_2 + (A_1 - A_2)/[1 + (t/t_0)^{A_3}]$$

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INFLUENCE OF CONTROLLED ATMOSPHERE TREATMENT ON STORAGE PESTS AND QUALITY OF SUN-DRIED FIG FRUITS

Sen[1]* F, KB Meyvaci[1], F Turanli[2], U Aksoy[1]

[1]Ege University, Faculty of Agriculture, Department of Horticulture, 35100 Izmir, Turkey
[2]Ege University, Faculty of Agriculture, Department of Plant Protection, 35100 Izmir, Turkey
*Corresponding author's e-mail: fatih.sen@ege.edu.tr

ABSTRACT

Sun-drying of fruit is a local practice especially under mild climatic conditions. In western Turkey, fig drying destined for the world market is a commercial practice. Prevention of storage pests attacking figs at the orchard while drying and later during storage is of major concern for the dried fig industry. Before the phase-out of methyl bromide (MB) was the most widely used fumigant to control storage pests due to its efficacy and low cost. After the ban, various alternatives are tested. A trial is designed to test controlled atmosphere in a pilot chamber developed by EcO2 (EcO2 B.V., AG Numansdorp, The Netherlands). Short term controlled atmosphere (CA) (%12±0.5 CO_2, %1±0.5 O_2, balance N_2 fumigation at elevated temperature (41±1°C) is tested on dried figs to control major storage pests, fig moth (*Ephestia cautella*), Indian meal moth (*Plodia interpunctella*), and dried fruit beetle (*Carpophilus* spp.), Dried fruit treated with MB (60 g/m^3 for 24 h) as control were compared with fruit fumigated with CA after the treatments and during 6 months storage under ambient conditions to assess quality changes. The tested CA treatment is recommended as a post-harvest MB alternative for dried figs since it provided 100% control of the pest species tested and required circa 26 h for the completion of the whole process which is comparatively shorter than most MB alternatives. CA application had no negative impact on dried fig quality, on the contrary had a positive effect by slowing down sugar formation on the outer skin at storage.

Key words: *Ficus carica* L., fig moth, Indian meal moth, dried fruit beetle, heat treatment, dried fruit quality

INTRODUCTION

Dried fig production and commercial sun-drying based on a single cultivar, (*Ficus carica* Sarilop (= Calimyrna)) is an important activity in the western part of Turkey. Dried fig trade may continue all year long if fruit quality is kept well and storage pest infestation is prevented. Storage pests especially the fig moth (*Ephestia cautella*; Pyralidae: Lepidoptera) and *Carpophilus hemipterus* play important role in the quality and consequent trade volume of dried fig (Turanlı, 2003). Large populations can develop before being discovered and considerable damage may occur.

Due to ban on MB, alternatives are developed but all having some limitations that prevent it from being a real substitute (Bell, 2000; Damarli et al., 1998; Johnson et al., 2000; Fields and White, 2002; Schneider et al., 2003; Aksoy et al., 2004). Controlled atmospheres

(CA) can be an alternative to MB under certain conditions. Adults of dried fruit beetle, *Carpophilus hemipterus* (L.) is relatively tolerant to heat and exposure of 20-60 min is required to achieve complete mortality at 50°C (Al-Azawi et al., 1984). *Ephestia cautella*, *Ephestia kuehniella and Plodia interpunctella* are accepted as the most tolerant species (Fields and White, 2002). Navarro et al. (2004) recommended heat treatment at 50-55°C as a MB alternative for disinfestations of dates. Higher temperatures increase efficacy of insecticide applications that target the respiratory system and for short exposure periods, low-oxygen application at higher temperatures can be considered (Donahaye et al., 1996).

The objective of this study was to investigate the potential use of controlled atmosphere (CA) with reduced oxygen level at elevated, but safe for product quality, temperature for the control of *E. cautella, P. interpunctella* and *Carpophilus spp.*

MATERIAL AND METHOD

The research was carried out on extra quality sun-dried fig (*Ficus carica* L. cv. Sarılop) fruits in a pilot fumigation chamber designed by EcO2 (EcO2 B.V., AG Numansdorp, the Netherlands). Controlled fumigation conditions were tested with lots each containing 1 ton (40 boxes) of dried fig fruit. The initial stage of the treatment comprised of increasing fig fruit temperature to 41°C and lowering O_2 concentration to 1±0.5 % followed by an increase of CO_2 to 12±1%. The safe maximum temperature level (<45°C) is decided based upon the results obtained in a series of tests undertaken to assess fruit quality changes at various temperatures (data not shown). Dried fig fruit were kept for further 16 hours at tested conditions for further penetration into the fruit tissue. The whole system was monitored and modified when necessary by a computer. The temperatures were recorded in the chamber atmosphere, inside boxes and inside the fig fruit. Fruit fumigated under controlled atmosphere conditions were stored at ambient storage conditions for six months, and quality changes were compared with methyl bromide treated control (60 g/m³ for 24 h) fruit.

Existing natural infestation levels were recorded as the number of larvae of mixed population of *Ephestia cautella* and *Plodia interpunctella*. The dried fruit beetles, *Carpophilus* spp. was evaluated as the number of infested fruits. During the experiments, culture jars containing fruits infested by different stages of storage pests larvae of lepidopteran pests and *Carpophilus* spp. were placed inside the chamber. The tests were carried out as 5 replicates, each replicate containing 7 fig fruit infested with each pest group. Culture jars were examined 6, 12, 24 hours and 14 days after the treatment in the laboratory to evaluate the effects of applications on mortality of storage pests.

Dried fig fruit quality was evaluated after the treatments and at 2 months intervals during 6 months storage under cold storage conditions. Samples were dried in a vacuum oven to a constant weight (AOAC, 1990), and fruit moisture content was calculated based on the percentage of weight loss. Water activity was measured at 25°C by a water activity meter (TH 500, Novasina, Pfaeffikon, Switzerland). The surface color of dried figs was measured on opposite sides of 25 fruits randomly taken by a colorimeter (CR-300, Minolta Co., Osaka, Japan), and average scores were recorded in terms of CIE-L* a* b* values.

A Nippon FHR-1 penetrometer possessing a conical tip (base diameter 12 mm and length 10 mm) was used to measure firmness, and the results were expressed as Newton (N). Total soluble solids content (TSS) was determined with a refractometer (ATC-1, Atago, Japan) and expressed as g/100 g.

Sugaring on the outer surface was evaluated visually on a 1–5 scale, each class describing the surface area covered by white sugar crystals (1; no sugaring to 5; complete

cover with sugar). The sugaring index of the sample was calculated using the following formula: (sugaring class value (scale) x number of fruits within each class)/total number of fruits (Aksoy et al., 2004).

Fluoresence on dried fig fruit were evaluated visually under long wave (360 nm) UV light. 8 plates, each containing 10 fruits showing BGYF were prepared to assess if the tested treatments have any effect on BGYF since BGYF fruit are removed during processing to decrease aflatoxin contamination. The BGYF area was marked on each fruit with a board marker and the intensity of BGYF was evaluated as very soft: 1, soft: 2, medium: 3, strong: 4, very strong: 5. Half of the plates underwent the aforementioned treatments together with the other fruits in the experiments. The individual treated and non-treated fruits were examined by two panelists already trained on BGYF. Additionally, 8 panelists were asked to compare treated and non-treated samples in respect to BGYF intensity.

The experiments were conducted as completely randomized design with five replicates (except BGYF test). All computation and statistical analyses were done using SPSS (SPSS, Inc., Chicago, IL, USA) package version 19.0. Significant differences between the means for each storage period were determined by Duncan's multiple range tests at $P \leq 0.05$. Standard deviation of the mean (SD) was also calculated from the replicates.

RESULTS AND DISCUSSION

Mortality of test insects
The natural infestation level of fruit samples was 10.0 % before the treatments. The *Carpophilus* spp. infested fruit rate was 7.4%. 100 % mortality was obtained in tested stages of the targeted stored pests 6 hours after the CA treatment. The total fumigation process including heating, CA exposure and aeration lasted for 26 hours.

Fruit quality
Dried fig fruit colour L* (lightness) value was lower ($P \leq 0.05$) and b* (yellowness) value higher ($P \leq 0.01$) in Controlled Atmosphere (CA) treated fruit compared to MB treated. This difference in colour (b*) continued till the second month however equalized later towards the end of the storage (Table 1). This difference could also be due to the sampling as variations are seen in 0-2-4 month results of MB treated fruit. Colour a* value of CA treated fruit were similar to MB treatments throughout the storage. Fruit colour may change during drying and storage due to a number of chemical and biochemical reactions. In most cases, Maillard reaction, a non-enzymic browning, occurs in dried fruit (Roos and Himberg, 1994; Perera and Baldwin, 2001). The darkening continue in storage however the rate depends both on the substrate and on that is related to the substrate and the storage conditions (Perera, 2005). Turkish dried figs are known for their light colour therefore colour is accepted as a major quality attribute.

CA treatment had marked effect on water activity ($P \leq 0.05$) and moisture contents ($P \leq 0.01$) of treated fig samples (Fig 1A and Table 2) possibly as a result of enhanced moisture loss at elevated temperatures. The moisture destined to the chamber atmosphere is further channeled out prior to inlet of nitrogen gas letting lower moisture content in the chamber. However, this difference was lost after two months of storage since moisture transfer continues until fruit moisture reaches to equilibrium with the exposed atmosphere. Loss of moisture from dried fig fruit may harden the texture and thus reduce palatability if at severe rates (Sen et al., 2008; Sen et al, 2010) however the reduction rate was not at levels that could have considerable effect on firmness (Table 2). Similarly, the effect on TSS was also

negligible. Lower water activity levels obtained in CA treatment allowed dried fruit to be at safer levels in respect to microbial growth even at the initial stage of processing (Fontana, 1987).

Table 1. Effect of MB (60 g/m3 for 24 h) and CA (1% O_2, 12% CO_2, balance N_2 at 41°C for 16 h) treatments on surface color (CIE L*, a*, b* values) of dried fig fruit measured after treatment and during storage.[z]

Storage period	L* value		a* value		b* value	
	MB	CA	MB	CA	MB	CA
0. Month	58.43±1.16 a[x]*	56.68±2.56 b	4.68±0.21[NS]	5.36±0.19	24.25±0.83 b**	28.29±1.07 a
2. Month	59.43±1.36[NS]	58.29±2.39	4.41±0.16[NS]	4.86±0.18	22.82±0.78 b*	25.02±0.99 a
4. Month	58.20±1.98[NS]	56.95±2.24	4.66±0.24[NS]	5.21±0.33	21.65±0.83[NS]	22.74±0.87
6. Month	55.05±1.21[NS]	56.08±2.45	5.15±0.16[NS]	4.39±0.22	16.28±0.55[NS]	18.25±0.61

[NS], *, **, Nonsignificant or significant at $P \leq 0.05$, or 0.01, respectively.
[z] Results are the means of five replicate samples ±SD.
[x] Means for each experiment were separated within columns by Duncan's multiple range test, P <0.05.

Table 2. Effect of MB (60 g/m3 for 24 h) and CA (1% O_2, 12% CO_2, balance N_2 at 41°C for 16 h) treatments on surface water activity, TSS and firmness of dried fig fruit measured during the storage period.[z]

Storage period	Water activity		TSS (%)		Firmness (N)	
	MB	CA	MB	CA	MB	CA
0. Month		0,633±0,007				
	0,659±0,009a[x]*	b	60.0±0.3[NS]	61.2±0.3	7.69±0.13[NS]	7.51±0.24
2. Month	0,634±0,004[NS]	0,620±0,003	62.0±0.8[NS]	62.0±0.8	6.90±0.22[NS]	7.07±0.28
4. Month	0,598±0,012[NS]	0,607±0,008	62.3±1.3[NS]	63.3±0.8	7.23±0.11[NS]	7.29±0.21
6. Month	0,589±0,004[NS]	0,582±0,005	60.9±0.8[NS]	62.7±1.3	7.66±0.13[NS]	7.55±0.14

[NS], *, Nonsignificant or significant at $P \leq 0.05$.
[z] Results are the means of five replicate samples ±SD.
[x] Means for each experiment were separated within columns by Duncan's multiple range test, P <0.05.

If dried figs are stored under unfavourable conditions that trigger moisture loss at storage, dissolved substances are transported parallel with the outer flow of water and sugar crystals form on the skin (Meyvaci et al., 2003). CA and MB treated dried figs displayed similar sugaring tendencies during storage. Incidence and severity of sugaring increase with storage temperature and time (Mitcham et al., 2012). However CA treated fruit had less sugaring compared to MB (Fig. 1B). The crystallization rate of sugars was slowed down possibly due to initial heating of the fruit.
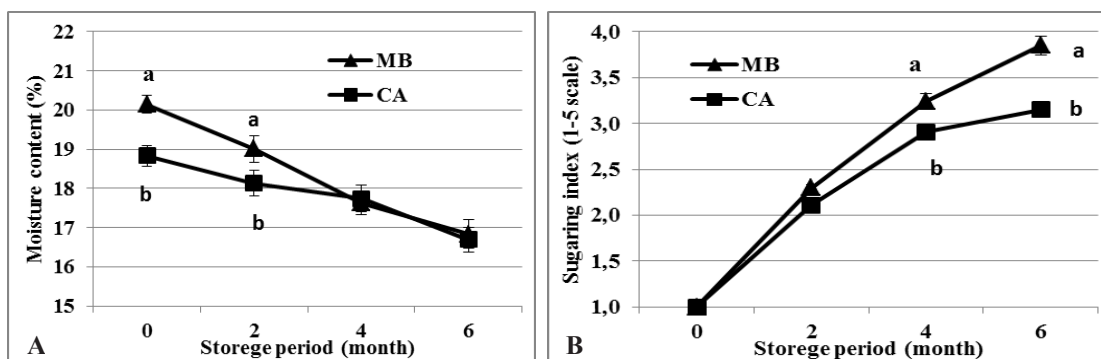
Fig. 1- Effect of MB (60 g/m3 for 24 h) and CA (1% O_2, 12% CO_2, balance N_2 at 41°C for 16 h) treatments on surface moisture content (A) and sugaring index (B) of dried fig fruit measured after treatment and during storage.[z]

Bright greenish yellow fluorescence

BGYF fruit irrespective of the intensity or size of the fluorescence are known to be highly correlated with aflatoxin contamination (Steiner et al., 1985. Thus BGYF fruit are removed during processing to reduce aflatoxin levels. CA or MB treatments had no reducing or modifying effect on intensity or area of bright greenish yellow fluorescence on the fruit surface. This result shows that CA treatment prior to BGYF removal does not create any bottlenecks in dried fig processing.

CONCLUSION

The tested CA+heat treatment was effective in providing complete mortality of major dried fig storage pests, fig moth (*Ephestia cautella*), Indian meal moth (*Plodia interpunctella*), and dried fruit beetle (*Carpophilus* spp.) and had no negative impact on fruit quality of fruit stored for four months under ambient conditions. Therefore, CA treatment can be recommended to the sector as a MB alternative since it requires comparatively short duration and can be easily adapted to mobile systems. During operations, energy and gas costs need to be considered.

ACKNOWLEDGMENTS

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LABORATORY STUDIES ON THE APPLICATION OF HERMETIC STORAGE FOR PRESERVING ARABICA COFFEE

Glory C. Sabio[*1], Joel V. Dator[1], Marianne C. Austria[1], and Vicky G. Mesa[2]

[1] Former employees of the Bureau of Postharvest Research and Extension (BPRE),
Central Luzon State University (CLSU), Munoz, Nueva Ecija, Philippines
[2] Philippine Center for Postharvest Development and Mechanization (PhilMech)
(formerly BPRE), CLSU, Munoz, Nueva Ecija, Philippines
[*]Corresponding author's e-mail: gcsabio@yahoo.com

ABSTRACT

Laboratory storage trials of up to 9-month duration were carried out to observe and compare the use of gastight SuperGrainbags (SGB) with plastic woven polypropylene bags (PPB) as storage containers for preserving dried (10-10.8 % m.c.) parchment Arabica coffee. Trials were done under simulated field condition (17.8-21°C; 67.2-85.4% r.h.) using incubators, and at ambient room condition (25.7-31.5°C; 59.7-71.8% r.h.). Four (4) replicate bags (10 kg capacity) each filled with approximately 7 kg of dried Arabica coffee were prepared for each kind of storage container and condition for destructive sampling and analyses at predetermined storage periods. Parameters observed were changes in m.c., microbial load, Ochratoxin A contamination, insect infestation, weight loss and sensory attributes (appearance, aroma, flavor and body or mouth feel). Results of the storage trials except the sensory attributes of Arabica coffee are presented and discussed in this paper.

Key words: SuperGrainbags, Polypropylene bags, Arabica coffee, Simulated field condition, Ambient room condition, moisture content, microbial load, Ochratoxin A, insect infestation, weight loss, sensory attributes

INTRODUCTION

Coffee is one of the biggest dollar earner crops grown in the Philippines. The Philippines was the 4th largest coffee exporter in the world before coffee farms were wiped out by the dreaded "coffee rust" disease in 1889 (Albert, 2007). It re-emerged as a coffee exporter again in the 1970's, generating at least US$150 million a year up until 1986. But, the export market started to dry up in the 1990's once more when coffee farming became not profitable because of highly erratic market price, lack of technologies, poor farm to market roads, and lifting of the ban on coffee importation (Asia Pulse News, 2002; Figaro Foundation Corporation, 2006; Albert, 2007). Many farmers sold their coffee farms then or shifted to other crops. However, there was a recent surge in international as well as local demand for coffee beverage. Coffee is being claimed to offer various health benefits and positive functional effects on

concentration, alertness and body endurance among others (Philippine Herbal Medicine, 2006). Cognizant of the promises of a vibrant market to the Philippines' coffee industry, the government and some private organizations started in 2002, to aggressively campaign and tap available resources for the revival of the coffee industry (Asia Pulse News, 2002 ; Asia Pulse News, 2007).

There are four important commercial varieties of coffee grown in the Philippines: Arabica (*Coffea arabica* L.), Robusta (*Coffea canephora* Pierre ex A. Froehner), Liberica (*Coffea liberica* W. Bull. ex Hiern.) and Excelsa (*Coffea excelsa* A. Chev.) (Philippine Herbal Medicine, 2006*)*. Arabica coffee, which accounts for 5-10% of the country's total production (Anenias, 2001) is highly demanded in the market. It is sought by premium coffee buyers worldwide for its elegant and complex flavor. Arabica is twice more expensive than Robusta coffee, therefore increased production of this variety is mainly promoted not only for local consumption but for export as well. Arabica coffee is largely produced in the province of Benguet, Luzon Island, Philippines.

In anticipation of increased coffee production, BPRE explored the development and local application of hermetic storage for coffee at the village level. Coffee farmers and even traders require adequate storage technologies to preserve the qualities of stored coffee and prevent losses especially when prolonged storage becomes inevitable such as when there is a glut in supply and the coffee farmers have to wait for better market price. Prolonged storage of bagged coffee in parchment state and more so as green coffee beans in warm climates under ambient conditions, even when properly dried, could result to gradual loss of beans' taste and aroma, color and density, and mold infection and contamination with toxins and insect infestation (Aronson et al., 2005). The successful use of hermetic storage in preserving the qualities of coffee beans has been reported in Costa Rica (Aronson et. al., 2005) and India and Kenya (Ministry of Agriculture and Rural Development, 2002). The lethal hypoxic atmosphere formed could minimize several oxidative processes that could adversely affect the bean quality and kill microbial and insect pests (Aronson et al., 2005). Furthermore, hermetic storage promotes organic way (no chemical insecticides or pesticides used) of protecting coffee. Many consumers nowadays, local or abroad, are health conscious and are looking for organic products. By choosing organic coffee, small-scale farmers who depend on traditional farming systems are supported and the non-use of hazardous chemical fertilizers and pesticides protects the environment as well. Hence, this study was conducted.

This study aimed at developing an appropriate hermetic storage technology for preserving the qualities and minimizing losses of Arabica coffee during storage and transport at the village level. The specific objectives were to:

a) assess the efficacy of gastight storage containers in maintaining moisture content of coffee, preventing mold growth and toxin contamination, controlling insect infestations, and weight loss in stored coffee, and

b) determine the combined effects of hermetic storage using gastight polyethylene (PE) plastic bags and moisture content on the organoleptic qualities of Arabica coffee

The first objective was investigated under Study l: Laboratory Storage Trial of Arabica Coffee, while objective 2 was observed under Study 2: Sensory Evaluation of Stored Arabica Coffee. The results of Study 1 are presented and discussed in this paper.

MATERIALS AND METHODS

a) Preparation of test coffee:

The required volume of Arabica coffee that was used in the storage experiment was purchased through a middleman from farmers in neighboring farms in Mankayan, Benguet who harvested coffee berries within the first two weeks of December 2009. Procured coffee stock was already depulped and sundried to moisture content ranging from 12-14%. Purchased coffee was transported in gastight containers to BPRE headquarters without delay and sundried further down to 10-12% m.c., rebagged, tempered overnight then used for the experiment on the following day.

Coffee samples for sensory evaluation were gathered from representative samples during the scheduled sampling period then dehulled using a laboratory huller. The resulting green beans were aspirated, packed in 1.2 kg (to come up with 1 kg ground coffee) polyethylene bags then brought to the processor for roasting and grinding. The coffee beans were roasted medium dark at 218°C. The ground coffee samples were brought back to the BPRE laboratory then turned over to the College of Home Science and Industry, CLSU on the following day for sensory evaluation.

b) Storage trial set-up:

Extended storage trials of up to 9-month duration were carried out to observe and compare the use of hermetic or gastight storage containers called SuperGrainbags (SGB) made of transparent multi-layered (with gas barrier between layers), polyethylene plastic (0.078mm) material with plastic woven polypropylene bags (PPB) for storing parchment coffee under two different conditions namely: a) Simulated Field Condition (Benguet) (17.8-21°C; 67.2-85.4% r.h.) using incubators, and b) Ambient room condition (Control) (25.7-31.5°C; 59.7-71.8% r.h.).

Four (4) replicates, each one filled with approximately 7 kg of parchment coffee, were prepared for every kind of storage container and condition for destructive sampling and analyses at the end of every predetermined storage period (Table 1).

c) Assessment criteria and frequency of observation:

The effectiveness of the storage containers tested in preserving parchment coffee under the specified storage conditions were based on the parameters listed and sampling schedule shown in Table 2.

All parameters except sensory evaluation were observed and analyzed at the Food Protection Department (FPD) laboratories of BPRE. Moisture contents of coffee were measured immediately after collecting samples using a Dole moisture meter calibrated through oven method. The weight loss that referred to quantity loss or reduction in weight of stored coffee was calculated by weighing the prepared replicate bags of Arabica coffee for observation at pre-determined storage periods (e.g. after 1, 2, 3, 4 to 9 months) and compared these weights with the initial weight of coffee recorded at the start of storage in replicate samples using the formula:

$$\text{Percent weight loss} = \frac{\text{initial weight - weight at end of storage}}{\text{Initial weight}} \times 100$$

Table 1. Coffee storage trial protocol

Storage condition	Storage containers	No. of replicates (7 kg @ rep) per storage/sampling period (months)						Total	
		0	2	3	4	6	9	No. of replicates	Wt (kg)
Simulated Field Condition (Incubators, 18-21°C; 68-85% r.h.)	SGB[a]		4	4	4	4	4	20	160
	PPB[b]		4	4	4	4	4	20	160
Ambient Room Condition (Control, 26-32°C; 58-72% r.h.)	SGB[a]		4	4	4	4	4	20	160
	PPB[b]		4	4	4	4	4	20	160
Total no. of replicates analyzed		4[c]	16	16	16	16	16	84	672

[a]SuperGrainbags
[b]Polypropelene bags
[c]Representative samples randomly collected from stock of test coffee at the start of storage to determine the initial condition for future reference.

Table 2. Test parameters and frequency of observation.

Parameters	Schedule of sampling and analyses (months)
1. Changes in moisture content	0, 2,3,4,6,9
2. Sensory evaluation/organoleptic tests: taste, aroma, color, and body (mouth feel)	0, 2,4,6,9
3. Microbial load	0, 3, 6,9
4. Ochratoxin	0, 3,6,9
5. Insect infestation	0,3,6,9
6. Weight loss	0, 2,3,4,6,9

Microbial infection was determined from thoroughly mixed replicate samples per storage container, condition and duration. Three subsamples of 10 randomly selected coffee seeds each were directly plated in potato dextrose agar for observation. Assays were done starting from 3 up to 7 days after plating. Ochratoxin A contamination in coffee samples was analyzed by the Laboratory Services Division, FPD, using the Immunoaffinity Column (IAC)-Liquid Chromatograph (LC) Method (AOAC). Insect infestation was determined by sieving 500 g sample gathered from each replicate sample. All insects sieved (dead or alive) were then sorted, counted and identified. Representative specimens were properly mounted, and stored.

The temperature and r.h. under the Simulated field and Ambient room conditions were monitored daily throughout the experiment. Monitoring of changes in color of stored coffee, CO_2 and O_2 concentrations inside representative SuperGrainbags was attempted but discontinued because of inadequacy of equipment.

d) Statistical Analyses: Gathered data were subjected to Analysis of Variance (ANOVA) using Split plot in Completely Randomized Design.   Statistical significance at 5% level was tested by comparing the F test statistic.

RESULTS AND DISCUSSION

The main species of insect collected from coffee samples stored both in SuperGrainbags (SGB) and in Polypropylene bags (PPB) was the coffee berry borer, *Hypothenemus hampei* (Ferrari 1867). The incidence of *H. hampei* in the Philippines has been reported earlier by Morallo-Rejesus and Baldos **(**1980). *H. hampei*, a native of Africa, is recognized as one of the most harmful pests of coffee worldwide with Arabica as its preferred host plant (Pest CabWeb 2002; Garcia, 2010). All insects sieved from samples were dead adult insects. Infestation in samples stored in SGB and PPB remained comparable with no significant changes all throughout the storage trial. *H. hampei,* which is considered as field pest, apparently failed to survive the drying process of coffee and probably the storage condition too. The presence of psocids in some samples was occasionally noted however, their occurrence is most likely due to cross infestation.

The moisture content of Arabica coffee stored in SGB and in PPB significantly decreased generally comparably through time (Table 3).

Table 3. Combined effect of storage container and time on m.c. (%) of Arabica coffee.

Container	Storage period (months)			
	0	3	6	9
SGB[a]	10.275a	9.705b	9.275c	8. 48d
PPB[b]	10.375a	9.500bc	8.315d	8.09d

[a]SuperGrainbags
[b]Polypropelene bags
Note: Values in a row or in a column followed by the same letter are not statistically different at 5% level.

This is despite of the fact that the decrease in m.c. of coffee stored in SGB were more gradual and lesser than those stored in PPB. Storage condition showed no significant effect on the moisture content of coffee stored in SGB under Simulated and Ambient room conditions (Table 4).

The moisture content of coffee stored in PPB under Simulated room condition was similar to those stored in SGB. However, those stored in PBB under Ambient room condition exhibited significantly lower moisture content than those stored under simulated field condition.

Table 4. Combined effect of storage conditions and containers on m.c. (%) of Arabica coffee.

Condition	Containers	
	SGB[c]	PPB[d]
Simulated field condition[a]	9.440a	9.683a
Ambient room condition[b]	9.428a	8.458b

[a] Incubators, 17.8-21°C; 67.2-85.4% r.h.
[b] Control, 25.7-31.5°C; 59.7-71.8% r.h.
[c] SuperGrainbags
[d] Polypropelene bags
Note: Values in a row or in a column followed by the same letter are not statistically different at 5% level.

Decrease in weight loss of Arabica coffee stored in SGB became significant only at 9 month storage while those stored in PPB incurred significant decrease starting at 6 month of storage (Table 5).

Table 5. Combined effect of storage container and time on weight loss from an initial value of 7 kg per bag over time of Arabica coffee.

Container	Storage period (months)			
	0	3	6	9
SGB[a]	7.000a	6.940ab	6.915ab	6.890b
PPB[b]	7.000a	6.900ab	6.715c	6.565d

[a] SuperGrainbags
[b] Polypropelene bags
Note: Values in a row or in a column followed by the same letter(s) are not statistically different at 5% level.

These results indicate that weight loss of coffee resulting from loss of moisture content could be less when kept in SGB. Consequently, the potential monetary gain could be higher when SGB container is used for storing coffee. Furthermore, water absorption and adsorption that could result to fast deterioration of coffee whether during storage or transport may be minimized if not prevented with the use of sealed containers.

Microbial analysis of test coffee stored in both SGB and PPB showed the constant presence of 3 species of fungi, the *Penicillium citrinum*, *Aspergillus niger* and *Aspergillus flavus,* from the onset up to the end of the storage trial both under Simulated field condition and Ambient room condition (Tables 6 and 7).

Table 6. Percentage frequency of isolation of fungi in Arabica coffee stored in PPB[a] and SGB[b] containers under simulated field condition (17.8 -21°C; 67.2-85.4% r.h.).

Fungal species/Storage container	Storage period (months)						
	0	3		6		9	
		PPB	SGB	PPB	SGB	PPB	SGB
Aspergillus niger	3.74	21	19	21	21	21	15
Aspergillus flavus	2.80	8	13	27	40	16	3
Aspergillus oryzae	0	0	0	6	11	3	1
Aspergillus terreus	0	0	0	0	0	3	9
Mucor sp.1	0	11	10	16	12	3	9
Mucor sp.2	0	0	0	8	19	3	10
Mucor sp.3	0	0	0	0	0	3	3
Penicillium citrinum	93.46	99	94	10	3	99	80
Penicillium funiculosum	0	0	0	50	39	0	0
Unknown	0	0	0	0	0	0	0

[a] Polypropelene bags
[b] SuperGrainbags

Table 7. Percentage frequency of isolation of fungi in Arabica coffee stored in PPB[a] and SGB[b] containers under ambient room condition (25.7-31.5°C; 59.7-71.8% r.h.).

Fungal species/Storage container	Storage period (months)						
	0	3		6		9	
		PPB	SGB	PPB	SGB	PPB	SGB
Aspergillus niger	3.74	20	34	34	10	48	31
Aspergillus flavus	2.80	24	31	40	18	14	13
Aspergillus oryzae	0	0	0	7	6	6	4
Aspergillus terreus	0	0	0	0	0	6	3
Mucor sp.1	0	15	13	14	9	12	7
Mucor sp.2	0	0	0	6	5	8	15
Mucor sp.3	0	0	0	0	0	1	0
Penicillium citrinum	93.46	83	95	34	26	29	63
Penicillium funiculosum	0	0	0	0	13	2	2
Unknown	0	0	0	0	0	0	2

[a] Polypropelene bags
[b] SuperGrainbags

Damon (2000) mentioned that some authors reported that due to physical damage caused by *H. hampei*, attacked mature berries become vulnerable to infection and further pest attack. *P. citrinum* was more frequent than the two latter species in the samples but it is not a known Ochratoxin A producing fungus. *A. niger,* on the other hand, has been reported (Abarca et al., 1994; Accensi et al., 2001) as an Ochratoxin producing fungus.   Mean percentage infection of coffee samples by this organism did not significantly differ between those stored in SGB and PPB whether under simulated field or ambient room condition up to 3 months (Table 8) but from 6 months onward, those stored in SGB under both conditions, and in PPB under Simulated field condition registered significantly lower infection by *A. niger* compared to those stored in PPB under Ambient room condition. A. *flavus* is associated with *Aflatoxin* production in food.   There were other fungal species that were encountered as storage progressed but these are regarded as common storage contaminants.

Table 8. Mean Percentage Infection of *Aspergillus niger* in Arabica coffee beans stored in SGB[a] and PPB[b] containers at different storage conditions.

Storage Condition	Storage Container	Storage Period (months)			
		0	3	6	9
Simulated Field Condition[c]	SGB	4.0	18.5[BCD]	21.0[BCD]	15.0[CD]
	PPB	4.0	21.0[BCD]	20.8[BCD]	21.0[BCD]
Ambient Room Condition[d]	SGB	4.0	34.0[AB]	10.0[D]	30.8[BC]
	PPB	4.0	20.0[BCD]	34.0[AB]	47.8[A]

[a]SuperGrainbags
[b]Polypropelene bags
[c]Simulated field condition, 17.8 -21°C; 67.2- 85.4% r.h.
[d]Ambient room condition, 25.7-31.5°C; 59.7-71.8% r.h.
Note: Values in a row or in a column followed by the same letter (s) are not statistically different at 5% level.

Analysis of test coffee stocks for Ochratoxin A contamination revealed the presence of <0.3 µg/kg (the detection limit of the method used) Ochratoxin A in initial samples (Table 9). Except in two samples, this level remained in all up to the end of the 9-month storage period. One of the samples kept in SGB under Ambient room condition for 6 month exhibited 0.66 µg/kg Ochratoxin A. The other sample that was stored in PPB under Simulated field condition for 6 months contained 1.1µg/kg Ochratoxin A. Even so, these levels of Ochratoxin A are still far below the safe level of 5µg/kg in roasted coffee beans and ground roasted coffee set by the Commission Regulation (EC) No 1881/2006.

SUMMARY AND CONCLUSIONS

The application of hermetic storage containers using SuperGrainbags (SGB) for preserving dried (10-10.8 %m.c) parchment Arabica coffee was observed and compared with the ordinary plastic woven polypropylene bags (PPB). Laboratory storage trials of up to 9-month duration were conducted under Simulated Field Condition using incubators (17.8-21°C; 67.2-85.4% r.h.), and Ambient Room Condition (25.7-31.5°C; 59.7-71.8% r.h.). The parameters observed were changes in moisture content, microbial load, Ochratoxin A level, insect infestation and weight loss.

Table 9. Levels[a] of Ochratoxin A contamination (ug/kg) in coffee bean samples stored in SGB[b] and PPB[c] under different storage conditions and time.

Storage container	Replicates	Storage time (months)						
		0	3		6		9	
			ARC[d]	SFC[e]	ARC[d]	SFC[e]	ARC[d]	SFC[e]
PPB	R1	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
PPB	R2	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
PPB	R3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
PPB	R4	<0.3	<0.3	<0.3	<0.3	1.1	<0.3	<0.3
SGB	R1	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
SGB	R2	<0.3	<0.3	<0.3	0.66	<0.3	<0.3	<0.3
SGB	R3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
SGB	R4	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3

[a]Ochratoxin level of < "value" stands for the detection limit of the method used.
[b]SuperGrainbags
[c]Polypropelene bags
[d]Ambient room condition (25.7-31.5°C; 59.7-71.8% r.h.)
[e]Simulated field condition (17.8 -21°C; 67.2-85.4% r.h.)

Dead adults *Hypothenemus hampei*, was the predominant insect pest collected from Arabica coffee samples stored in SGB or in PPB under Simulated field condition or Ambient room condition. The moisture content of Arabica coffee stored in SGB decreased gradually and lesser than those stored in PPB through time but the changes were significant and generally comparable. Storage condition showed no significant effect on the moisture content of coffee stored in SGB but for coffee stored in PPB, those kept under Ambient room condition exhibited significantly lower moisture content than those stored under Simulated field condition. Significant weight loss in Arabica coffee stored in SGB was only noted at 9 month but those stored in PPB incurred significant weight loss starting from 6 months. *Aspergillus niger*, an Ochratoxin producing fungus, was constantly present in samples kept in SGB and PPB both under Simulated field condition and Ambient room condition from the onset up to the end of the storage trial. However, percentage *A. niger* infection of coffee stored in SGB under both storage condition, and in PPB under Simulated field condition, were significantly lower compared to those stored in PPB under Ambient room condition. Nevertheless, the level of Ochratoxin A in coffee stored both in SGB and PPB under

Simulated and Ambient room conditions generally remained at <0.3 µg/kg up to the end of the 9-month storage period. Only two samples registered increases but the levels were far below the safe level of 5µg/kg in roasted coffee beans and ground roasted coffee set by the Commission Regulation (EC) No 123/2005 as cited in Food and Agriculture Organization of the United Nations (2012).

The effectiveness of hermetic storage using SGB in reducing fluctuations in moisture content and weight loss of coffee was evident. This shows SGB may be used by Arabica coffee farmers, as well as coffee traders and processors in preserving the quality and reducing potential monetary losses too during storage of Arabica coffee, particularly when extended storage becomes inevitable. Present findings likewise provide impetus in pursuing studies that would improve the use of organic or pesticide free hermetic storage not only for preserving the qualities and reducing losses of Arabica coffee but for the other commercially important coffee varieties as well. The inclusion of consumer's preference test in the sensory evaluation of hermetically stored coffee would also be worthwhile.

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EVALUATION OF SILO BAGS FOR TEMPORARY STORAGE OF WHEAT

Bhadriraju Subramanyam[1],*, Lakshmikantha H. Channaiah[1], Carlos Campabadal[1], John Lawrence[1], Leandro Cardoso[2], and Dirk E. Maier[1]

[1]Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA
[2]National Institute of Agricultural Technology (Instituto Nacional de Tecnología Agropecuaria), Rute 226, Km 73.5, 7620, Balcarce, Argentina
*Corresponding author's e-mail: *sbhadrir@k-state.edu*

ABSTRACT

Trials were conducted to evaluate the effectiveness of silo bags for temporary storage of hard red winter wheat under field conditions in Kansas, USA. Newly-harvested wheat with moisture content of $11.6 \pm 0.2\%$ was stored in four silo bags, each of 50 metric tonne capacity for a period of four months starting August 24 to December 14, 2010. The dimensions of the silo bags were approximately 20.0 m long and 3.0 m in diameter. The average temperature of the top and bottom layers of grain in bags was 32.3 and 26.7°C, respectively, in August. In December, average temperature of the top layer was -3.1°C and the temperature of the bottom layer was 13.0°C. During the study period, relative humidity of the top and bottom layers of grain fluctuated between 46.7 to 55.9%. The carbon dioxide concentration ranged from $0.53 \pm 0.05\%$ to $1.45 \pm 0.25\%$ during the study period. The analysis of wheat quality at the beginning and at end of the storage period clearly indicated that the parameters such as moisture content, test weight, falling number, and kernel weight were unaffected during storage in silo bags. The fungal concentration and mycotoxin levels did not change significantly during storage. The 30 adults of lesser grain borer, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae), confined in PVC cylinders and placed in silo bags in August increased by approximately 10 fold at the end of the storage period. The population growth of *R. dominica* in PVC cylinders in silo bags was 14 to 19 times lower when compared with growth in PVC cylinders placed in a laboratory growth chamber at 28°C and 65% r.h. Despite low carbon dioxide levels, silo bags can be used for temporary wheat storage when there is limited storage space during years of over production.

Keywords: Silo bags, modified atmospheres, wheat, fungi, mycotoxins, wheat quality, *Rhyzopertha dominica*

INTRODUCTION

Wheat in the United States is typically stored for six to nine months on farms (Martin et al., 1977). In years of bumper harvest, wheat storage on farm and at grain elevators is a huge challenge and this has forced farmers to sell their crops at a lower price which usually prevails

at the harvest time. Under such situations the low wheat prices have caused an increased demand for more storage capacity. Producers and elevator managers will overfill their bins/silos and are forced to set up temporary storage structures. Overfilling bins/silos makes grain quality management difficult. There is an urgent need to conduct research on alternative grain storage systems and evaluate their effectiveness in maintaining stored-grain quality, and their efficacy in minimizing fungal and stored-product insect problems. The use of silo bags for storing grain has been adopted successfully in recent years in Latin American countries, especially Argentina (Abalone et al., 2011a). In these relatively airtight silo bags, the elevated carbon dioxide (CO_2) and decreased oxygen (O_2) may create modified atmospheres detrimental to insects and fungi (Fleurat-Lessard, 1990; Adler et al., 2000; Navarro, 2006). More recently, silo bags are being used in the USA and Canada, primarily for temporary storage of grain. In 2010 we cooperated with a Kansas elevator operator using silo bags for storage of wheat. Our objectives were to determine environmental conditions inside silo bags holding wheat, and to evaluate impact of short-term storage of wheat (4 months) on wheat quality parameters, fungal infection, and effects on the lesser grain borer, *Rhyzopertha dominica* (F.) (Bostrichidae: Coleoptera) populations.

MATERIALS AND METHODS

Hard red winter wheat (11.6 ± 0.2% moisture content) harvested in the 2010 crop year was stored in four silo bags, each of 50 tonne capacity, for a period of four months starting August 24 to December 14, 2010 in Manhattan, Kansas. The dimensions of the silo bags were 20 m long and 3 m in diameter. Grain temperature and relative humidity inside the silo bags were recorded using HOBO® data logging units (Onset Computer Corp., Bourne, Massachusetts, USA). Two data loggers were used at each of three sampling points within each bag. At each point a logger was placed at the top (dorsal) side of the bag and at the bottom (1 to 1.5 m depth) of the silo bag. Levels of CO_2 in each bag were monitored monthly using a CO_2 detection device (SilCheck Company, Buenos Aires, Argentina) at each of the three sampling points.

Grain samples were collected at the beginning of storage (August 24), on October 23 and December 14, 2010. Grain samples were collected at each of three sample points using a 1.52 m long grain probe at the top (0 to 0.5 m), middle (0.5 to 1.0 m) and bottom (1.0 to 1.5 m) grain layers. At the top, middle, or bottom locations, the probe sample collected 160 g. In each bag, all top samples were pooled, as were middle and bottom samples. Wheat quality parameters measured included test weight, protein content, falling number, kernel weight, kernel diameter, and kernel moisture. Kernel weight, diameter, and moisture from 300 kernels were determined using Perten's single kernel characterization system, SKCS 4100 (Perten Instruments, Hägersten, Sweden). All wheat quality evaluations (except mycotoxins) were performed at the Wheat Quality Laboratory in the Department of Grain Science and Industry, Kansas State University, using official approved methods.

Samples were also analyzed for total fungal counts (cfu/g) using Dichloran-Glycerol (DG 18) agar media (Oxoid Limited, Hampshire, United Kingdom). The levels of aflatoxin, fumonisin, and vomitoxin in wheat samples were quantified using the AOAC (Association of Analytical Chemists, Gaithersburg, Maryland, USA) approved method based on an Enzyme Linked Immmunosorbent Assay (ELISA) kit (AgraQuant® mycotoxin ELISA test kits, Romer Labs Inc., Union, Missouri, USA). In order to assess how insect populations would develop inside silo bags, PVC cylinders of 0.3 m long and 9 cm in diameter were used in only two out of the four bags. One end of the cylinder was glued with a 200 μm heavy duty mesh. We used

R. dominica as the test species as it is an economically important pest insect associated with wheat in Kansas. Organic hard red winter wheat (Heartland Mills, Marienthal, Kansas, USA) was used to fill 80% of each cylinder (890 g), and 30 unsexed adults of mixed ages, reared at 28°C and 65% r.h. on the same wheat, were added to each cylinder. After adult introduction, the open end of the cylinder was glued with the mesh. Into each silo bag at each of three sampling points two cylinders with insects (30 adults) were inserted by making an entry point in the bag, which was later sealed with duct tape. There were a total of six cylinders per silo bag. One cylinder was at the top (0 to 0.5 m depth) and the other at the bottom (1.0 to 1.5 m depth) at each of the three sampling points per bag. Three cylinders from each of the two bags were sampled on October 23, 2010, and the remaining three on December 14, 2010. Control treatments included nine cylinders with wheat and insects placed in a growth chamber at 28°C and 65% r.h., and sampled in August, October, and December. Wheat in cylinders was sifted and the number of adult progeny produced enumerated (live and dead for October and December samples).

The percent CO_2 data without transformation (because data were normal) by sampling location on bag and month were subjected to two-way analysis of variance (ANOVA) to determine significant differences ($P = 0.05$). Based on this analysis, data were pooled by sampling point and subjected to one-way ANOVA and means among months were separated by Bonferroni *t*-tests (SAS Institute 2005). The wheat quality parameters data (untransformed) were subjected to two-way ANOVA to determine significant differences by month and sampling depth. If wheat quality parameters were not influenced by sampling depth, data were pooled and differences among months were determined by one-way ANOVA and Bonferroni *t*-tests. Individual fungal counts and mycotoxin data were analyzed using two-way ANOVA to determine differences among months and sampling depth. Additionally at each month, differences among depths for fungal counts and each mycotoxin were determined by one-way ANOVA and Bonferroni *t*-tests. The insect data by month from each of the two bags and the control treatment were analyzed as one-way ANOVA followed by Ryan-Einot-Gabriel-Welsch (REGWQ) test for mean separation after transforming insect count data to logarithmic scale.

RESULTS AND DISCUSSION

The temperature of the top and bottom layers of grain in the bags averaged 32.3°C and 26.7°C, respectively, in August; in December it was -3.1 and 13.0°C, respectively. The average relative humidity of the top and bottom layers was 52.7 and 55.9%, respectively in August. In December the corresponding values were 46.6 and 51.7%. The CO_2 levels were significantly different among storage months ($F = 5.25$; df = 4, 45; $P = 0.0015$), but not among sampling points in the bags ($F = 1.03$; df = 2, 45; $P = 0.3670$). The interaction of months and sampling points was also not significant ($F = 0.72$; df = 8, 45; $P = 0.6744$), indicating that the differences in CO_2 levels among the three sampling points remained consistent across the storage months. Therefore, the sampling location data were pooled and the one-way ANOVA showed significant differences among months ($F = 4.28$; df = 4, 15; $P = 0.0166$). The CO_2 levels were similar between August and October, and dropped significantly in December (Table 1), perhaps due to the onset of cold weather. However, the levels observed (0.53 to 1.45%) were too low to exert any adverse effects on biological organisms. Newton (1991, 1993) reported that levels of CO_2 should be about 60% to effectively control stored product insects and mites. Levels of 10 to 30% of CO_2 can be toxic to insects provided the level of O_2 is 0.5 to 2.6% (Krishnamurthy et al., 1986).

Table 1. Carbon dioxide (CO_2) levels in silo bags

Month	Mean ± SE CO_2 level (%)[a]
Aug	0.98 ± 0.23ab
Sep	1.45 ± 0.25a
Oct	1.38 ± 0.11a
Nov	1.10 ± 0.17ab
Dec	0.53 ± 0.05b

[a] Means (n=4) followed by different letters are significantly different ($P < 0.05$).

The test weight of the wheat was not affected by storage time (Type III SS (1 missing value) $F = 1.66$; df = 2, 80; $P = 0.1957$) and sampling depth ($F = 0.03$; df = 8, 80; $P = 1.000$). The storage time and sampling depth interaction also was not significant ($F = 0.03$; df = 16, 80; $P = 1.000$). The protein content, falling number, kernel weight, kernel diameter, and kernel moisture were significantly different among storage times or months (F range = 4.45 to 285.04; df = 2, 81; $P \leq 0.0131$) but not at the sampling depths (F range = 0.14 to 0.81; df = 8, 80; $P \geq 0.5943$). The storage time (df = 8, 81) and sampling depth interaction (df = 16, 81) was also not significant (F range = 0.19 to 0.1.52; $P \geq 0.1635$). Since quality parameters were similar among sampling depths, data were pooled by depth, and differences among storage times for each of quality parameters (except for test weight) were analyzed using one-way ANOVA. However, one-way analysis showed only protein and moisture to be different among August, October, and December (Table 2). The lack of differences among storage times for falling number, kernel weight, and dry matter was perhaps a result of pooling data across sampling depths, which eliminated any variance due to sampling depths.

Table 2. Mean ± SE wheat quality parameters by storage month

Month	Test weight (kg/hl)[a]	Protein (%)[a]	Falling number (sec)[b]	Kernel weight (mg)[a]	Kernel diameter (mm)[a]	Kernel moisture (%)[b]
Aug	79.0 ± 1.8	12.6 ± 0.2	411.1 ± 8.5c	27.8 ± 0.4	2.6 ± 0.01	11.6 ± 0.2a
Oct	79.2 ± 0.3	13.1 ± 0.2	553.1 ± 10.3a	28.2 ± 0.3	2.6 ± 0.02	10.8 ± 0.4b
Dec	77.8 ± 2.1	12.9 ± 0.1	490.0 ± 5.3b	27.7 ± 0.2	2.6 ± 0.02	11.3 ± 0.1a

[a] Means (n=4) among months for each of the variables was not significant (F range = 0.2 to 1.96; df = 2, 9; $P > 0.1966$).
[b] Means (n=4) within months followed by different letters are significantly different ($P < 0.05$).

Fungal counts varied by storage time ($F = 13.76$; df = 2, 27; $P < 0.0001$) and by sampling depth within bags ($F = 119.10$; df = 2, 77; $P < 0.001$), but the interaction of sampling time and depth was not significant ($F = 0.19$; df = 4, 27; $P = 0.9409$). Therefore, differences among sampling depths and each month were determined. Results showed significant differences among the three depths during August, October, and December (F range = 33.0 to 55.5; df = 2, 9; $P < 0.0001$) (Table 3). In general, the fungal counts at 1.0 to 1.5 m depth were significantly higher than those at 0 to 0.5 m and 0.5 to 1.0 m depths.

Table 3. Mold propagules (cfu/g) by storage month and sampling depth within silo bags

Month	Sampling depth (m)	Mean ± SE cfu/g[a,b]
Aug	0-0.5	127.5 ± 4.3b
	0.5-1.0	145.0 ± 8.9b
	1.0-1.5	218.8 ± 10.9a
Oct	0-0.5	138.8 ± 4.3b
	0.5-1.0	162.5 ± 7.5b
	1.0-1.5	242.5 ± 13.8a
Dec	0-0.5	157.5 ± 2.5b
	0.5-1.0	173.8 ± 5.5b
	1.0-1.5	257.5 ± 12.5a

[a]cfu/g, colony forming units per gram.
[b]Means ($n = 4$) within months followed by different letters are significantly different ($P < 0.05$).

The aflatoxin level did not vary by storage time ($F = 1.26$; df = 2, 27; $P = 0.2989$) but varied by sampling depth ($F = 11.23$; df = 2, 27; $P = 0.0003$). The sampling time and sampling depth interaction was not significant ($F = 0.47$; df = 4, 27; $P = 0.7590$). In the case of fumonisins and vomitoxin, both sampling time and sampling depth were significant (F range = 3.74 to 46.48; df for both variables = 2, 27; $P \leq 0.0369$), but the interaction of sampling time and depth was not significant ($F > 0.36$; df = 4, 27; $P > 0.6952$). One-way ANOVA by storage month showed both the fumonisins and vomitoxin levels to be greater in December compared with August or October (Table 4).

Table 4. Mycotoxin (mean ± SE) levels by storage month and sampling depth within silo bags

Month	Sampling depth (m)	Total aflatoxins (ppb)	Fumonisins (ppm)[b,c]	Vomitoxin/DON (ppm)[b,c]
Aug[a]	0-0.5	0.50 ± 0.05	0.07 ± 0.02b	0.02 ± 0.02b
	0.5-1.0	0.20 ± 0.16	0.28 ± 0.06b	0.11 ± 0.05b
	1.0-1.5	0.56 ± 0.27	0.51 ± 0.11a	0.41 ± 0.02a
Oct[a]	0-0.5	0.10 ± 0.10	0.11 ± 0.51b	0.11 ± 0.05b
	0.5-1.0	0.25 ± 0.18	0.40 ± 0.07a	0.25 ± 0.07b
	1.0-1.5	0.73 ± 0.23	0.55 ± 0.09a	0.57 ± 0.06a
Dec	0-0.5	0.12 ± 0.19b	0.16 ± 0.05c	0.17 ± 0.07b
	0.5-1.0	0.30 ± 0.19b	0.47 ± 0.08b	0.31 ± 0.08b
	1.0-1.5	1.10 ± 0.30a	0.73 ± 0.08a	0.73 ± 0.07a

[a]Mean ($n = 4$) aflatoxin levels within months were not significant ($F > 1.99$; df = 2, 9; $P > 0.0835$; one-way ANOVA).
[b]Fumonisin and vomitoxin levels within months were significant (F range = 8.35 to 32.15; df = 2, 9; $P < 0.0089$; one-way ANOVA).
[c]For each month, means followed by different letters are significantly different ($P < 0.05$).

This trend was observed with afltaoxin only in December samples; samples from August and October had similar aflatoxin level, irrespective of the sampling depth. The increased level of fungal and mycotoxin contamination is difficult to explain because the

moisture of samples taken at all depths was essentially similar as determined in the laboratory from samples collected in the field (see Table 2). Wheat samples from the bottom contained slightly more broken kernels, dust, and infected kernels (visual observation) compared to top samples. Damaged and broken kernels are more likely to be contaminated by fungi and mycotoxins (Shotwell et al., 1985). Further, the samples at the bottom were close to the ground and understanding changes in ground temperature and humidity as they relate to temperature differences and moisture migration patterns within the silo bag may shed light on why the bottom samples had higher fungal counts and mycotoxin levels. During the storage period the silo bags were damaged by pests such as rodents. Additionally, the silo bags were extensively damaged by the cadelle beetle, *Tenebroides mauritanicus* L. Damage by these pests at the ground and silo bag interface may have resulted in moisture seeping into the bottom of the bag resulting in increased fungal and mycotoxin levels

The number of *R. dominica* adults within PVC cylinders showed a significant increase during October and December with each of the two bags and in the control treatment (F = 2006.20; df = 8, 18; $P < 0.001$) (Table 5). The 30 introduced adults in the control treatment at 28°C and 65% r.h. increased to 102 and 140 fold by October and December, respectively. However, PVC cylinders with insects inside the two silo bags showed only a 7 to 10 fold increase in *R. dominica* numbers in October and December. Therefore, *R. dominica* adults in PVC cylinder in silo bags were 14 to 19 times lower than those observed in cylinders placed at 28°C and 65% r.h. The reduced numbers found in silo bags was not due to the CO_2 levels observed but due to the onset of cooler weather during September, October, and November which may have prolonged the insect's development time.

Table 5. Adults of *R. dominica* in PVC cylinders in silo bags and in a growth chamber

Treatment	Month	Mean ± SE[a] numbers of adults/cylinder
Bag C	Aug	30.0 ± 0.0f
	Oct	198.0 ± 7.2e
	Dec	276.0 ± 25.2cd
Bag D	Aug	30.0 ± 0.0f
	Oct	226.7 ± 3.4de
	Dec	308.2 ± 13.6c
Chamber (28°C/65% r.h.)	Aug	30.0 ± 0.0f
	Oct	3066.0 ± 103.2b
	Dec	4206.7 ± 173.0a

[a]Means (n=3) followed by different letters are significantly different ($P < 0.05$).

Levels of CO_2 in interstitial spaces of wheat stored in silo bags are influenced by changes in grain temperature, moisture, and storage time (Abalone et al., 2011a). For wheat at 12 to 13% moisture, the level of interstitial O_2 was more than 12% and that of CO_2 was less than 7% after six months of storage (Abalone et al., 2011b). Our four month study showed that silo bags can be used to store hard red winter wheat without appreciable loss of wheat quality, but the CO_2 levels observed were not high enough to arrest insect and fungal development and prevent mycotoxin contamination. The low CO_2 concentrations observed may be due to gaps in sealing at both ends of the bags or loss of CO_2 due to damage by rodents and the cadelle beetle larvae. Wheat in silo bags should be stored on leveled ground with good drainage and on ground free of stones and gravel. Protecting against rodent damage

for bags placed outdoors on farms or at elevators will be difficult, but protection against insect attack, especially by species capable of penetrating the silo bags, can be prevented by spraying an approved insecticide on the outside of bags.

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NON-CHEMICAL TREATMENTS FOR POTENTIAL DISINFESTATION OF TWO MAJOR ARTHROPOD PESTS OF SOUTHERN DRY-CURED HAMS

Thomas W. Phillips[1], Md. Mahbub Hasan[1,2], Michael J. Aikins[1], Salehe Abbar[1] and Barbara Amoah[1]

[1]Dept. of Entomology, 123 Waters Hall, Kansas State University, Manhattan, KS 66506 USA
[2] Dept. of Zoology, Rajshahi University, Rajshahi, Bangladesh
*Corresponding author's e-mail: *twp1@ksu.edu*

ABSTRACT

We explored the feasibility of controlled atmospheres (CA) for controlling arthropod pests infesting dried-cured ham facilities in the USA using low oxygen (O_2), high carbon dioxide (CO_2) and Ozone (O_3). Studies were also conducted on Sessional treatment of food-grade materials as deterrent to ham mite infestation. Results showed that both low oxygen and higher CO_2 levels required longer exposure (144h) to kill 100% of all stages of red legged ham beetle, *Necrobia rufipes* DeGeer (Coleoptera: Cleridae) and ham mite *Tyrophagus putrescentiae* Schrank (Astigmata: Acaridae) at 23°C. In addition, both these trials had no any significant mortality effect against the ham beetle and ham mites especially at short exposures ranging from 12 to 48 h. Ham beetles showed more tolerant to higher CO_2 (75.08%) and low pressure (25mmHg) than ham mites. Our CA trials also showed that the egg stages of both species were usually found to be more tolerant than other stages tested. The ozone trials were shown more promising among the all CA executed in controlling both the insect pests. The results suggest that O_3 has potential in controlling ham beetle and ham mites particularly with higher concentration (≈155 ppm) at 24h exposure. Food-grade coatings showed such as certain oils and glycols showed great promise in both preventing disinfestation of ham and in repelling mites from treated hams.

SESSION 5

POSTERS

CONTROL OF BROWN-LEGGED MITE ADULTS USING NITROGEN ATMOSPHERE

Radek Aulicky*, Zuzana Kucerova, Vaclav Stejskal

Crop Research Institute, Drnovská 507, 161 06 Prague 6, Czech Republic
*Corresponding author's e-mail: *aulicky@vurv.cz*

ABSTRACT

Aleuroglyphus ovatus has a worldwide distribution and is a common pest of various stored products. Modified atmosphere may be nont-toxic option for control of the mites in value added products; such as seeds, spices and dry/dried fruits. However, there is few published information on the efficacy of modified atmosphere on mites and there is no information on *Aleuroglyphus sp.* Therefore this study brings the first report on efficacy of modified atmospheres (N_2) on Brown-legged mite (*Aleuroglyphus ovatus*). We tested the efficacy of modified 100% gaseous nitrogen atmospheres on mite adults' survival. We found that 100% nitrogen caused 100% imago mortality after 33 hours; LT_{50} =8.88 (8.18-9.53) and LT_{90}= 21.62 (20.04-23.61). The results were obtained due to support of research grant (QI101B088) provided by Ministry of the Czech Republic.

Key words: nitrogen, N_2, atmosphere, stores, mites, *Aleuroglyphus ovatus*

INTRODUCTION

Brown-legged mite, *Aleuroglyphus ovatus* (Troupeau, 1878) is a small acaroid mite (Hughes, 1976; Kucerova and Stejskal, 2009). *A. ovatus* has a worldwide distribution and is a common pest of various stored products such as grain products, seeds, stored wheat and barley (Athanassiou et al., 2005; Stejskal and Hubert, 2008; Hubert et al., 2009). When optimal temperatures are reached (Xin and Shen, 1964; Yan et al., 1992), *A. ovatus* has capacity for rapid reproduction and population increase (Aspaly et al., 2007; Xia et al., 2009). Elevated populations of storage mites negatively influence the quality of stored commodities by disseminating moulds (Hubert et al., 2004), causing allergenic reactions (Fernandez-Caldas et al., 2000; Stejskal and Hubert, 2008) or leads to pulmonary and urinary acariasis (Xia et al., 2009). Effective control of storage mites is not easy. Although structure of *A. ovatus* pheromone is identified (Shibata, et al., 1998), no monitoring product is commercially available. Mite populations are generally difficult to suppress by residual pesticides (Collins, 2006; Nayak, 2006; Hubert et al., 2007) or fumigants such as methyl bromide or phosphine (Bowley and Bell, 1981; Şen et al., 2009). In addition, usage of toxic insecticides is associated with medical risks, occurrence of pesticide residues in food or with interaction with construction materials in the treated building. The non-chemical alternatives to pesticides are modified atmospheres for control of pests (Emekci et al., 2004; Navarro, 2006). Because of the limited information on efficacy of modified atmospheres on mites

(Navarro et al., 1985; LungShu et al, 1998; Navarro, 2006) we have explored the laboratory efficacy of N_2 atmosphere on adults of *A. ovatus*.

MATERIALS AND METHODS

Mites

The specimens of Brown-legged mite, *Aleuroglyphus ovatus* (Troupeau, 1878) were taken from the cultures kept at the Crop Research Institute, Prague; the culture originated from the Central Science Laboratory Sand Hutton (York, UK). The mites were mass-reared in frit-chambers plugged with rubber. The rubber was pierced with a steal tube (5 mm diameter). Both ends of the glass tube were covered with muslin. The rearing diet (100 g) consisted of 44 g oat flakes, 44 g wheat germ, 12 g yeast. The frits were placed into Secador desiccators (P-Lab, Praha, Czech R.) and kept at 85% RH and 25°C in darkness. Before the experiment, adults were collected with a fine brush from the surface of the rubber or the sides of the frit and counted under a Stemi 2000-C dissection-microscope.

Testing apparatus and experimental procedure

We tested *A. ovatus* in a N_2 apparatus that was composed from 10 small plastic boxes (Lock&Lock HPL834, 3900ml) serially connected by plastic hoses (PVC, transparent, diameter 6/9mm, Deutsch & Neumann GmbH). Nitrogen atmosphere (100 % N_2 –5.0, purity (% obj.): ≥ 99,999) was delivered from the pressured metal cylinder (Linde Gas a.s.) using outlet valve C200/2B-3SS (Linde Gas a.s.). We checked oxygen content using oxygen sensor GMH 3691 (Greisinger electronic GmbH). Each plastic box (in 10 repetitions) contained 50 specimens of experimental mites in vials. We maintained 75% r.h. of air by saturated solution of NaCl in each plastic box. We measured the temperature and humidity by data loggers (TinyTag Ultra 2, Gemini Data Loggers Ltd., UK). Nitrogen exposure times were: 1 - 36 h. After exposure we placed the treated mites in chambers maintained at 75% r.h. and 27°C. Mortality check was executed 24 h after MA-exposure termination. Results were analyzed by logistic regression mortality model ($\chi2$ – test) for LT_{50} and LT_{90} using statistic program XLSTAT.

RESULTS AND DISCUSSION

Fig. 1 shows the obtained statistical model describing dependence of *A. ovatus* mortality on exposure time by 100% nitrogen atmosphere (75% r.h. and 27°C). We estimated lethal time parameters as follows: LT_{50} =8.88 (8.18-9.53) and LT_{90}= 21.62 (20.04-23.6). We found that 100% nitrogen atmosphere caused 100% imago mortality after 33 hours. Since our study is the first report showing an acaricidal effect of N_2 on *A. ovatus*, we cannot directly compare our results with data obtained by other MA- scientists. On the basis of the results presented here, the tested nitrogen atmosphere could be potential candidates in the control of the *A. ovatus* in food and feed commodities.
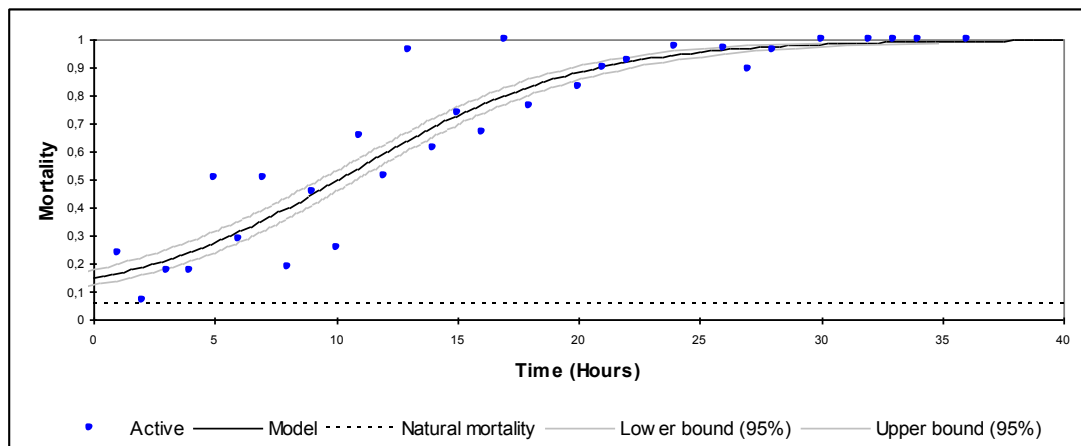
Fig. 1- Mortality of *Aleuroglyphus ovatus* (model parameters, n=10, Intercept±SE-3.33±0.23; Slope±SE 3.43±0.2, Lethal time (h), LT_{50} (95% CL) 8.88 (8.18-9.53); LT_{90} (95% CL) 20.04 (20.04-23.61); $\chi2 = 619.1$, df 1; P <0.0001)

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STORAGE OF MALTING BARLEY WITH DIFFERENT MOISTURE CONTENTS IN HERMETIC SILO-BAGS

Ricardo Bartosik*, Dario Ochandio, Leandro Cardoso, Diego de la Torre

National Institute of Agricultural Technologies, Rute 226 km 73.5, Balcarce, (7620) Argentina
*Corresponding author's e-mail: *rbartosik@balcarce.inta.gov.ar*

ABSTRACT

The storage of dry malting barley (12%) in silo-bags is a well adopted practice in Argentina, with no deleterious effects on the malting process. However, sometimes farmers have to store barley at moisture contents higher than 12%, implying a higher risk for the malting quality and, eventually, a monetary loss for farmers (barley has to be traded as feed). In that sense, it would be convenient to have a monitoring tool that would allow for testing the storage condition of the barley in the silo-bag and making a quick risk assessment. The objective of this study was to quantify the effect of storage moisture content on the germination of barley and to determine its correlation with the CO_2 concentration, as an indicator of storage risk. The tests were carried out in two silo-bags filled with barley at moisture content ranging between 13 and 18%. Grain samples were collected, at the beginning of storage and every 15 days during five months of storage and submitted to the lab for performing the germination test. Carbon dioxide and grain temperature were measured in the silo-bag with the same frequency. For the temperature values registered in summer time in Argentina, it is not safe to store malting barley with moisture over 14%. With moisture content lower than 14%, barley can be stored in silo-bags for 5 months without affecting the germination. Carbon dioxide measurement is an effective indicator for detecting grain spoilage risk caused by excessive moisture content or for detecting potential problems derived from water infiltration in the bag during storage. However, the evolution of CO_2 should be used as a spoilage indicator during storage rather than a single reading at a particular moment.

Key words: grain storage, carbon dioxide, biological activity, germination test, grain temperature.

INTRODUCTION

Argentina is the largest producer of barley in South America, with an estimated production of 4 million tonnes in 2011/2012 (Agrositio, 2012). The main destination of barley is the malting industry. Quality requirements of barley include a germination test (GT) of 98.5% with a tolerance to 95%, grain size, protein content and a low percentage of shelled and broken kernels (Savio and Cattaneo, 2008).

Barley is stored either in permanent storage structures (bins or flat storage) or in silo-bags. The silo-bag is a hermetic storage system with a self-modified atmosphere. The silo-bag plastic cover is made with a three-layer polyethylene of 235 µm thickness which prevents the free exchange of gases between the ambient atmosphere and the interstitial space. Thus, the

bulk biotic respiration (grains, fungi and insects) produces the increase of CO_2 concentration and the reduction of O_2 concentration (Bartosik et al., 2008a).

Some studies on dry barley storage (12% moisture content (m.c.) or less) in silo-bags have been reported (Ochandio et al., 2008; Massigoge et al., 2010). The results showed that commercial quality was not affected after 12 months of storage. On the contrary, storage of barley with higher m.c. negatively affects the malting process due to a drop in germination (Cardoso et al., 2010). In this sense, Darby and Caddick (2007) mentioned that the safe storage time for barley stored in silo-bags at 14% m.c. and 35°C would be reduced to only 1 month.

During rainy years it is common to harvest barley with a m.c. higher than 12.5% (trading tolerance), that must be stored wet in silo-bags for a few months before it is conditioned to the safe storage m.c. This represents a risk of quality loss if the grain remains stored in the silo-bag for a long period of time. Bartosik et al. (2008a) showed that for wheat stored in silo-bag the CO_2 concentration increased with the grain m.c., and that the increase of biological activity in the wet grain can produce commercial quality loss. Massigoge et al. (2010) hypothesized that it would be possible to use the CO_2 concentration to estimate the risk of commercial quality loss for barley stored in silo-bags.

The objective of this study was to quantify the effect of grain m.c. on the germination of barley and to determine its correlation with the CO_2 concentration in the silo-bag as an indicator of spoilage risk.

MATERIALS AND METHODS

The tests were carried out in the district of Balcarce, Buenos Aires province, Argentina. Two silo-bags (A and B) with approximately 180 tonnes of malting barley with m.c. from 12.9 to 17.9% in each bag were selected. Three sampling sites were determined for each silo-bag based on grain m.c. data (high, low and intermediate) obtained from samples collected during the bagging operation (Table 1).

Table 1. Initial moisture content (m.c.) for 3 sampling sites for silo-bags A and B

	Initial m.c. (%)		
	Site 1	Site 2	Site 3
Silo-bag A	A1: 13.8	A2: 13.4	A3: 12.9
Silo-bag B	B1: 17.9	B2: 15.4	B3: 13

The study began on January 13 (15 days after bagging) and lasted about 135 days. Carbon dioxide concentration, grain temperature, m.c. and germination were determined. The sampling procedure consisted of measuring the CO_2 concentration with a portable gas analyzer (PBI Dan Sensor, CheckPoint, Denmark) and perforating the plastic cover with a needle. A wooden stick with three temperature sensors at different heights was inserted into the grain mass (diagonally from top to bottom and towards the center of the silo-bag) for measuring grain temperature at about 0.1, 0.7 and 1.4 m from the surface of the grain. Temperature values were obtained between mid-morning and noon. Later, at each sampling site, a grain sample was collected using a standard torpedo probe, separated in three different levels (0.10, 0.75 and 1.6 m depth, corresponding to the upper, middle, and lower layer, respectively, being the total height of the silo-bag of 1.7 m). After sampling the silo-bag, the openings were sealed with a special tape in order to restore the hermeticity. The described

sampling procedure was repeated approximately every two weeks during the storage period. The collected grain samples were placed in sealed plastic bags and submitted to the Grain Postharvest Laboratory of INTA (Balcarce Research Station), where the grain m.c. was measured with a moisture meter (GAC 2100, Dickey-John), and to the Seed Laboratory where GT was conducted as recommended by ISTA (2008), by pre-chilling the seeds for 48 h and then placing the seeds for germination during 7 days at 20°C under light conditions (four replicates of 50 seeds were considered).

RESULTS AND DISCUSSION

The grain temperature (excluding the peripheral grain layer in the bag) at the beginning of the study (mid-January) was 22°C for both silo-bags (Figures 1 and 2). The maximum temperature was registered at the end of January (24 and 26°C for silo-bags A and B, respectively), then the temperature constantly declined until mid-April (fall). In subsequent samplings it was observed that the temperature stabilized, ending with 16°C in both bags at May 31st. These ranges of temperature and their evolution are consistent with those reported by Ochandio et al. (2009) and Cardoso et al. (2010) for similar locations and storage period.
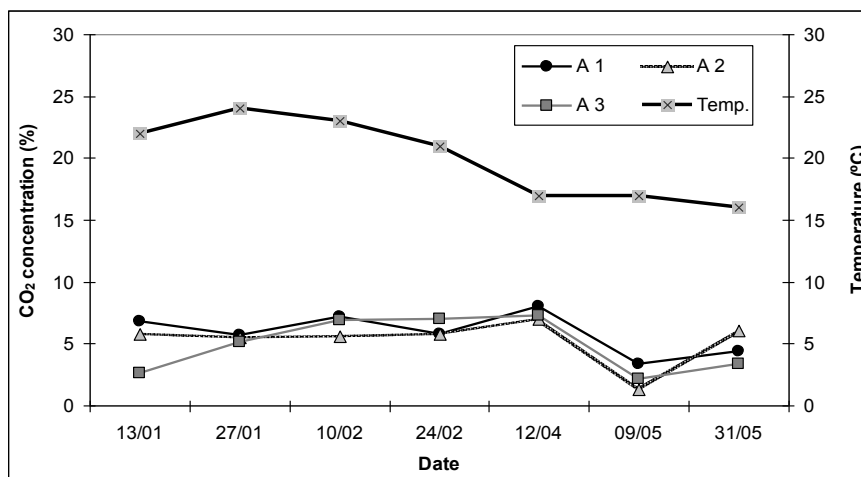
Fig. 1- CO_2 concentration (%) for the three sectors of the silo-bag A (A1, A2 and A3) and temperature (Temp., °C) of silo-bag A during the storage period.

Figure 3 shows that the germination in the three sectors of silo-bag A (A1, A2 and A3) and sector 3 of silo-bag B (B3) remained clearly above the commercialization tolerance (95%) during the study period.

For the range of grain temperature observed in this study the values of germination are consistent with the recommendations realized by Darby and Caddick (2007), who suggest a safe storage time of 9 and 6 months for barley grain stored with 13 and 14% m.c., respectively (at 25°C or less). These authors also mentioned that with temperature of 35°C and m.c. of 14%, deterioration of quality occurs very quickly.
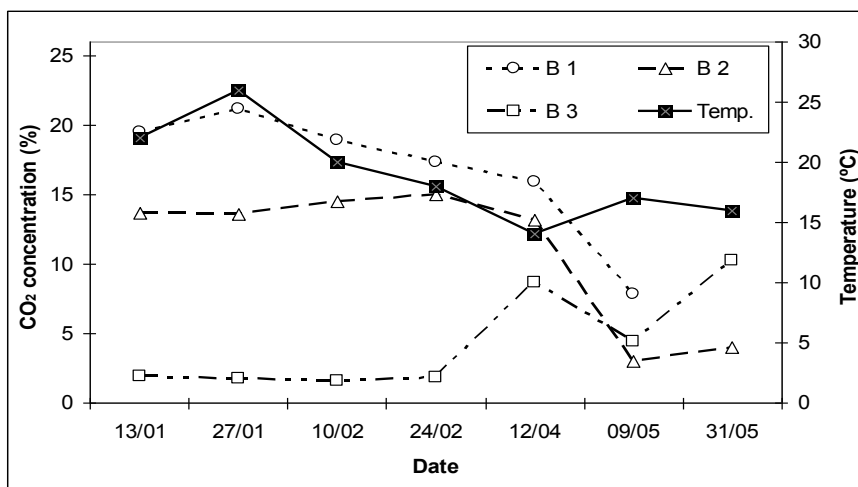
Fig. 2- CO$_2$ concentration (%) for the three sectors of the silo-bag B (B1, B2 and B3) and temperature (Temp., ºC) during the storage period.

In sectors 1 and 2 of the silo-bag B (B1 and B2), with m.c. of 17.9% and 15.4%, respectively, the values of germination after 15 days of storage was below 98% (Figure 3). During the first month of storage the germination in sector B2 continued the decreasing tendency, but with values still higher than 95%. After February 10th a rapid decrease of germination was observed, falling to 76.1% at end of test. In sector B1 (17.9% m.c.) the germination fell to 30% after the first month of storage. In late February, the germination was lower than 10% in this sector.
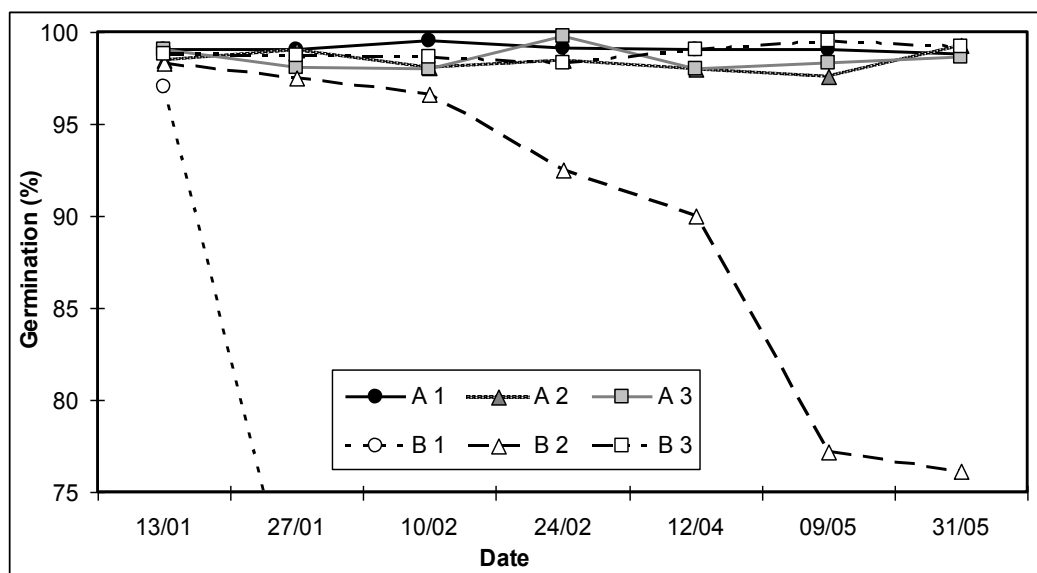
Fig. 3- Germination values for the three sectors of the silo-bag A (A1, A2 and A3) and the silo-bag B (B1, B2 and B3) during the storage period.

There was a positive correlation between CO_2 concentration and grain m.c. When the m.c. was under 13.8% (silo-bag A, and sector 3 of silo-bag B) the CO_2 concentration was about 3-8%. When the grain m.c. was higher, the CO_2 concentration of the silo-bag sector also was higher. For instance, in sector B1, with 17.9% m.c., the CO_2 concentration rose up to 20%, while in sector B2, with 15.4% m.c., the CO_2 was about 15% (Figures 1 and 2). This result is consistent with the results obtained by Crocce (2009) for silo-bags with wheat at different m.c. values.

The maximum CO_2 concentration for each sampling site is reached, in general, during the first month of bagging. In general, biological activity also responded to grain temperature. In the silo-bag, grain temperature is influenced by the ambient temperature throughout the storage season (Bartosik et al., 2008a). Barley is bagged in early summer, so the maximum grain temperature is reached during the first months of storage. During fall, the grain temperature decreased and hence the biological activity. The CO_2 concentration measured in the silo-bag is the result of a balance between the respiration rate (CO_2 production), and the CO_2 lost from the silo-bag by permeability of the system - through openings in the plastic cover or through the natural permeability of the plastic material to the gases. This explains the reduction in the measured CO_2 concentration in May, following a decrease in the grain temperature. The same relationship among grain temperature and CO_2 concentration was reported by Crocce (2009), who observed a decreasing tendency of biological activity during cold season for wheat stored in silo-bags. This tendency was stronger with grain at 13% m.c. or higher.

However, in sector 3 of silo-bag B (B3) there was an increase in the biological activity after February, contrary to the decreasing trend of the grain temperature (Figure 2). The CO_2 concentration increased from 2% to about 10%, implying that the biological activity did not correspond to the grain m.c. (13%). This increase in biological activity could be related to water entering to the system through perforations of the plastic bag, which caused spoilage problems in that sector of the grain mass. The increase of CO_2 in the autumn, due to spoiled grain in the bottom of silo-bag, was also reported by Massigoge et al. (2010) and Cardoso et al. (2010) for dry barley stored in silo-bags.

Bartosik et al. (2008b) suggested that CO_2 monitoring could be used as an early indicator of spoilage problems in silo-bags. In this study, a relationship between CO_2 concentration and grain quality could be established. In sectors B1 and B2 (m.c. of 17.9 and 15.4%, respectively) there was an immediate evidence of biological activity, which resulted with a substantial decrease in the germination. During the entire storage period the CO_2 concentration fluctuated between 20 to 13% in summer, and then decreased to 5 to 10% in fall. On the other hand, the sites in which m.c. was below 14% (A1-A3 and B3) and the CO_2 concentration was from 2 to 7% at the beginning of storage, the germination did not decrease. Furthermore, in the site B3 there was an increase the CO_2 concentration in May (Figure 2), but no negative effect in the germination was observed. It could be hypothesized that the increase in the biological activity could come from a localized spoiled area from water entering through some perforation in the bag, as it was explained before. This localized spoiled grain would generate enough biological activity to affect the CO_2 concentration of the sector, without affecting the germination of the barley. This would indicate that the evolution of the CO_2 concentration rather than isolated readings of CO_2 should be taken into account when CO_2 is used as a storage quality parameter. If only a single CO_2 reading taken in a specific moment is used to estimate storage risk, the storage risk could be over or underestimated.

These results indicate that, for the temperature values registered in summer time in Argentina, it is not safe to store malting barley with m.c. over 14%. With m.c. lower than 14%, barley can be stored in silo-bags for 5 months without affecting the germination.

Carbon dioxide measurement is an effective indicator for detecting grain spoilage risk caused by excesive m.c. or for detecting potential problems derived from water infiltration in the bag during storage. However, the evolution of CO_2 should be used as a spoilage indicator during storage rather than a single reading in particular moment.

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SESSION 6

Sealing techniques and MA / fumigation engineering

Chairpersons:
Ronald T. Noyes, USA
Ricardo Bartosik, Argentina
Aydın Suzu Tuncbilek, Turkey

INVESTIGATION OF THERMOSIPHON PIPES TO DISTRIBUTE PHOSPHINE GAS THROUGH GRAIN SILOS FROM A GROUND LEVEL INTRODUCTION POINT

Chris Newman[1, 3], James Newman[1, 2], Hui Cheng[1, 2], Yonglin Ren[1, 2, 3]

[1] Cooperative Research Centre for National Plant Biosecurity.
[2] Post-harvest Biosecurity Laboratory, School of Biological Sciences, Murdoch University, Western Australia.
[3] Department of Agriculture and Food, Western Australia.
*Corresponding author's e-mail: *chris.newman@agric.wa.gov.au*

ABSTRACT

A system of recirculating phosphine gas by a thermosiphon pipe and applying aluminium phosphide at ground level was tested on twelve 1472 m³ silos. The Ground Level Application System (GLAS) introduces aluminium phosphide (AlP) tablets or blankets into a reaction chamber duct at the base of a sealed silo which is connected by a thermosiphon pipe to the headspace of the silo. The experiment demonstrated the effect created by ambient conditions on movement of phosphine gas through the silos. Air in the pipe expanding under passive heat exchange from the sun becomes less dense and moves up the pipe, during the day. This air movement lifts the phosphine gas as it is released from the AlP in the reaction chamber, into the grain profile via the headspace. After sunset the air cooled and move down the pipe into the reaction chamber. Thermosiphons work due to differences in temperature between internal and external silo environments. In any 24 hr period there will be parity in temperatures at least twice when the air in the pipe does not move. During these periods, the released phosphine gas must diffuse from the reaction chamber directly upward into the grain bulk through perforated duct covers. This prevents potentially explosive concentrations developing in the reaction chamber. This system has implications for improved worker safety and reduced selection for phosphine resistance in the resident stored product insects.

Key words: Thermosiphon, grain storage, phosphine, aluminium phosphide, recirculation, sealed silo.

INTRODUCTION

Thermosiphoning is passive heat exchange which circulate liquid or air without a mechanical pump. In grain storage it consists of a pipe connected to the headspace and base openings of the structure. As air in the pipe expands under passive heat exchange from the sun, it becomes less dense and buoyant and moves the gas/air mixture upwards in the pipe drawing air into the pipe from ducts at the base of the silo. The process is continual provided there is a temperature differential between ambient and the stored commodity, which sets up a circulation of the internal grain void space air mixed with the gas introduced into the system.

Previous work on the use of thermosiphon pipes to enhance circulation of phosphine gas:

- Boland (1983)[1] gas distribution in a 22 metre high concrete cell
- South Australia Cooperative Bulk Handling (2000)[2] trial on a horizontal grain store
- Newman (2006)[3] trial in 75 t sealed silo circulating phosphine from a GLAS point
- Ball (2003 Personal communication) Thermosiphon distributed fumigation, 1200t silo, South Australia. (Data published in this paper)

The standard technique to fumigate grain silos is to load Aluminium phosphide (AlP) into the headspace where the phosphine gas evolves and is then conducted throughout the silo by diffusion and internal thermal convection air currents. Without a recirculation system it can take many days to reach threshold concentrations throughout large well sealed silos >200t.

In large grain silos, complete gas distribution can be achieved by powered recirculation but this is not commonly fitted in Australia when the silo is constructed on farms. Powered recirculation needs continuous energy for approximately four days for the fan to distribute the phosphine throughout the grain bulk as it is liberated from the AlP formulation.

Initial thermosiphon fumigation Balaklava, South Australia
An investigative thermosiphon trial on one 1200 t silo in 2008 was undertaken by Australian Fumigation, South Australia (Fig. 1). The existing 100mm PVC headspace pressure relief pipe fitted on the south side was utilised as the thermosiphon, bypassing the pressure relief valve at ground level and connecting it to the aeration intake seal plate with a 90mm id flexible tube. Blanket formulation AlP was loaded into the headspace of the silo at a rate of 1.4g/m³. Phosphine gas reached a concentration of 200 ppm at all 8 monitored points in the silo approximately 66 h after application of the AlP into the headspace and remained above this level for the 15 days of the monitored period. It was observed the headspace concentrations did not reach the peaks associated with a top loaded fumigation because the gas was conducted into the grain bulk by air currents caused by the thermosiphon pipe soon after liberation from the blankets. The results of this trial indicated a further investigation of the process.

MATERIALS AND METHOD

Two sites were selected to provide a range of climatic and commodity types for adequate testing of the thermosiphon system. Site 1, Arthur River in Western Australia has four 1200t aerated silos 'sealed in construction' arranged in a group. Site 2, Balaklava in South Australia has four 1200t 'retro sealed' aerated silos arranged on an east-west line. The GLAS tested in this project introduces AlP at the base of a sealed silo into a reaction chamber which is connected to a thermosiphon pipe attached to the headspace. The reaction chamber is also the aeration duct or plenum formed in the concrete base of the silo to enable effective distribution of air across the silo floor but is of sufficient volume to accommodate the blankets of AlP and allow a safe headspace in which the phosphine will generate. The plenum consists of two channels 0.6m×0.2m in profile and 10.3m in length providing a volume of 1.24 m³ each. The perforated steel plates that cover the channel and support the grain allow diffusion of the gas into the grain when air movement stops in the thermosiphon pipes. The two channels are cast in a 'V' formation so a fumigation door on each side of the transition section enables the blankets of AlP to be probed into the opposite channel (Fig. 2).
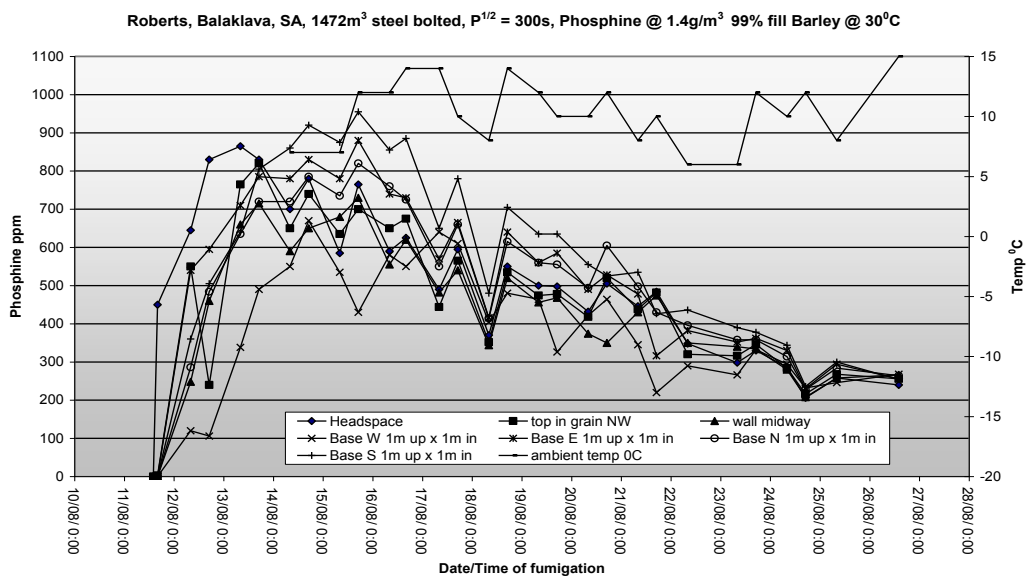
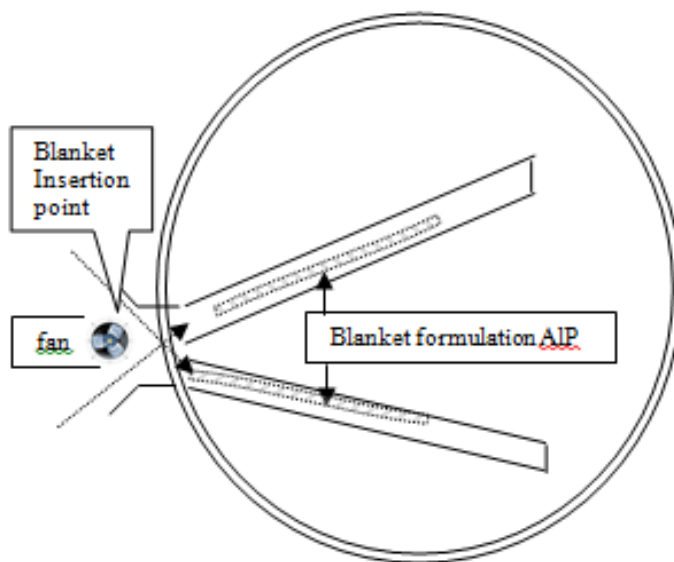Fig.1- Initial thermosiphon fumigation in 1200t bolted steel silo

Fig. 2- Plan of aeration ducts/phosphine reaction chambers in silo base.

Site 1 Arthur River, Western Australia

At the Arthur River site the silos are located in a group of four which causes strong shading during the day. To avoid structural changes to the silo wall, entry into the silos was limited to the aeration transition section with the consequent reduced effect on the amount of sun falling on the pipes of the two western silos. Two parallel thermosiphon pipes were installed as a rigid fixing (Fig. 3) on three of the silos, the fourth silo was left as constructed in order to conduct a standard top loaded fumigation as a control. The pipes complete with pressure relief valves were fitted at the SSW of Silo 1, ESE of Silo 2 and SSW of Silo 3. Ground level phosphine introduction points were created in the aeration transition duct by manufacturing a small door sealed with rubber strips and locked with bolts and wing nuts.

One black pipe and one white pipe of 100mm diameter were fitted to compare the relative efficiency of black versus white to move the air from the bottom to the top of the pipes under diurnal sun conditions. Black PVC pipes are not manufactured in Australia so the alternative is to paint a white pipe with the attendant problems of maintenance at height as the paint weathers. The aim of this installation was to discover if it was possible to use white pipes only to conduct the air.
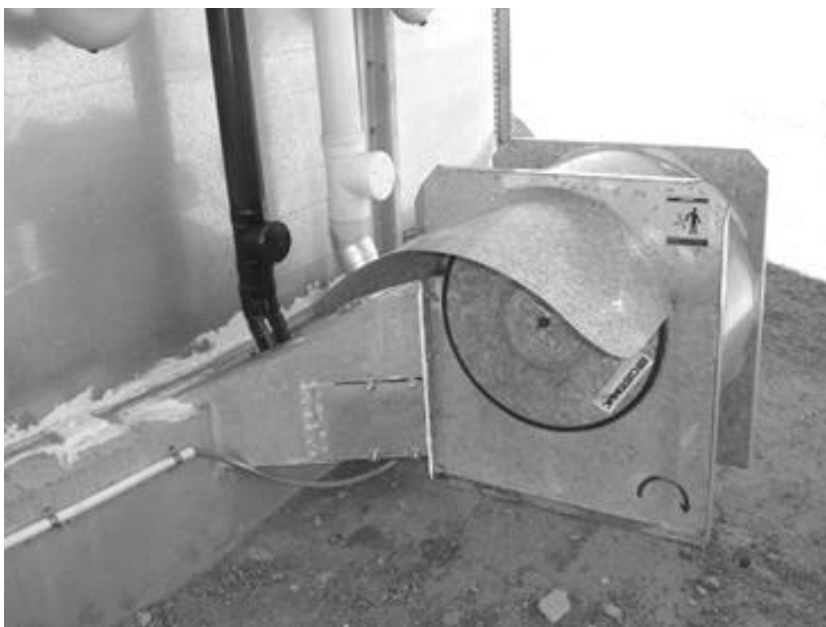
Fig. 3- Thermosiphon pipes fitted into aeration transition

Gas monitoring tubing of 1mm i.d. was attached to a steel cable with cable ties and installed centrally in the silo with an eye bolt through the roof near the peak and tensioned with a turnbuckle to the internal unloading auger motor housing. Monitoring points were located above the silo floor at 1, 4, 8, 12, 14 m and in the headspace. The six monitoring tubes were drawn through a PVC fitting in the neck of the top inspection hatch and conducted to ground level within a 25 mm i.d. UV stable PVC black irrigation pipe to provide long term protection from weather and parrots.

After one year of operation it was found that the drag on the lines by the outloading grain had stripped them from the cable. A new set of internal lines were assembled, covered

with heat shrink tubing and refitted which has proved successful in preventing further damage.

A monitoring tube was inserted into the left branch of the plenum and one tube into each of the thermosiphon pipes and stainless steel tubing of 1mm i.d. was inserted at four points around the base of the silos 0.5 m above silo floors and probed 0.3 m into the grain

Site 2 Balaklava South Australia

At the Balaklava site an additional 100mm black painted PVC pipe was added to the north side of each of the four 1200t silos to increase the period of sun exposure and air recirculation in the silo. The pipes enter near the peak of the roof and connect into the outloading auger inspection plate with flexible 90mm pipes to complete the air circuit across the silo. The transition section of the aeration system was modified to fit a sealable fumigation door on each side (Fig. 4) to provide access to the aeration plenums.

Prior to grain loading, monitoring points were installed on a central tethered cable. The monitoring line consisted of five lengths of 1mm id nylon tubing bundled with a 3mm stranded support cable encased in heat shrink tube. The tubing lines were spaced on the cable at 1 m, 3 m, 8 m and 10 m above floor height and in the headspace, and were passed through a PVC fitting in the apex of the silo and terminated outside the silo at ground level. The cable was attached by an eye bolt through the roof structure near the apex, then was tethered and tensioned to the gearbox of the outloading sweep auger at the base. When the outload sweep is used the operator releases the turnbuckle to avoid twisting the monitoring lines. Stainless steel tubing of 1mm i.d. was inserted at four points around the base of the silos one metre above silo floors and probed 0.3 m into the grain. Nylon tubing monitoring lines were inserted into each branch of the plenum.
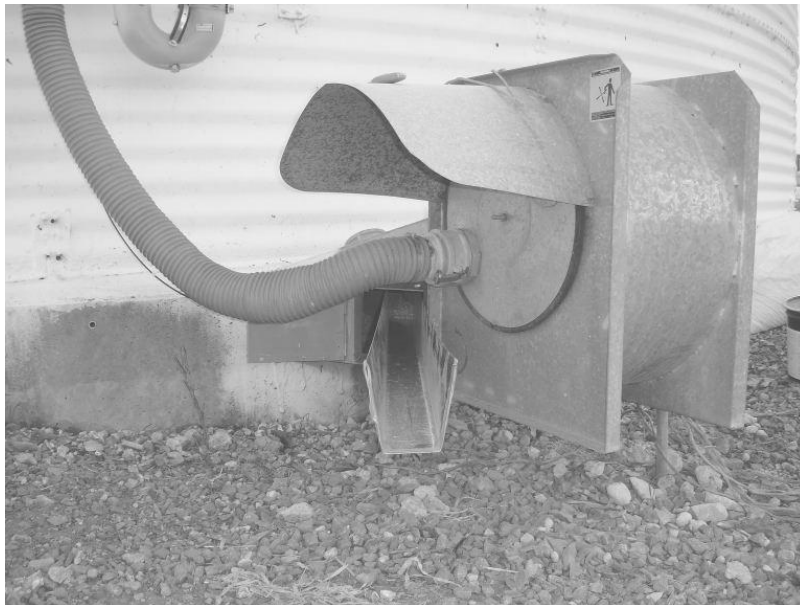
Fig. 4- South thermosiphon pipe enters via the aeration fan seal plate. Fumigant access door cut into aeration transition duct with probing channel in place.

RESULTS

Fumigations Autumn 2010, Site 1 Arthur River Western Australia
The fumigation conducted in May 2010 provided the opportunity to study ground level thermosiphon (GLAS) fumigation under cool conditions. The silo contained approximately 300 t of grain giving it a 75% headspace reducing monitoring to three points on the centre line at 8.1 m (headspace) 4 m and 1 m above floor level. A complication was that the black thermosiphon pipe had fractured at the wall to roof joint and had to be capped for a fumigation to proceed leaving one 100mm white PVC pipe to conduct the gas from the plenum.

Silo 3 Fumigation (1200t / 1472m³)
Two thousand grams of blanket formulation of AlP were probed into the plenum/phosphine reaction chamber and monitoring commenced 40 minutes later. This silo has the thermosiphon pipe located on the SSW side so in winter sun exposure is limited to approximately 2 hrs per day in cool conditions. There was limited movement of gas into the headspace due to the absence of a long period of sun on the thermosiphon pipe so it took 5 d for phosphine to reach 200 ppm at all monitored points in the silo. Gas values stayed above 200 ppm for the remaining 5 d of the monitored period; most gas readings were trending upward at the final reading.

The gas slowly diffused upwards into the grain with limited effect from the thermosiphon pipe. The ambient temperatures were low but the sun acting on the north side of the silo caused air to rise, drawing phosphine gas from the plenum at high concentrations past the north monitoring point which was located 0.5 m from the silo floor and 0.3 m into the grain.

The experience and data from this fumigation demonstrated the critical requirement of positioning the thermosiphon pipe on the north wall of the silo for maximum sun exposure. To augment air movement in silos 1 and 3, black painted 100 mm PVC pipes were fitted on the NNE and connected to the inside of the silo through the outloading auger inspection plate with a 90 mm flexible pipe. The fumigation also demonstrated that the evolving phosphine gas, with limited assistance from the thermosiphon pipe, diffused upwards into the grain above the plenum grating without reaching dangerous concentrations.

Silo 3 Tests of plenum concentrations
The white pipe warms more slowly than black which reduced the time available for the air gas mixture to be drawn from the plenum. To discover what effect this would have on the development and distribution of the evolving gas and enable field testing of the high concentrations in the plenum, a dilution technique was used.

A Canary Co 'SiloChek' has a range 0-2000ppm. To measure high values a peristaltic electric diaphragm pump was used to draw the phosphine/air mixture from the reaction chamber into a Tedlar® gas tight bag. Two hundred ml of this gas was drawn into a 1000ml laboratory grade syringe and then a further 500ml of air was drawn in before being injected into another Tedlar® bag. The diluted Tedlar® bag was attached to a Canary monitor and the final displayed concentration multiplied by 3.5. This technique shows that the concentrations did not exceed 5000 ppm.

The gas remaining in three of the Tedlar® bags was transported to the DAFWA phosphine resistance laboratory and tested to obtain a cross reference using a gas chromatograh. The results are shown in Table 1. From this limited test it is suggested the concentrations in the reaction chamber as indicated by the Canary monitor in the field could

be elevated by approximately 1600 ppm making the highest recorded concentration 6220 ppm, compared to 17,900 ppm at the phosphine explosive limit.

Site 2 Balaklava South Australia Winter 2010

Silo 2 Fumigation (1200t / 1472m³) top loaded
The silo was 65% filled with wheat at 8.9% mc. The silo pressure test exhibited a halving pressure (P½) of 120 seconds. To compare GLAS and top loaded fumigations 2000g of AlP were added to the headspace of this silo providing 1.35g of phosphine/m³. Monitoring commenced 18 hrs later using a combination of a Spectros non dispersible infra red automatic data logging unit and a hand held Canary Company 'SiloChek' electronic cell. The Spectros monitor had four operating monitoring points which were attached to the plenums and to the North thermosiphon pipe on silos 2 and 4. All other points were monitored using the 'SiloChek'. For these experiments a gas value of 200ppm was adopted as the threshold at which the fumigation period commenced.

Table 1. Comparison of electronic monitor field readings with gas chromatograph analysis

	Canary (ppm)	GC (ppm)	GC/Canary
Base N	2600	3950	1.52
Plenum L	3031	4880	1.61
Plenum R	2450	4090	1.67
Average	2694	4307	1.60
SD			0.08
SD %			4.73

Phosphine gas reached 200ppm at all monitored points in the silo within 26 hrs after application of the AlP to the headspace (Fig. 5). All points recorded concentrations between 200 and 1400 ppm for the remaining 10 days of the monitored period.
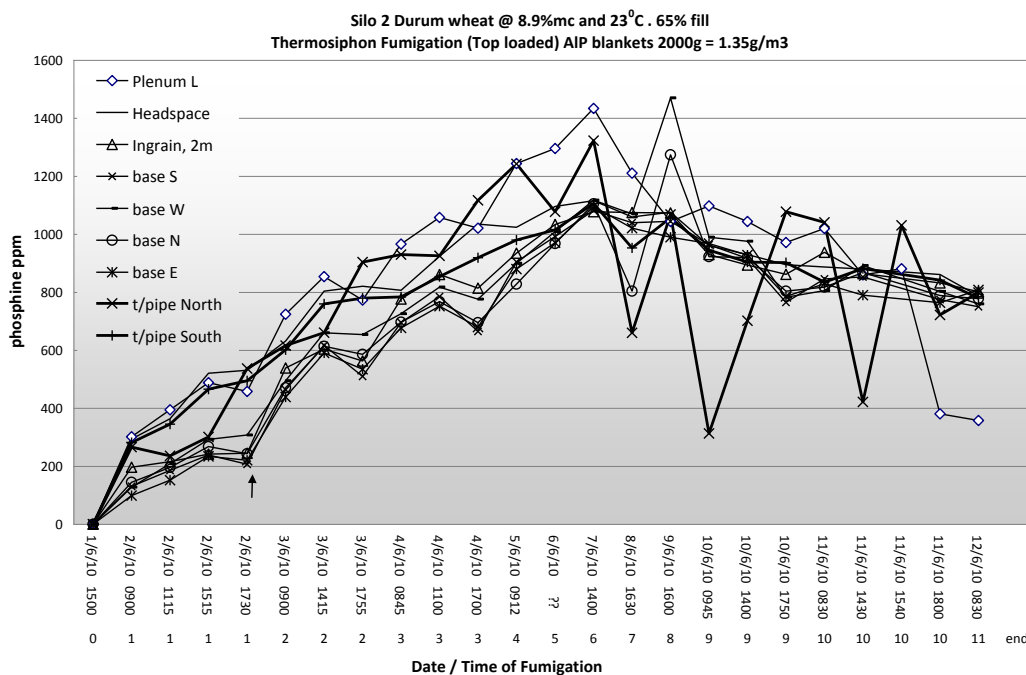
Fig. 5- Top loaded Thermosiphon fumigation.

Phosphine concentrations show a co-related rise and decay at all points in the grain bulk as the gas is drawn into the grain bulk soon after release from the AlP. This is in comparison to a non recirculated silo where there is an immediate high concentration of phosphine gas at the AlP insertion point and a slower rise at all other points at the gas diffuses though the grain bulk with some assistance from internal air currents. The graph also shows large oscillations of concentrations in the thermosiphon pipes due to the diurnal and nocturnal variations of temperatures.

Silo 4 (1200t / 1472m³) Ground level application

The silo was filled to 95% with wheat at 9.5% mc. The silo pressure test showed a halving pressure (P½) of 180 seconds. The dosage of 2000g of AlP were probed into the aeration plenums of this silo providing 1.35g of phosphine /m³ (Fig 6).

Fig. 6- probing AlP Blanket formulation into the aeration duct/phosphine reaction chamber

Monitoring commenced 18 hrs later and the threshold concentration of 200ppm was reached in 48 hrs. Concentrations were recorded between 200 and 800 ppm for the remaining nine days of the monitored period (Fig. 7). The concentrations followed a similar path of co-related rise and decay as in silo 4 and large oscillations of phosphine concentrations in the thermosiphon pipes. The slower time to threshold at all points was most likely due to the ground level application technique.
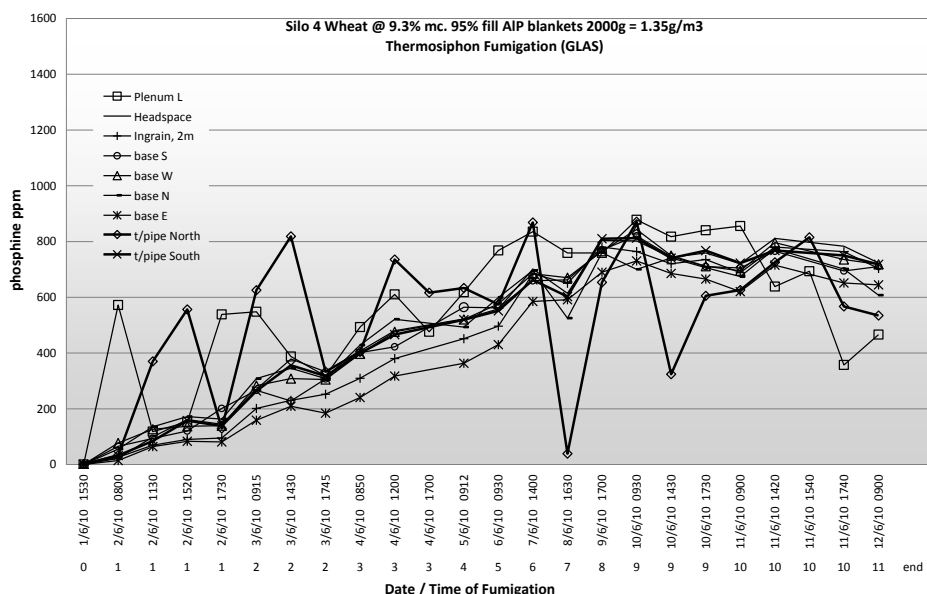
Fig. 7- Winter GLAS Thermosiphon fumigation
Fumigations Autumn 2011

Site 2 Balaklava South Australia

Fumigations commenced on all four 1200t silos in March with warm days and cool nights. The fumigations were recorded with hand held Drager electronic gas monitors with a maximum reading of 2000 ppm. Aluminium phosphide blankets containing 1000 g of phosphine were probed into each plenum branch of each silo (2000g total dosage) and monitoring commenced the following morning.

Rapid peaks of concentration were observed which were caused by diurnal movement of phosphine in the thermosiphon pipes and plenum. After sunset the air in the thermosiphon pipes ceased to move and phosphine generated from the AlP blankets diffused into the grain above the perforated duct cover plates. At sunrise the thermosiphon pipes warmed and concentrations escalated rapidly as the gas generated overnight was drawn out in the first hour. Sunrise occurred at approximately 07:00 and due to low overnight ambient temperatures of ~5ºC it took a further 15 minutes for the south thermosiphon pipe to warm and cause air to rise. From repeat monitoring, the concentration was observed to rise after 07:20 from zero through to ~600ppm in 45 minutes. High concentrations of phosphine were observed moving up the south thermosiphon pipe 20–24 h after application of the AlP but time to reach 200 ppm of phosphine at all monitored points varied between 60 and 144 h.

As an example of a fumigation in the four silos the record of Silo1 is presented in Fig. 8. Low levels of phosphine were recorded across the base approximately 24 hrs after application of the AlP and in the headspace and all other points after 32 hrs. This shows the mixing of the internal air by the current emanating from the thermosiphon pipes. The fumigation period commenced 111 hrs (4.6 d) after application when all points in the silo reached 200 ppm and remained above this concentration for a further 6 days when monitoring was terminated. The concentration of phosphine throughout out the grain bulk remained between 200 and 500ppm at all points in this period.
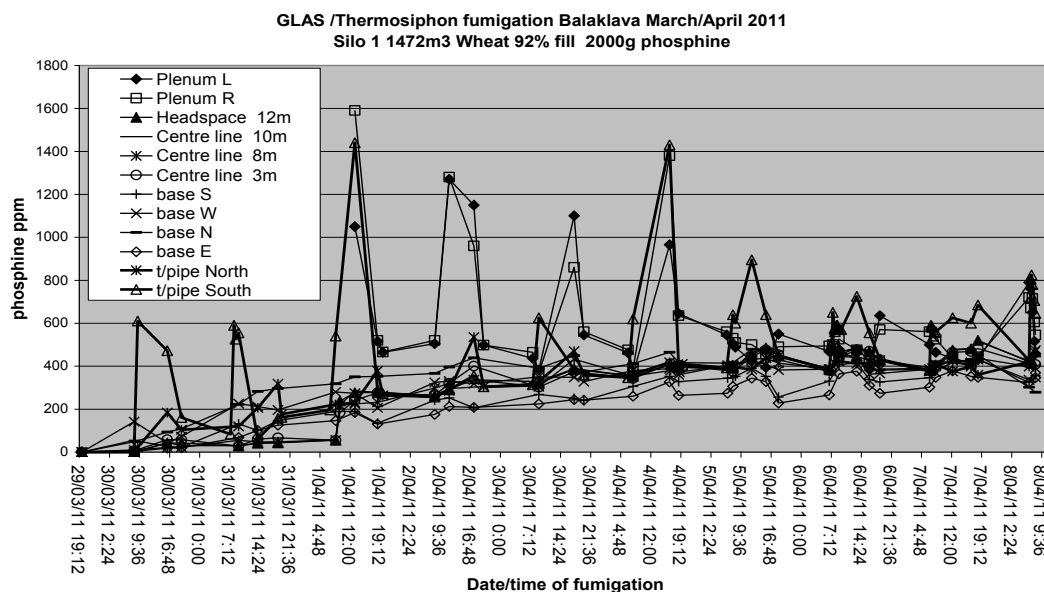
Fig. 8- Example of autumn GLAS thermosiphon fumigations

Fumigations Summer 2012

Fumigations commenced in February 2012 under generally warm ambient conditions. Gas concentrations were taken with a Drager X am 5000 electronic phosphine monitor with an upper limit of 2000 ppm. Some points in silos at Balaklava were recorded using a Spectros non-dispersible infra red monitor. Drager phosphine indicator tubes (500 – 10000 ppm) were used on some silos soon after application of the AlP to record the concentrations developing that were beyond the range of the X am 5000.

Site 1 Arthur River Western Australia

All silos on this site had been subjected to aeration after grain loading creating a stable temperature throughout the silos and reducing the internal thermal air currents. An on site temperature logger provided precise data which shows the influence of the sun on movement of gas in the thermosiphon pipes. From two fumigations on this site it was observed there is an approximate correlation between rising ambient temperatures and rising phosphine gas values as the released gas is drawn from the reaction chamber via the thermosiphon pipes and into the headspace (Fig. 9). It was also observed that the peak concentrations in the plenum occur when there is a reversal of air in the thermosiphon pipe and the gas that has liberated into the grain bulk is drawn down into the plenum or is drawn down from the headspace through the thermosiphon pipe into the plenum.
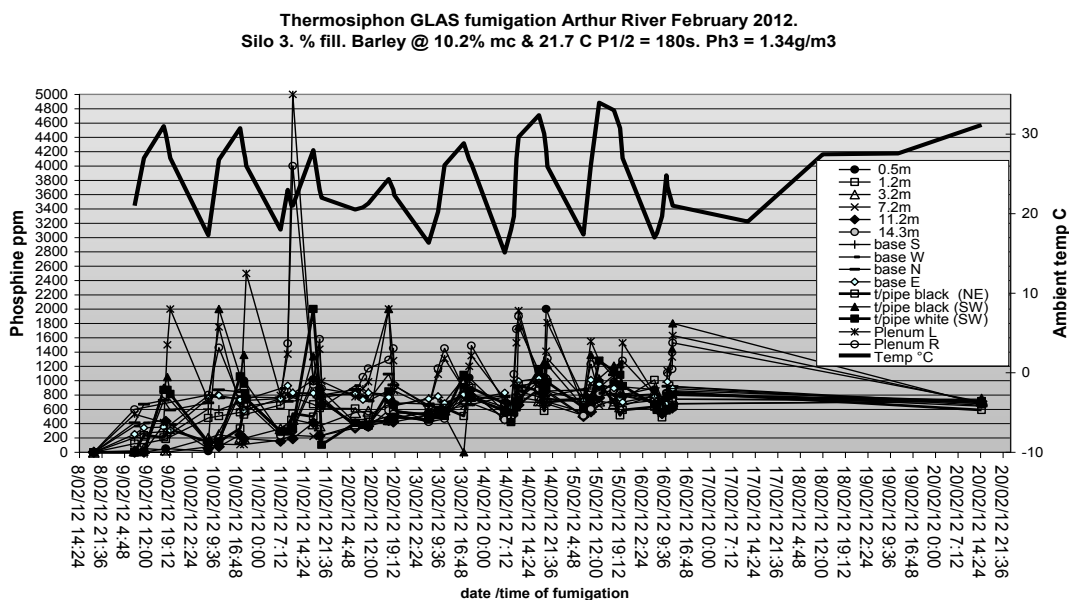
Fig. 9- Example of summer fumigation GLAS

The addition of a black painted 100 mm thermosiphon pipe at the approximate NNE point was effective with gas shown to move up these pipes into the headspace within a day after loading of the AlP. For the gas to be drawn up this pipe it needs first to emanate from the reaction chamber and move across the silo floor before penetrating into the auger channel, seeping around the closed grain control slide plates. Thermosiphon pipes are attached to

unloading augers on six of the seven silos tested in this experiment and it is recorded that although they do not exhibit the dramatic peaks and falls of the unimpeded pipes, they are a significant contributor to the circulation of phosphine in the silo

The phosphine gas in this silo reached 200 ppm at all monitored points 63 hrs after application of the AlP into the ground level reaction chamber and remained above this level for the remaining nine days of the fumigation. Drager phosphine indicator tubes (500–10000 ppm) were used to record gas concentrations in the plenums when the Drager X am electronic monitor was observed to reach maximum concentration rapidly after connection. The highest recorded concentration was 5000 ppm 63 hrs after application of the AlP.

Non recirculated fumigation

As a direct comparison to thermosiphon gas distribution, silo 4 with no thermosiphon pipes fitted, was top loaded with 2000 g of AlP. From experience a silo top loaded with AlP, the slowest point to reach the required concentration is the base of that silo. Monitoring points of stainless steel tubing 1mm i.d. was inserted at four points around the base of the silo one metre above ground level and probed 0.3 m into the grain. The silo contained a 50% load of Canola at 7.8% m.c. and achieved a P½ of 180 s. Phosphine gas readings started to appear on the Drager monitor after 4 days and reached 100 ppm after nine days. The gas did not reach 200 ppm at all points before monitoring ceased at 12 days.

Site 2 Balaklava South Australia

Blanket formulation AlP was probed into the reaction chamber at the base of three silos and gas concentrations were recorded at all monitored points 24 - 36 h after application. The threshold concentration of 200 ppm was reached between 90 – 108 h and remained above this level for the monitored period.

A Spectros phosphine monitor was attached to four points on silos 1 and 2 including the plenum to record the concentrations that develop at the critical times as phosphine gas is generating and the airflow changes direction. A large difference was recorded in the concentrations between the two plenums; this may be due to the monitor tube placement in proximity to the AlP.

After probing blanket formulation of AlP into the reaction chamber of silo 2 it was found that one monitor tube was blocked and a new line was probed. This tube appears to have lodged in the blanket formulations presenting high readings for most of the monitored period. The Spectros monitor records automatically at three hour intervals and is able to analyse concentrations ~10,000 ppm. The placement of the monitoring tube provided an opportunity to observe the gas release in relation to safety of the GLAS system. The highest phosphine concentration observed was 10,690 ppm (Fig. 10).

CONCLUSIONS

The results from 10 experiments on large silos under a range of climatic conditions from warm to cool showed that thermosiphon pipes attached to GLAS are successful in delivering the evolving phosphine gas from ground level reaction chambers into the headspace and creating a recirculating current within the grain bulk to achieve even distribution. Thermosiphon fumigations do not typically display the high peaks in the headspace after application of the AlP and the tailing off concentrations at all other points in the silo. Lower co-related phosphine concentrations were noted throughout the grain bulk whether the AlP was top loaded or applied at ground level.
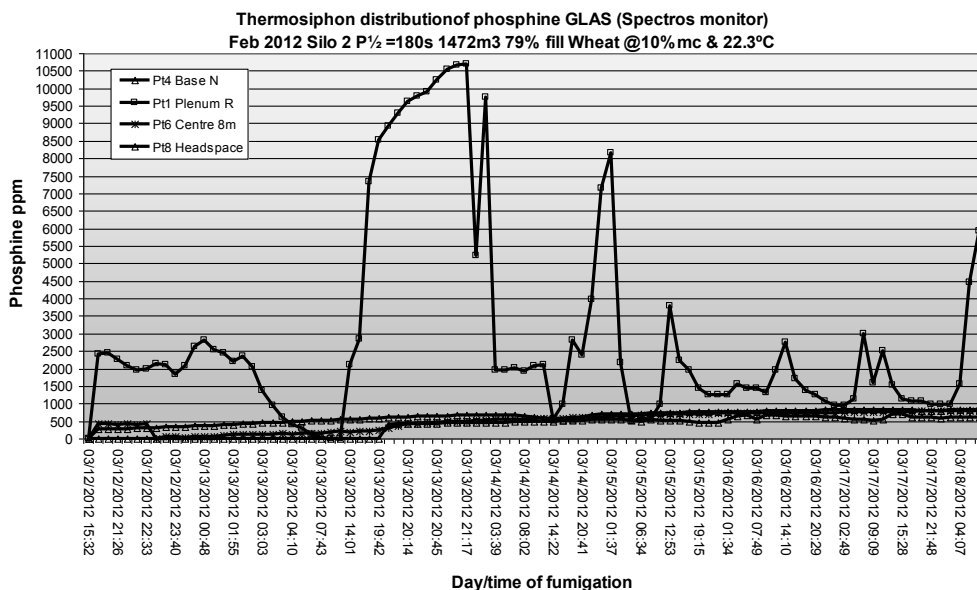
Fig. 10- Analysis of concentrations of Ph3 in reaction chamber, GLAS fumigation

A sealed silo exhibiting a minimum P½ =180s is essential to ensure the required concentration × time (Ct) product is achieved to control all life stages of the target species. The concentration of phosphine gas and time needed varies according the susceptibility of the resident population of insects. The threshold concentration chosen for these experiments is 200 ppm but the time factor will depend on the resistance of the endemic insect population to phosphine.

The test on a non thermosiphon (check) silo showed the concentrations did not reach 200 ppm 12 d after application of the AlP. This has implications for time of outturn and may contribute to development of phosphine resistance if there are surviving insects in this zone.

The top loaded fumigations reached the threshold concentration more quickly than a ground level fumigation which can affect outturn timing and can be critical to meet shipping schedules. GLAS fumigations have significant safety advantages for the application of large quantities of AlP but the slower time to minimum concentration must be taken into account when determining an outloading date.

The thermosiphon process works when there is a difference in temperature between the internal and external silo environment. In any 24 hr period there will be parity in the temperatures at least twice when the air in the pipe will cease to move. It is necessary to ensure that during these periods released phosphine gas can diffuse from the reaction chamber into the grain to prevent potentially explosive high concentrations developing in the reaction chamber.

There is an observable correlation between rising ambient temperatures and the increase in gas concentrations moving up the pipes after sunrise. In particular as sunlight warms the thermosiphon pipes the gas concentration can be observed climbing rapidly in the first 60 minutes, accounting for the spikes seen on the graphs, before settling to a lower level during the day. It is also observed that the gas concentrations in the plenum will increase around

sunset as the air in the thermosiphon pipe stalls and then reverses pushing phosphine into the reaction chamber and through the grating into the grain bulk at the base.

Safety in the use of phosphine is critical; part of these experiments was to ensure that concentrations would not build to the lower flammability limit. Intensive sampling of the concentrations emanating from two phosphine reaction chambers found maxima between 6220 and 10,690 ppm which remains well below the lower flammability limit of 17,900 ppm. It is necessary to ensure that the circulation of air in the thermosiphon system is not impeded and annual checking of the pipe work must be undertaken prior to loading AlP. If using a grated or perforated plenum as the phosphine reaction chamber, it is essential to ensure this perforated flooring is not blocked with fines or dust from previous crops. When using aeration to cool grain prior to fumigation it is essential to ensure the incoming air humidity will not cause moulding of the grain adjacent to the plenum grating which could create a resistance to airflow. It is likely that phosphine will penetrate the moulds but any air restriction will impede the development of the Ct in the grain bulk.

Shading or poor positioning of the pipe which reduces exposure to the sun has a direct impact on the speed of gas delivery and admixture. A pipe oriented to approximately north is more likely to be successful but a south facing pipe will have some positive benefit on gas delivery. To avoid making structural changes when retrofitting a thermosiphon pipe to an established silo the best entry point will be through the outloading machinery and aeration in point which may not be the best position. When constructing a new silo it is possible to take this into account and orient the silo aeration or unload systems to enable favourable positioning of the thermosiphon pipe.

Retro fitting ground level application points to large silos fitted with aeration and a plenum is possible but the fitting of the thermosiphon pipe to the peak of the roof presents the risk of working at heights and should be undertaken by a professional team using an elevated work platform or industrial rope access techniques.

ACKNOWLEDGEMENTS

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THE MEASUREMENT AND CONTROL OF LOW OXYGEN AND HIGH CO_2 ATMOSPHERES

David Bishop*

Technical Director, International Controlled Atmosphere Ltd, Transfesa Road, Paddock Wood, Kent, United Kingdom.
*Corresponding author's e-mail: *davidB@ICAstorage.com*

ABSTRACT

Low oxygen and high CO_2 storage is standard practice in the fruit storage industry which has developed over many years to provide reliable and competitive systems with simple to operate computer based interfaces.

Recent developments in dynamic CA for fruit storage has resulted in the need to measure oxygen to even lower levels of sensitivity which could be appropriate for the MA atmospheres used for crop de-infestation.

Many lessons have been learnt about the use and operation of high accuracy gas analysers in an agricultural environment. This experience is discussed in its application to the de-infestation of stored products.

The construction, sealing and testing of low oxygen fruit stores have many similarities with the requirements for fumigation: the current construction and testing procedures will be discussed.

Key Words: Oxygen, Carbon Dioxide, Gas Analyser, Controlled Atmosphere, Oxygen sensors

BACKGROUND

The establishment of low oxygen and enhanced Carbon Dioxide (CO_2) atmospheres is used extensively for the commercial long term storage of fresh fruit and vegetables.

The significant difference between fresh produce storage and the use of low oxygen for product de-infestation is that fruit and vegetables respire, helping to create their own low oxygen atmosphere, requiring that product generated carbon dioxide is removed without increasing the oxygen concentration. For pest eradication the product is essentially inert with the atmosphere modification relying totally on external equipment with no requirement for carbon dioxide removal.

To illustrate the similarities and differences between the two applications, Table 1 shows some typical controlled atmosphere conditions for various products. These conditions are regularly established in many thousands of CA storage rooms throughout the world.

Recent advances in the use of dynamic controlled atmosphere substitute the need for "recipe" levels of oxygen with "variable" oxygen concentrations. The fruit in the storage room is monitored using various methods to detect anaerobic stress and the oxygen is maintained at a threshold just above the measured stress point. This can require the maintenance of oxygen down to a level as low as 0.2%.

Table 1. Typical CA conditions for various fresh produce

Product	Oxygen %	Carbon Dioxide %	Temperature °C
Apples (Gala)	1	< 1	0.5
Apples (Bramley)	1	5	4.5
Pears (Conference)	2	<1	-0.5
Onions	3	5	0
Strawberries	-	15	0
Bananas	3	3	14

With CA fumigation techniques requiring oxygen at 0.5% and lower it can be seen that the established measuring and control techniques widely used in the produce CA industry can be readily used for de-infestation.

OXYGEN SENSORS

With the low oxygen levels required in these applications it is important to use accurate and stable analysers to make the measurements. There are 3 different sensor principles that have been used in this industry; their characteristics are summarised in table 2.

Table 2. Comparison of oxygen sensors

Principle	Minimum reading O_2	Typical sensor cost €	Typical sensor life Years	Comments
Paramagnetic	0.2%	1500	20+	Sensitive to flow and vibration
Zirconia	10 ppm	600	6	Sensor heated to 300+ C, Logarithmic output
Electrochemical cell	0.02%	60	2	Life reduced when CO_2 high

ICA design and manufacture a range of oxygen analysers used both in ICA equipment and incorporated as OEM analysers by many CA equipment manufacturers. In recent years we have exclusively used electrochemical sensors due to the high quality of their performance and their cost effectiveness. The bi-annual replacement of the cells is a disadvantage but is a quick and simple procedure.

CARBON DIOXIDE SENSORS

Sensors using the principle of Infra-Red adsorption are the only practical choice for carbon dioxide measurement. Infra-red radiation in the specific band that is adsorbed by CO_2 is generated and passed through a chamber containing the flowing gas sample to be measured

The amount of radiation adsorbed by the CO_2 is measured and converted to a measurement of CO_2 concentration. High quality and accurate sensors are readily available with ranges from of 0-1000ppm to 0-20%. Surprisingly the availability of sensors with the range 0-100% CO_2 is more difficult with limited choices and at a higher cost.

GAS SAMPLING

It is important that care is taken over sampling the storage room atmosphere and that the analyser is presented with a representative sample of the room content. The prevention of leaks and blockages are essential for long term successful operation. Samples can be satisfactorily obtained through tubes of over 100m in length if the correct precautions are taken.

The tube should be robust and if fitted outside not subject to UV degradation. Black UV stabilised nylon tubing is the most satisfactory. The tube should be run in a single length from the inside of the room to the analyser manifold as tube fittings can easily be a point of leakage.

If the contents of storage room are stored at a higher dew point than the minimum ambient temperature then condensation will occur in the sampling line. To prevent this condensation causing blockage the internal tube diameter should be at least 6mm and the tube installed with a constant slope and without dips that can cause a water trap. If ambient temperatures are expected to be below zero then blockage by freezing can occur.

In de-infestation applications a leakage in the sampling line is fail safe in that the oxygen will be reading higher on the analyser that it is in the room. In fresh produce storage this error can cause severe damage to the stored crop if the oxygen falls below the anaerobic level.

AUTOMATION

Gas measurement, room sampling and machinery control can all be automated with computer based control systems. Features of these systems should include data recording, alarms, automatic analyser checking and calibration. It is common for the room environment to be maintained at the required Oxygen and CO_2 level with the automatic operation of the atmosphere generating machinery. Temperature measurement and recording can also be incorporated to maintain the complete record for audit purposes. The ICA6000 system is designed for this purpose and is independent of any machinery manufacturer. It has excellent communication facilities, includes build in analysers and sampling systems and is very simple to install and operate.

ROOM SEALING AND TESTING

The construction of storage rooms for controlled atmosphere produce storage is a specialist area that needs close attention to detail. Because these rooms require to be refrigerated they are commonly made from sectional insulating panels. All the panel joints are taped and sealed with a flexible elastomeric coating. The doors have to be specially designed and made for Low Oxygen use and include all round gaskets or pneumatic seals.

An essential part of the specification for a new room is a pass on a standard leak test. It is good and normal practice that this test is repeated every year before the storage season.

The standard UK test is to pressurise the room using a small blower to a pressure of 200 Pa (20mm water). The blower is then sealed off and the time taken for the pressure to fall to 130 Pa is recorded. An acceptable time for low oxygen rooms is 10 minutes but in practice a good room would hold up for 30 minutes or more. In N America the standard is slightly different in that the time is measured for the pressure to fall to half the original value which should be longer than 30 minutes for a low oxygen room.

CONCLUSIONS

The need for lower oxygen levels, easier operation and more cost effectiveness is common in both the Produce Storage and Crop Protection applications of Controlled Atmosphere. A sharing of expertise and common equipment supply should be able to help achieve these objectives.

COMPUTER SIMULATION OF GAS CONCENTRATION IN THE INTERSTITIAL ATMOSPHERE OF A WHEAT SILO-BAG FOR TYPICAL AGRICULTURAL AREAS OF ARGENTINA

Alien Arias Barreto[1], Rita Abalone[1], A. Gastón[1,2*], R. Bartosik[3]

[1] Fac. de Cs. Exactas Ingeniería y Agrimensura. UNR. IFIR (CONICET/UNR)
[2] CIC- UNR. Fac. de Cs. Exactas Ingeniería y Agrimensura. UNR.
Av. Pellegrini 250. (2000) Rosario. Argentina
[3] National Institute of Agricultural Technologies (INTA). Ruta 226 km 73,5 (7620). Balcarce. Argentina
*Corresponding author's e-mail: *analiag@fceia.unr.edu.ar*

ABSTRACT

A validated mathematical model was used to determine the change in concentration of CO_2 in a silo-bag holding wheat from summer to winter for a typical productive region in the North (Saenz Peña, Chaco Province), Center (Pergamino, Buenos Aires Province) and South (Balcarce, Buenos Aires Province) of Argentina. Initial moisture content of grain was set to 12, 14 and 16% w.b. and bagging temperatures to 25ºC and 40ºC. For standard conditions (12% w.b., 25ºC) CO_2 level increased to 4% V/V and O_2 decreased to 15.5% V/V in Balcarce while to 6% V/V and 13.9% V/V in Saenz Peña. For moist grain (16% w.b., 25ºC and 40ºC), O_2 depleted to less than 1% at the three locations. Evolution of grain mean temperature in combination with CO_2 and O_2 levels achieved in the silo-bags demonstrate that for the climatic conditions of Argentina insect activity is controlled in airtight silo-bags.

Keywords: grain storage, hermetic storage, wheat, silo-bags, atmosphere composition, modeling

INTRODUCTION

In 2010 more than 40 million tonnes of grains were stored in hermetic systems (silo-bags) in Argentina. This technique, originally used for grain silage, consists in storing dry grain in hermetically sealed plastic bags. The respiration process of the biological agents in the grain ecosystem (grain, insects, mites and microorganisms) increases carbon dioxide (CO_2) and reduces oxygen (O_2) concentrations, promoting a suitable environment for grain conservation.

Gas concentration in grain bags depends on the balance between respiration of the ecosystem, the entrance of external O_2 to the system, and the loss of CO_2 to the ambient air. The transfer of gases depends on the gas partial pressure differential and the effective permeability of the plastic cover (openings and natural permeability of the plastic layer to gases). Grain type and condition, moisture content (m.c.), temperature, storage time and O_2 and CO_2 concentrations affect the grain respiration rate.

A novel technology for monitoring grain storability in silo-bags based on CO_2 detection

was implemented by The National Institute of Agricultural Technologies of Argentina (INTA), Balcarce Experimental Station (EEA) (Bartosik et al., 2008; Cardoso et al., 2008). The procedure consists of comparing the measured CO_2 concentration at some locations in the silo-bag (local concentration value) with a referential value which represents adequate storage conditions. Additionally, it was observed that this referential value can change according to the season and climatic condition of a particular agricultural area. However, setting experimental field tests to cover the wide range of possible storage conditions is time consuming and costly.

The authors developed a 2D heat and mass transfer model which predicts grain storage temperature and m.c., O_2 and CO_2 concentrations as function of weather conditions, taking into account simultaneous O_2 consumption, CO_2 generation, and permeability to gas transfer of the plastic bag. The model was validated by comparison of predicted temperature, grain moisture content, mean O_2 and CO_2 concentrations with measured values in field tests (Gastón et al., 2009; Abalone et al., 2011a; b; c).

In the present work, the model was used to determine the referential mean O_2 and CO_2 concentration that corresponds to three productive region in the North (Saenz Peña, Chaco Province), Center (Pergamino, Buenos Aires Province) and South (Balcarce, Buenos Aires Province) of Argentina. The evolution of O_2 and CO_2 concentration during six months was simulated in silo-bags holding wheat. The effect of initial m.c. and bagging temperatures was investigated.

MATERIAL AND METHODS

Silo-bags
Silo-bags are 60 m long, 2.70 m diameter and 230 - 250 microns thick. The bags are made of a three-layer plastic, black in the inner side and white in the outer side with UV stabilizers. The plastic layers are a mixture of high density (HDPE) and low density polyethylene (LDPE). Approximately 200 tonnes of grains (wheat, corn and soybean) can be held in a bag; farmers usually store their production in bags during six to eight months.

Mathematical modelling
Stating the energy and mass balances for the grain and air phases in a control volume, a coupled system in terms of temperature T, grain moisture content W, oxygen O_2 and carbon dioxide CO_2 concentrations are derived. The balances take into account heat, water vapor, oxygen consumed and carbon dioxide released by respiration of the grain ecosystem, which is modeled by the complete combustion of a typical carbohydrate. Boundary conditions considered the interaction between the soil and the bottom layer of the silo-bag, solar radiation and convection to the surroundings. It was assumed that the silo-bag was impermeable to moisture transfer. Gas transfer through the plastic layer was modeled by defining an equivalent permeability of the plastic to O_2 and CO_2. This value is calculated by use of a resistance series model as the silo-bag is a mixture of high density (HDPE) and low density polyethylene (LDPE). A detailed description of the model is presented elsewhere (Gastón et al., 2009; Abalone et al., 2011a; b; c). The mathematical model was implemented using *COMSOL Multiphysics 4.2a* and solved numerically by the finite element method. Figure 1 shows the calculation domain, which represents a cross section of the silo-bag.
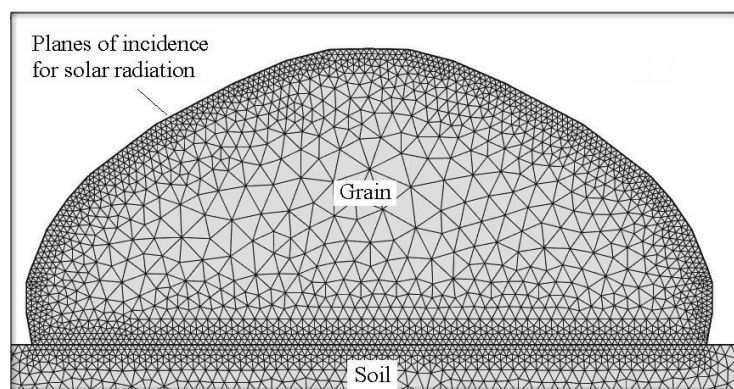
Fig. 1- Cross section of the silo-bag and discretization domain

RESULTS AND DISCUSSION

The model was applied to analyze the storage of wheat in a silo-bag from January to June (six months). Initial grain m.c. was set to 12, 14 and 16% w.b. and initial bagging temperatures to 25°C and 40°C. Climatic data corresponding to (1997-2004) years were considered for Balcarce (37.84S; 58.26W), in the Southwest of Buenos Aires Province; to (2001-2006) years for Pergamino (33.85S; 60.93W), in the North of Buenos Aires Province and to (1999-2006) years for Saenz Peña (26.78S), in Chaco Province.

Dependence of the rate of CO_2 production Y_{CO2} was evaluated by use of the correlation developed by White et al. (1982) which takes into account grain temperature, m.c. and storage time. Equivalent permeability of the plastic layer to O_2 was set equal to 9.75 $10^{-8} m^3 md^{-1} m^{-2} at^{-1}$ and to CO_2 equal to 3.22 10^{-7} $m^3 md^{-1} m^{-2} at^{-1}$, average plastic thickness L to 240 μm, effective diffusivity to CO_2 and O_2 to 3.97 10^{-6} $m^2 s^{-1}$ and 5.22 10^{-6} $m^2 s^{-1}$, respectively, wheat porosity to 0.38 and tortuosity to 1.53.

Figure 2 compares annual mean temperature and solar radiation at the three locations. In summer, mean ambient temperature in Saenz Peña is about 3°C higher than in Pergamino and 7°C higher than in Balcarce, while in winter about 6°C and 8°C higher, respectively. Solar radiation is comparable in Saenz Peña and Pergamino and about 9% higher than in Balcarce. In autumn irradiance at Saenz Peña is about 13% and 30% higher than in Pergamino and Balcarce.
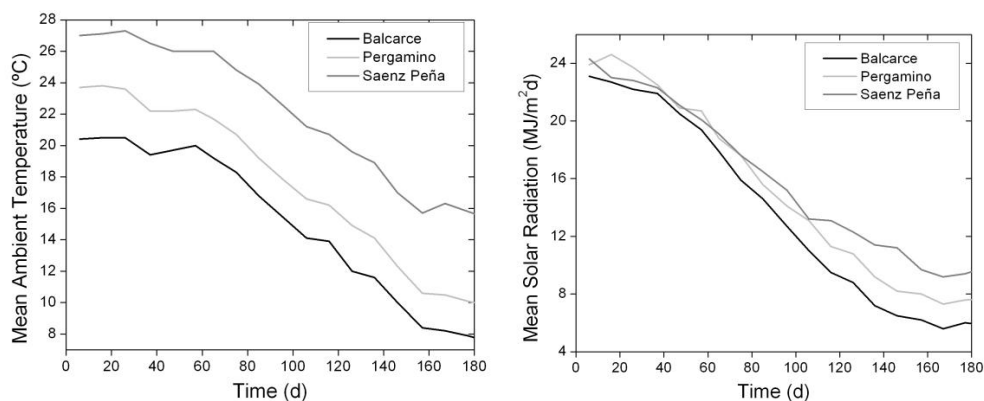
Fig. 2- Comparison of mean ambient temperature and solar radiation of agricultural areas

Results for each bagging condition and location were averaged over the eight years and a 90% confidence intervals for mean temperature, CO_2 and O_2 were constructed by applying a t-Student probability distribution.

Figure 3 shows mean gas concentration at the three locations for 25ºC initial bagging temperature. It can be appreciated that climatic conditions produce significant changes in referential levels after 180 days of storage, especially for 12% and 14% m.c. In Balcarce, CO_2 level increased to $(4.03\pm0.07)\%V/V$ and O_2 decreased to $(15.5\pm0.1)\%V/V$. An increment of 3ºC in ambient temperature shifts CO_2 and O_2 levels to $(4.6\pm0.1)\%V/V$ and $(14.8\pm0.1)\%V/V$ in Pergamino, and one of 8ºC to $(6.0\pm0.1)\%V/V$ and $(13.9\pm0.2)\%V/V$ in Saenz Peña.
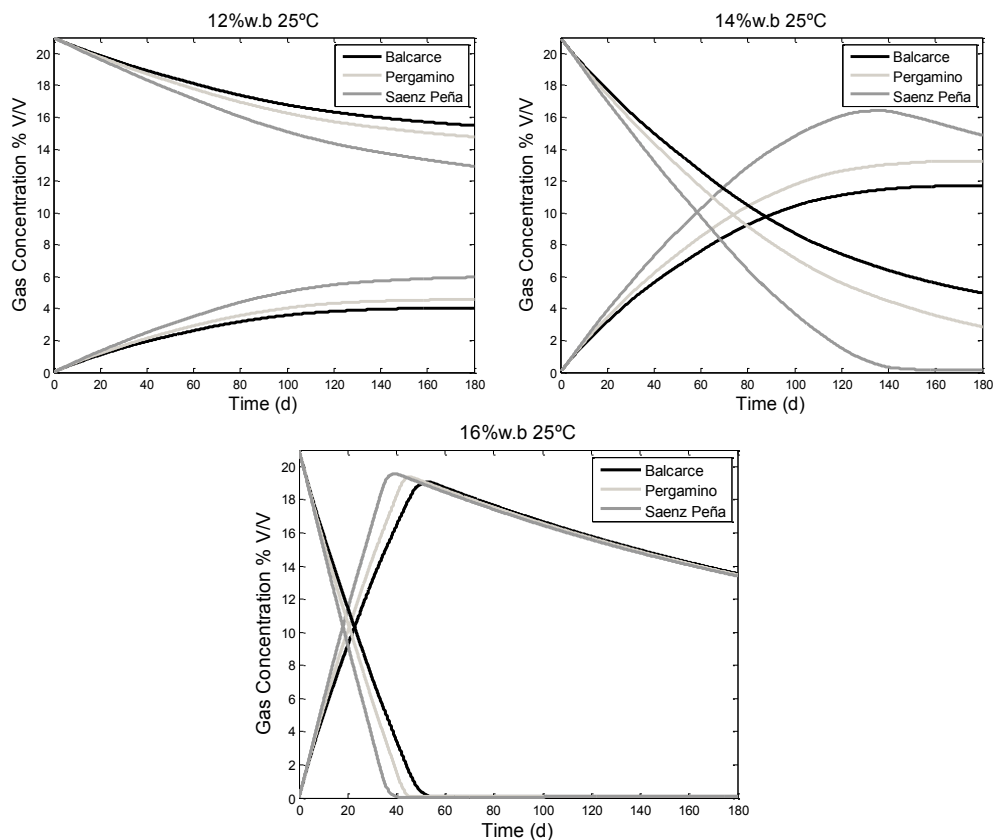
Fig. 3- Gas concentration evolution at Saenz Peña, Pergamino and Balcarce. Initial temperature 25ºC

Differences in concentration levels between summer (40 days) and winter (180 days) remained within 3% points approximately at the three locations. At 14% w.b. mould activity becomes important increasing CO_2 concentration to $(11.7\pm0.2)\%V/V$ and reducing O_2 concentration to $(5.0\pm0.3)\%V/V$ at Balcarce and to $(13.2\pm0.3)\%V/V$ and to $(2.9\pm0.4)\%V/V$ in Pergamino. Differences up 7% points between summer (40 days) and winter (180 days) were found. At Saenz Peña, O_2 is almost consumed after 140 day and remained at zero level thereafter because all O_2 entering through the plastic cover is consumed by respiration. CO_2 reaches $14.9\pm0.1\%$ V/V and then decays as results of permeation to atmosphere through the

plastic layer. For 16% w.b., O_2 depleted to less than 1% within 40 to 50 days (Saenz Peña and Balcarce) and CO_2 evolved as explained before.

Figure 4 shows results for 40ºC initial temperature. For 12% w.b., changes in CO_2 and O_2 are about 1 to 1.5%V/V points increase/decrease with respect to 25ºC. For 14% w.b. O_2 depleted to less than 1%V/V after 90 days in Saenz Peña, 140 days in Pergamino and 180 days in Balcarce while at 16% w.b. this conditions is achieved in about 20 days at the three locations.
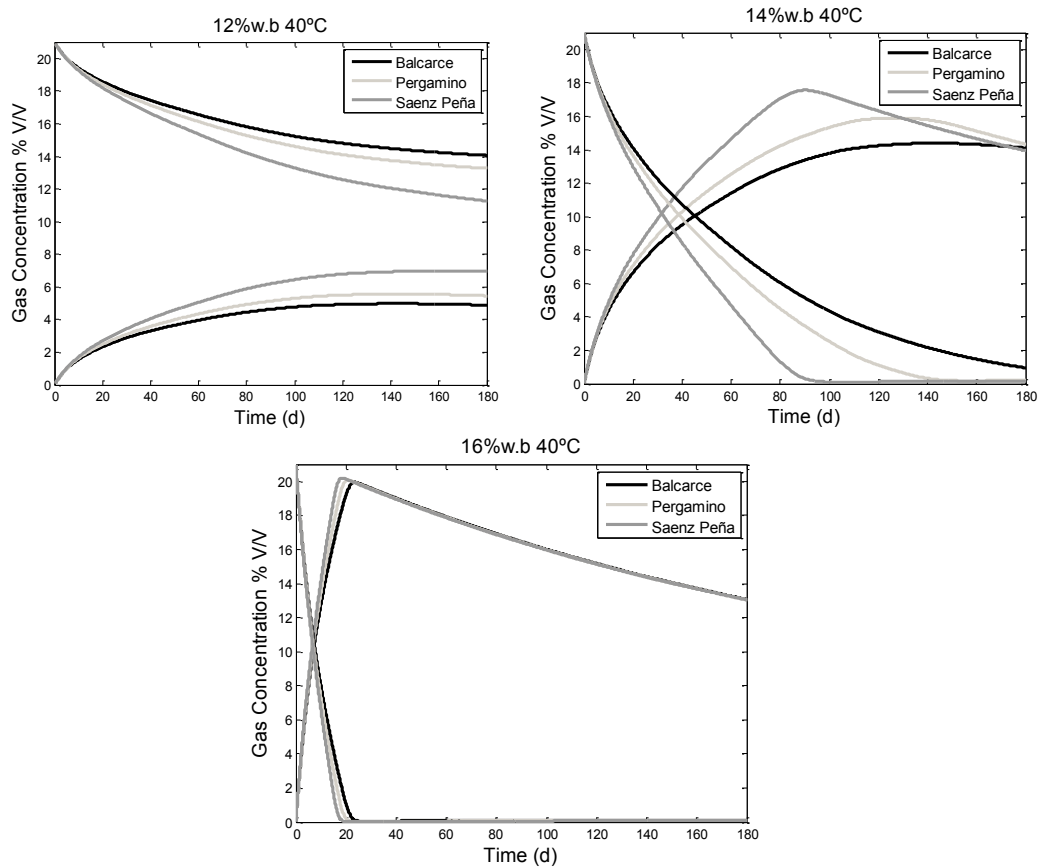
Fig. 4- Gas concentration evolution at Saenz Peña, Pergamino and Balcarce. Initial temperature 40ºC

Figures 5 and 6 illustrate the evolution of the mean temperature of the silo-bags. These results demonstrate that for the climatic conditions of Argentina, insect activity would be limited for dry and moist grain because mean grain temperature decreases below 17ºC during autumn and winter preventing insect infestation. Moreover, even in summer conditions, grain at 14% w.b. or higher, would limit insect activity as result of low O_2 and high CO_2 concentration in the silo-bag, if the silo-bag maintains at high air tightness level.

CONCLUSIONS

The effect of climatic conditions and grain moisture content on the evolution of gas concentration in silo-bags holding wheat was analyzed by use of a validated mathematical model. Referential mean O_2 and CO_2 levels were predicted for a typical productive region in the North (Saenz Peña, Chaco Province), Center (Pergamino, Buenos Aires Province) and South (Balcarce, Buenos Aires Province) of Argentina.
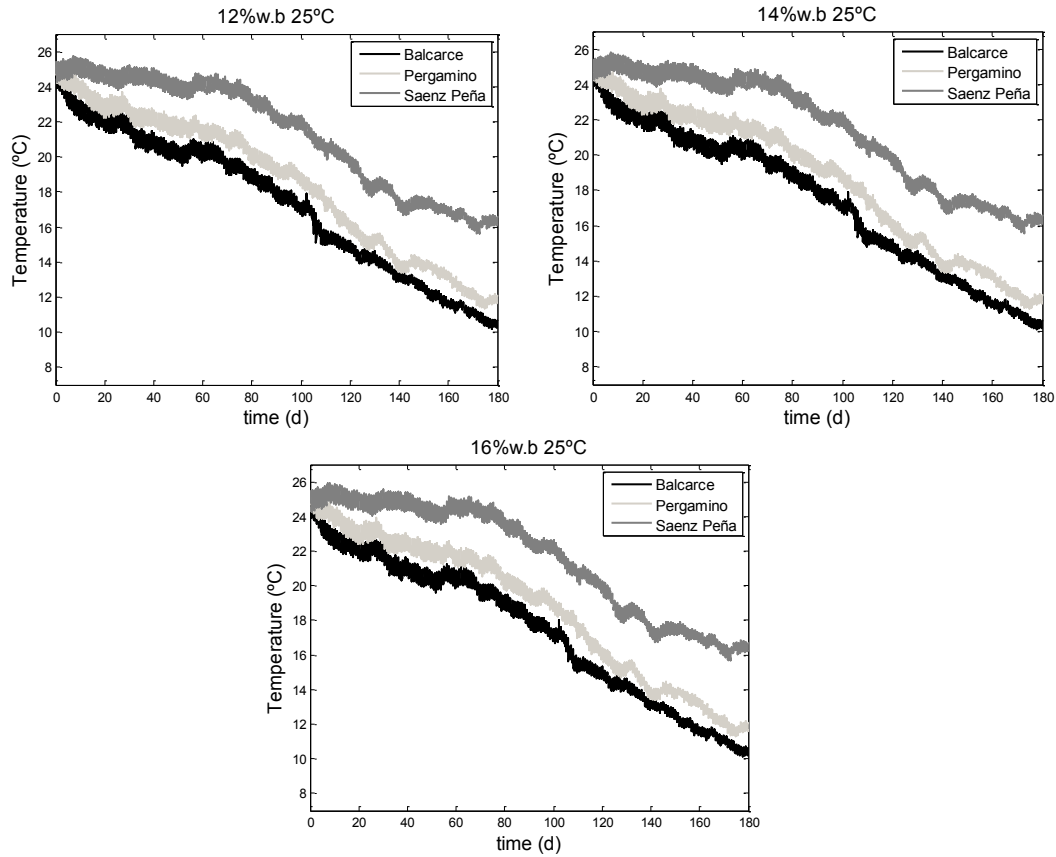
Fig. 5- Grain mean temperature evolution at Saenz Peña, Pergamino and Balcarce. Initial temperature 25ºC
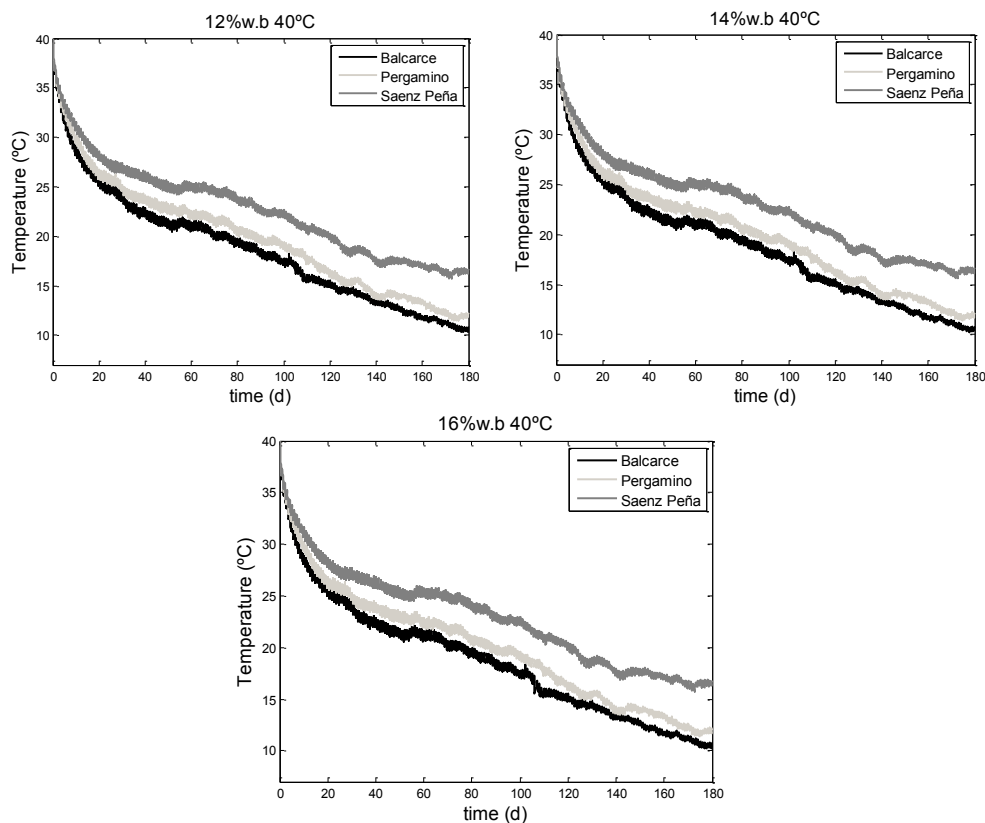
Fig. 6- Grain mean temperature evolution at Saenz Peña, Pergamino and Balcarce. Initial temperature 40ºC

Results showed that referential levels of O_2 and CO_2 strongly depend on initial moisture content and bagging grain temperature. Also, agricultural climatic condition produces significant changes, especially for dry and slightly moist grain. A difference of about 7 to 8ºC in mean ambient temperature between the northern (Saenz Peña) and southern location (Balcarce) shifts referential levels about 2%V/V points for 12% w.b. and about 4% V/V points for 14% w.b. At the three locations, O_2 depleted to less than 1% for 16% w.b.

Evolution of grain mean temperature in combination with CO_2 and O_2 levels achieved in the silo-bags demonstrate that for the climatic conditions of Argentina insect activity would be limited in silo-bags.

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AIR-TIGHTNESS LEVEL IN HERMETIC PLASTIC BAGS (SILO-BAGS) FOR DIFFERENT STORAGE CONDITIONS

Leandro Cardoso [1], Ricardo Bartosik [1*], Carlos Campabadal[2], Diego De La Torre [1]

[1]National Institute of Agricultural Technologies, Rute 226 km 73,5, Balcarce, (7620) Argentina
[2]International Grains Program Kansas State University, 1980 Kimball Ave, 102 IGP Bld, Manhattan, KS, (66503) US
*Corresponding author's e-mail: *rbartosik@balcarce.inta.gov.ar*

ABSTRACT

The silo-bag is a hermetic storage system used in more than 50 countries. As in any other hermetic storage system, achieving and maintaining a high hermeticity level is a key factor for successful storage.

The air-tightness of the bag can be affected by improper sealing of the end of the bag and perforations in the plastic cover. The evaluation of different sealing techniques and management practices and its effect on the air-tightness level is important for making recommendations for the appropriate use of the silo-bag system.

The objective of this study was to perform a pressure decay test to determine the initial air-tightness level of the silo-bag and its evolution after four months of storage in the field.

The pressure decay test was performed in 23 newly made commercial bags holding approximately 200 tonnes of grain each and was repeated in 13 of these bags after 4 months of storage in the field. In addition to the pressure decay test, soil conditions (bag setup on enhanced soil or setup over residues of the crop), sealing system and presence of ruptures were recorded at the beginning and after 4 months of storage.

In general, a low proportion of silo-bag achieved a good initial half pressure decay time (less than 35%), and in 75% of the bags the half pressure decay time decreased after 4 months of storage. Good sealing practices (especially thermo-sealing) and good soil preparation and good care of the plastic bag correlated with higher hermeticity level. Since the visual inspection is not satisfactory for determining the air-tightness level of a given silo-bag, it is recommended to perform a pressure test before a fumigation, controlled or modified atmosphere treatment.

Key words: modified atmospheres, controlled atmospheres, fumigants application, half pressure decay, pressure drop test, sealing system, ruptures.

INTRODUCTION

The silo-bag is a hermetic storage system used in more than 50 countries. In Argentina 40 million tonnes of grain were stored in silo-bags in 2011 (Bartosik, 2012). Most of the grain stored in silo-bags belongs to farmers. However, the commercial grain industry and grain elevators have shown a growing interest towards this storage system. Only about a 20% of

the grain was stored in silo-bags in 2006 (Vicini, 2006) but that proportion has markedly increased from that year.

Each silo-bag is 60 meters long and 2.8 meters in diameter with a plastic cover made of three layers (white outside and black inside) of 235 micrometers thickness (Cardoso et, al., 2008), which has an approximate storage capacity of 180 tonnes of soybean and corn.

The air-tightness created by the plastic of the silo-bag prevents the normal replenishment of oxygen (O_2), a gas used during the aerobic respiration of the biotic components of the bulk (fungi, insects and grain). The plastic also allows the retention of carbon dioxide (CO_2), a by-product gas generated during the respiratory process (Cardoso et al., 2008).

As in any other hermetic storage system, achieving and maintaining proper levels of air-tightness is a key factor for successful storage. Indeed, achieving high levels or air-tightness is critical for effective application of modified atmospheres, controlled atmospheres (Navarro, 1998), and control of biological activity (Darby and Caddick, 2007).

The air-tightness of the silo-bag can be negatively affected by improper sealing of the end of silo-bag and perforations in the plastic cover (Cardoso et al., 2010; Bartosik, 2012). The evaluation of different sealing techniques and management practices and their effects on the air-tightness level is important for making recommendations for the appropriate use of the silo-bag system.

The use of constant pressure test (or pressure drop test) is cited by Navarro (1998) as a valid methodology for determining the level of air-tightness of a storage structure. Darby and Caddick (2007) indicate that the pressure drop test (PDT) is relatively simple and fast.

The objective of this study was to perform a PDT to determine the initial air-tightness level of the silo-bag and its evolution after four months of storage in the field.

MATERIALS AND METHODS

The experiments were carried out in different farms and grain elevators of Balcarce area (Buenos Aires province, Argentine) and Manhattan area (Kansas, USA). The PDT was performed in 23 newly made commercial silo-bags holding approximately 200 tonnes of grain each, and was repeated in 13 of these bags after 4 months of storage in the field.

The procedure for performing the PDT consisted in generating 1200 Pa of negative pressure in the silo-bag. A PVC tube of 50 mm diameter and 1.6 m long was inserted in the silo-bag, close to the center (Figure 1). The inserted end of the tube has a sharp tip made of wood for allowing the penetration of the tube in the grain mass. Several small perforations were made in the tube for allowing the air passage. The other end of the tube came out from the bag and it was used to connect the bag to a vacuum generator connected by a flexible hose. In between the perforated insertion tube and the flexible hose a PVC closure valve was installed.

The vacuum generator in Balcarce was a centrifugal fan (Chicago Blower, 0.33 HP); in Manhattan a Dual Seal Vacuum Pump (General Electric, 1/2 HP) was used. The suction tube was sealed with tape and silicon sealant. The vacuum generator was turned on to generate -1200 Pa, measured with a digital manometer (Sper Scientific, China), and a water column manometer.

The valve was closed at -1200 Pa, or when pressure was constant at values lower than -1200 Pa for 10 minutes, when bags leaked. Time was recorded until pressure dropped to -100 Pa. Soil conditions (i.e., bag setup on enhanced soil or setup over residues of the previous

crop), sealing system and presence of ruptures were recorded at the beginning and every 15 days during storage.
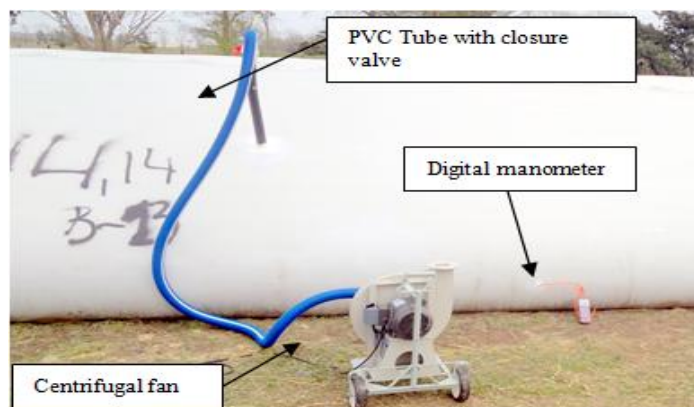

Fig. 1- Instruments used to carry out the pressure test in Balcarce.

From visual observations, two classifications for the bag closing quality were: 1) good sealing system (GSS) or 2) poor sealing system (PSS). Also, two levels of visual classifications regarding rupture risk of bag surface conditions were established: 1) low risk of rupture (LRR) and 2) moderate-high risk of rupture (HRR).

RESULTS AND DISCUSSION

Figure 2 shows silo-bags with good conditions regarding sealing (GSS) and ruptures (LRR) at the beginning of storage. Except for B8, all silo-bags in Fig. 2 were located in grain elevators. Silo-bags with poor sealing (PSS) and / or problems in their plastic cover (HRR) presented in Figure 3 were located on farms. One reason for better conditions at elevators is they dedicate the same site for silo-bags every year, which allows for better soil conditions (leveled, clean, etc) and other practices that favor silo-bag hermeticity. Also, the bag filling operator has better closure technology (i.e., closure tables or thermo-sealing). Contrastingly, when grain bagging is made on farms during harvest, silo-bags are usually placed on land with previous crop residues (sometimes it can be triturated, as in B8 and B16) or on land with flooding risk. Additionally, the sealing operation is implemented without proper care. Thus, simultaneous harvesting and bagging on farms result in more improvised locations and sealing of silo-bags than in grain elevators.

Figures 2 and 3 show that approximately 43% of silo-bags tested reached -1200 Pa during PDT; 63% of silo-bags which reached -1200 Pa were in GSS and LRR conditions (Figure 2). This clearly indicates the positive effect that good sealing techniques, proper soil conditions, and maintenance of the physical structure of the silo-bag have over its air-tightness level.

Darby and Caddick (2007) performed PDT in silo-bags, with range of half pressure decay (HPD) from -1200 to -600 Pa, indicating that a HPD of 5 minutes implies high level air tightness, a HPD of 3 to 5 minutes allows successful fumigation, and a HPD of less than 3 minutes indicates a poor air tightness level. Figure 2 shows that 4 of 11 silo-bags (36%) of the present study rated GSS and LRR, had a HPD (-1200 to -600 Pa) equal to or greater than 5 minutes (4 bags held 600 Pa after 300 seconds), and 5 out of 11 silo-bags (45%) had a HPD of

approximate 3 minutes or better (5 bags held 600 Pa after 180 seconds). The rest of the silo-bag in this condition failed the HPD time required for performing a successful fumigation. Darby and Caddick (2007) reported that some recently made silo-bags with no visual evidence of damage or bad sealing had an unsuccessful PDT. This was due to perforations in the bottom of the silo-bag during the bagging operation. A similar conclusion was made from the present study.
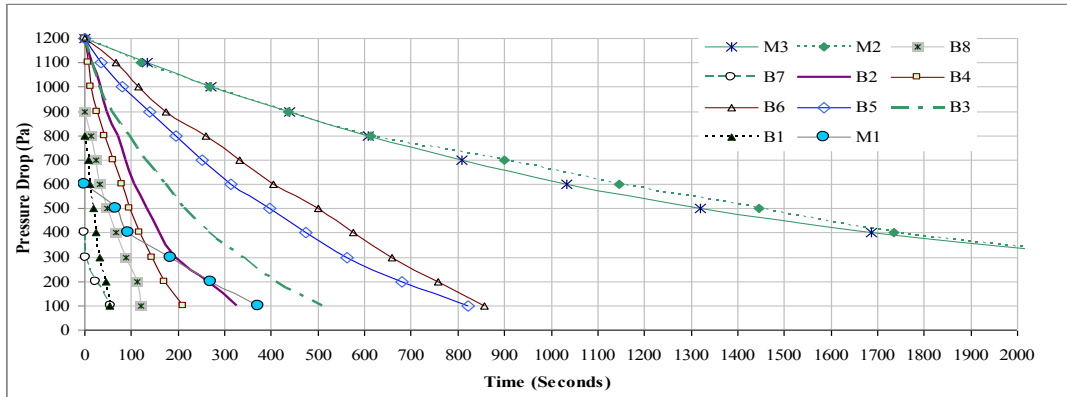

Fig. 2- Pressure drop (Pa) over time (seconds) for silo-bags (M = Manhattan, B = Balcarce) good sealing system (GSS) and low risk of rupture (LRR) at beginning of storage.
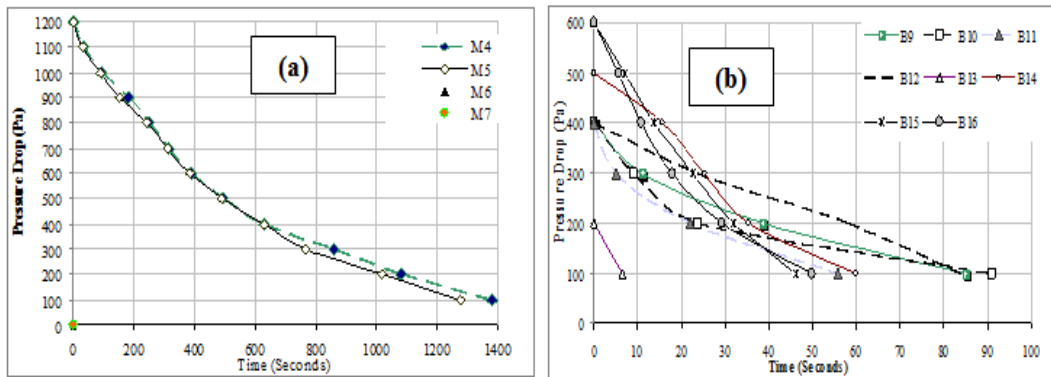
Fig. 3- Pressure drop (Pa) over time (seconds) for silo-bags (M = Manhattan, B = Balcarce) with moderate-high rupture risk (HRR) and: good sealing conditions (a) or poor sealing conditions (b).

Figure 3b shows that no silo-bags rated as PSS reached -1200 Pa. This indicates that closure system is critical for achieving high airtight levels. Figure 3a shows that 50% of silo-bags with GSS reached a PDT of 5 minutes or more, indicating that good sealing system produces a high airtight level. But, the other half did not reach -100 Pa.

Navarro (1998) proposed lower pressures; he performed PDT with HPD from -250 to -125 Pa. His HPD recommendations was that flexible structures of less than 500 m^3 (a silo-bag

is 260-270 m^3) require a minimum of 1.5 minutes for efficient use of fumigants, 3 minutes for controlled atmospheres systems and 5 minutes for modified atmosphere systems, including hermetic storage. According to these HPD thresholds, 3 out of 23 (13%) silo-bags classified as an airtight storage system (HPD pressure range of -200 to -100 Pa). Four silo-bags (17%) could be used for controlled atmosphere (HPD larger than 3 minutes), and 8 silo-bags (35%) could be used for successful fumigation (HPD larger than 1.5 minutes). This implies that the typical conditions of silo-bag storage have restrictions on the use of fumigants and, even more, controlled and modified atmospheres. However, there are precedents of successful fumigation (Cardoso et, al., 2009; Ridley et, al., 2011) and even implementation of controlled atmospheres in silo-bags (Milanesio, 2010) at the farm level when GSS and LRR conditions are achieved.

The range of pressure drop considered for computing the HPD test could cause differences in the characterization of the air-tightness level of the silo-bag. For instance, in silo-bag B5 (figure 2) the PDT was of 5:23 minutes when a HPD range of -1200 to -600 Pa was considered, and of 2:20 minutes for a -200 to -100 Pa HPD range. In general, when a lower range of pressure drop is used, HPD time is lower. The variation of HPD time according to the pressure range is more significant in those bags with high HPD. An over or underestimation of the air-tightness level of the silo-bag could be made according to the range of HPD test implemented..

Figure 4 shows that in 75% of the silo-bags, HPD time, and thus hermeticity, decreases after 4 months of storage (study only includes Balcarce silo-bags). Some silo-bags could not achieve the target pressure of -200 Pa. Darby and Caddick (2007) reported that maintaining tightness in silo-bags required continuous maintenance. In most cases, the loss of tightness was related to ruptures caused by animal (foxes, dogs, birds, rodents, etc) on the plastic covers. These problems are more common at the farm level than at the grain elevators. The use of electric fences to keep animals out of the silo-bag area was a suitable solution. In some silo-bags, HPD decreased substantially but no evidence of bag damage was observed. Surprisingly, the hermeticity of one silo-bag at the end of storage was greater than at the beginning. This is explained by the development of vegetation under the silo-bag that sealed ruptures in the bag floor, increasing the air-tightness.

Since visual inspection is not satisfactory for determining air-tightness of a given silo-bag, it is recommended that HDP tests be made before a fumigation or controlled or modified atmosphere treatment.
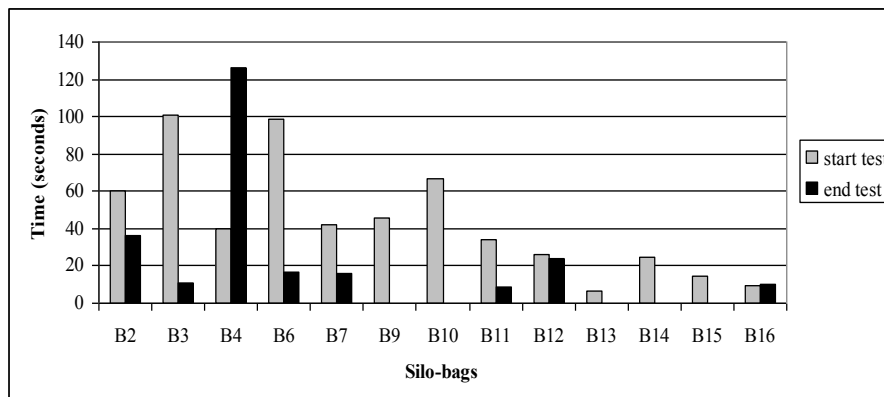

Fig. 4- Time (seconds) of half pressure drop (from 200-100 Pa) for 13 silo-bags in start an end test.

Results from Figure 5 indicate that any fumigation, controlled or modified atmosphere treatment will have better success at the beginning of storage than after several months. Since the plastic cover of the silo-bag prevents the entrance of insects, once the bag was treated, few chances of re infestation should be expected.

CONCLUSIONS

Pressure decay test were performed in several silo-bags, with the results related to the sealing and plastic cover conditions.

The pressure decay test was simple and its implementation was not problematic, either at the elevator or farm.

In general, a low proportion of silo-bag achieved a good initial half pressure decay (HPD) time (less than 35%); in 75% of the bags the HPD time decreased after 4 months of storage.

Good sealing practices (especially thermo sealing) and good soil preparation and good care of the plastic bag correlated with higher hermeticity level.

The pressure range at which the HPD test is performed affected the test result.

Fumigation, controlled or modified atmosphere treatments will have more chances to be successful at the beginning of storage than after several months.

Since the visual inspection is not satisfactory for determining the air tightness level of a given silo-bag, it is recommended to perform an HDP test before a fumigation, controlled or modified atmosphere treatment.

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MILL PRESSURIZATION TEST QUANTIFIES FUMIGANT LEAKAGE RATES DURING SULFURYL FLUORIDE AND METHYL BROMIDE FUMIGATION OF COMMERCIAL FLOUR MILLS

Johnselvakumar Lawrence[1], Dirk E. Maier*[,1], Bhadriraju Subramanyam[1], and Watcharapol Chayaprasert[1,2]

[1]Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas 66506, USA
[2]Department of Agricultural Engineering, Faculty of Engineering at Kamphaengsaen, Kasetsart University, Nakhon Pathom 73140, Thailand
Corresponding author's e-mail: *dmaier@k-state.edu*

ABSTRACT

During six fumigation trials in five commercial flour mills building pressurization tests were conducted after sealing by professional fumigators to quantify sealing quality or gas tightness of mills. The equivalent leakage areas (ELAs) of the mills were determined based on the pressurization test results. In order to account for the size differences of the mills, specific ELAs were calculated by dividing the ELAs by the corresponding mill volumes. The specific ELAs ranged from 0.041 to 0.108 cm^2/m^3. The half loss times (HLTs) determined from average fumigant concentration readings were between 2.88 and 47.48 h. In general, the fumigation trials in the structures that had lower specific ELAs yielded longer HLTs. These results suggested that specific ELAs can be used for quantification of sealing quality prior to structural fumigation and for predicting HLTs.

Key words: Flour mills, sulfuryl fumigation, sealing quality, pressurization test, gas tightness, half loss time, fumigation efficacy

INTRODUCTION

The concept of precision fumigation, especially with sulfuryl fluoride is designed to optimize the amount of fumigant usage based on gas tightness of a structure quantified by half loss time (HLT), species and stage of stored product insect to be controlled, and temperature of the structure being fumigated, among other things. HLT for a structure is assumed when sulfuryl fluoride is used for the very first time, because of lack of gas monitoring data that shows actual gas leakage rates from the structure. It is well known that gas leakage rates during structural fumigation depend on the sealing quality and prevailing weather conditions. Chayaprasert et al. (2012) have shown that for a pilot mill at Kansas State University (9628 m^3) treated with methyl bromide and sulfuryl fluoride, HLTs were inversely related only to wind speed and not any other weather conditions measured. The work of Chayaprasert et al. (2012) was based on a building pressurization test after sealing prior to each of three fumigations with methyl bromide and three with sulfuryl fluoride. This and other seminal work by Chayaprasert (2007), Chayaprasert et al. (2008) and Chayaprasert and Maier (2010)

showed that a simple building pressurization test can be used to gauge gas tightness of a structure based on computational fluid dynamics models and assumed weather conditions. These model simulations have been validated with limited field trials in commercial facilities. In commercial facilities, factors in addition to wind speed may also influence sulfuryl gas leakage rates. The present investigation was designed to provide additional field validation data to determine if building pressurization tests can be used to accurately assess sealing quality based on six fumigations in five commercial flour mills. The goal was to explore if building pressurization test can be generally used as a quantitative tool for measuring gas tightness of commercial flour mills prior to fumigation.

MATERIALS AND METHODS

Six fumigation trials were conducted in five different flour mill structures (Mills 1, 2, 3, 4, and 5) located in Indiana, Kansas, and Montana, USA. Details of the mill fumigations are given in Table 1. The volumes of the mill facilities ranged from 8,495 to 28,317 m^3. Mill 1A and Mill 1C were two buildings constructed adjacent to each other and were fumigated on the same day. Mill 2 was fumigated twice within a four month period. Sulfuryl fluoride (SF) was used in the first five fumigation trials. Methyl bromide (MB) was used to fumigate Mill 4 in the sixth trial. Due to high leakage rates, additional gas had to be introduced during fumigation.

Table 1. Mill volumes and fumigation details

Fumigation details	Fumigation trial					
	1	2	3	4	5	6
Mill ID	1A	1C	2	2	3	4
Mill volume (m^3)	8,495	9,911	28,317	28,317	13,592	14,158
Number of mill floors	5	4	6	6	7	7
Mill location	Indiana	Indiana	Indiana	Indiana	Kansas	Montana
Fumigation dates in 2011	Apr 22-23	Apr 22-23	May 28-29	Sep 3-4	Sep 4-5	Sep 17-18
Exposure time (h)	23	23	24	23.5	23.5	24
Fumigant	SF	SF	SF	SF	SF	MB
Initial fumigant amount (kg)	624	510	907	907	567	295
Top-up amount (kg)[a]	397	284	113	113	0	68
No. gas monitoring points	5	4	6	4	7	7

[a]Additional gas introduced during fumigation.

Ambient conditions inside the fumigated mill were recorded every 10 min using HOBO[®] H8 temperature loggers (Onset Computer Corporation, Bourne, Massachusetts, USA)). The outside weather conditions were recorded using a HOBO[®] U30 weather station. Five temperature loggers were installed at 1.52 m above each floor of the mill. The weather station was installed on the roof of the mill. The weather station monitored ambient temperature and relative humidity, barometric pressure, solar radiation, and wind speed and direction. However, only the wind speed and ambient temperature data were incorporated in the analysis of this study. A number of 4.3 mm inner diameter nylon tubes were placed in the mill for monitoring fumigant gas concentrations over time. Generally one tube was placed on each floor to measure gas concentrations at a height of 0.91 m. Fumigation concentrations

were recorded manually every hour throughout the exposure time. The Spectros Instruments Single Point Monitor (Spectros Instruments, Hopedale, Massachusetts, USA) was used for measuring gas concentrations in fumigations 1 to 4 and the Fumiscope (Key Chemical and Equipment, Clearwater, Florida, USA) was used in fumigations 5 and 6.

Prior to fumigant release after sealing the structure, a building pressure test was conducted to quantify sealing quality using the E3 blower door fan setup (Infiltec, Waynesboro, Virginia, USA) installed at an exit door. The mills were pressurized between 5 and 80 Pa depending upon their size and prevailing weather conditions. The airflow rate through the fan and the pressure differences between inside and outside of the mills were recorded. These values were used to quantitatively determine gas tightness of the mills using procedures described in Chayaprasert et al. (2012).

The pressurization test data were used to calculate the equivalent leakage areas (ELA), A_L (cm^2), of the mill structures. These ELAs quantitatively indicated the gas tightness of the structures. First, for each mill structure, the correlation between the airflow rates, Q (m^3/s), through the blower door fan and the pressure raises, p (Pa), in the structure was determined using Eq. 1 (ASHRAE, 2001):

$$Q = bp^n \tag{1}$$

where b is the flow coefficient and n is a dimensionless pressure exponent. The ELA was then calculated using Eq. 2 (ASHRAE, 2001):

$$A_L = 10,000 Q_r \frac{\sqrt{\rho / 2p_r}}{C_D} \tag{2}$$

where Q_r is the predicted airflow rate (m^3/s) at the reference pressure difference, p_r (Pa). The predicted airflow rate was calculated using Eq. 1 by assuming $p_r = 10$ Pa. The discharge coefficient, C_D, and the air density, ρ (kg/m^3), were assumed equal to 1 and 1.15, respectively.

To calculate the HLT the recorded gas concentration data from all monitoring points were averaged, yielding one gas concentration curve for each fumigation trial. The average data points where concentrations were increasing due to gas releases were discarded, resulting in sections of decreasing concentration curves. These sections of decreasing concentration curves were then fitted to the first-order kinetic equation (Eq. 3) (Cryer, 2008; Chayaprasert et al., 2008):

$$C_t = \frac{C_i}{2^{\frac{t}{HLT}}} \tag{3}$$

where, C_t is the current concentration (g/m^3) at the elapsed time t (h) and C_i is the initial concentration (g/m^3).

RESULTS AND DISCUSSION

The pressure difference and airflow rate relationships from the pressurization tests are shown in Fig. 1A and Table 2.

The steeper curves imply that higher airflow rates were needed to produce the same levels of pressure increase in the flour mills. These curves can be used for comparisons of gas tightness between the flour mills after correcting data for differences in mill sizes. The average fumigant concentration curves during all six fumigation trials are shown in Figs. 1B to 1D. The slopes of the concentration curves from fumigations 1 and 2 were steeper than

those of the other curves (Fig. 1B), implying shorter HLTs. On the other hand, the somewhat flat concentration curves of fumigations 3 and 4 in Mill 2 (Fig. 1C) suggested longer HLTs.

Table 2. Pressurization test results, average ambient conditions, and HLTs

Trial	b	n	A_L (cm^2)	A_L/V (cm^2/m^3)	Temp difference Mean $\pm$ SD (°C)	Wind speed Mean $\pm$ SD (m/s)	HLT (h)
1	0.077	0.697	919	0.108	4.67 $\pm$ 2.37	3.04 $\pm$ 1.15	3.19-4.72
2	0.058	0.672	653	0.066	3.15 $\pm$ 2.03	3.04 $\pm$ 1.15	2.88-9.19
3	----[a]	----[a]	----[a]	----[a]	9.37 $\pm$ 2.07	5.34 $\pm$ 1.43	47.48
4	0.049	0.990	1148	0.041	11.43 $\pm$ 5.34	3.56 $\pm$ 2.06	19.42-23.66
5	0.140	0.478	1009	0.074	12.81 $\pm$ 4.23	4.30 $\pm$ 1.27	10.15
6	0.099	0.692	1168	0.083	10.98 $\pm$ 3.59	3.74 $\pm$ 1.92	3.98-5.25

[a]Invalid pressurization test data.

This was actually the case as indicated by the calculated HLTs (Table 2). In fumigation 3, due to insufficient flow rate capacity of the blower door fan the pressure test could not be performed successfully. Except for fumigation 3, the ELAs (i.e., A_L) were between 653 and 1,168 cm^2. In order to account for the size differences of the mills, the specific ELAs (i.e., A_L/V) were calculated by dividing the ELAs by the corresponding mill volumes, V (m^3) (Table 1). A lower ELA value indicated higher gas tightness level. Based on the ELAs, the order of gas tightness levels, from the most to the least gas tight structures, were Mill 2 (fumigation 4), Mill 1C, Mill 3, Mill 4 and Mill 1A. The structures that had the longest to the shortest HLT values were Mill 2 (fumigation 3), Mill 2 (fumigation 4), Mill 3, Mill 4, Mill 1A and Mill 1C. Although the weather conditions during all fumigations were different, the similarity between the orders of the specific ELAs and the HLTs suggested that the specific ELA could be used for quantification of sealing quality prior to fumigation. The primary advantage of ELA is that the ELA of a structure can be determined before the fumigation while the HLT could not be calculated until some fumigant concentration readings are obtained. The air infiltration rate, q (m^3/s), into a building can be estimated based on the building's ELA, temperature difference between the inside and outside of the building, ΔT (°C), and prevailing wind speed, U (m/s) (ASHRAE, 2001):

$$q = \frac{A_L}{1,000} \sqrt{c_s \Delta T + c_w U^2} \tag{4}$$

where c_s and c_w are the stack and wind coefficients, respectively. Furthermore, HLT and the infiltration rate are related (Banks et al., 1983; Chayaprasert, 2007):

$$HLT = \frac{V}{q} \frac{\ln(2)}{3600} \tag{5}$$

where V is the volume of the building (m^3). Using Eq. 4 and 5, the HLT of a fumigant in a structure can be predicted in advance. Accurate prediction of HLT would help to optimize the amount of fumigant usage which is one of the focuses of the precision fumigation concept. However, the present study lacks proper replicate fumigations of the same structure under varying environmental conditions. The stack and wind coefficients are different for different

structures. The local wind and temperature conditions should be measured to use concepts described in this paper. The financial value of the pressurization test should be justified. In fumigation trials reported here structures that had lower specific ELAs yielded longer HLTs. These results suggested that specific HLTs and ELAs can be used for quantification of sealing quality prior to structural fumigation.
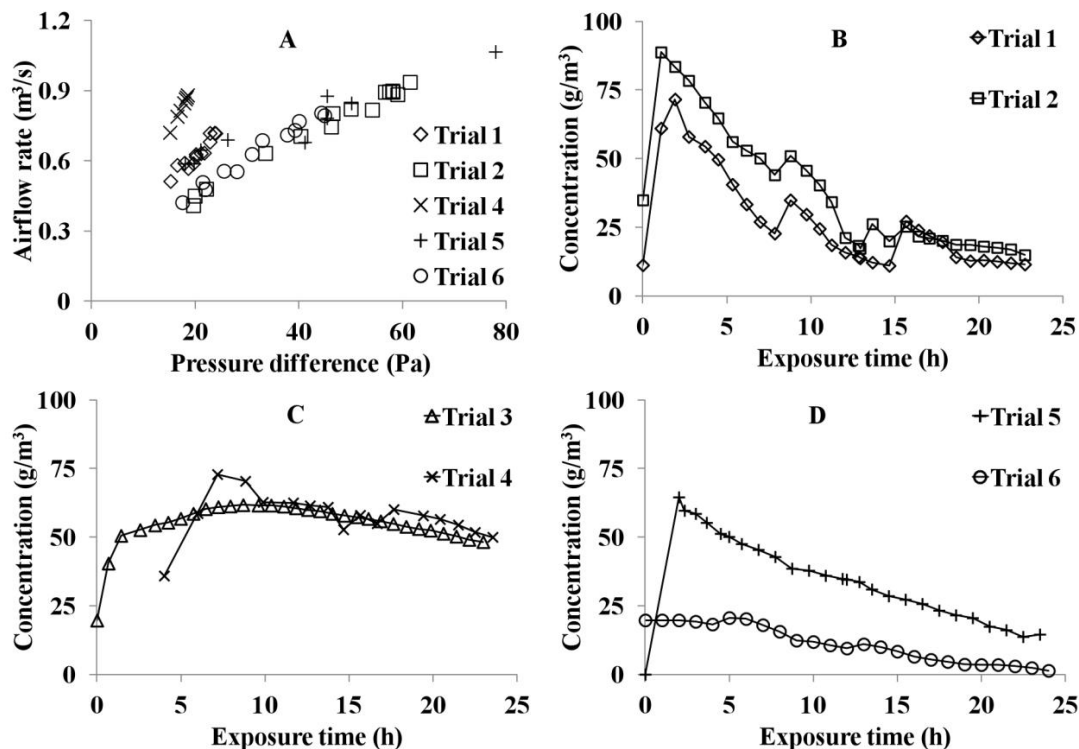

Fig. 1- (A) Pressure difference-airflow rate relationships from the pressurization tests. (B) Average gas concentrations during fumigations 1 and 2. (C) Average gas concentrations during fumigations 3 and 4. (D) Average gas concentrations during fumigations 5 and 6.

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AUTOMATIC ONLINE PHOSPHINE MONITORING SYSTEM FOR FUMIGATION

R. C. Naik[*] and R. D. Shroff

Uniphos Envirotronic Pvt. Ltd., A United Phosphorus Group Company, India.
[*]Corresponding author's e-mail: *naikrc@uniphos.com*

ABSTRACT

Fumigation is one of the most important procedures followed all over the world for infestation control of stored products. Though there are several fumigants used for the purpose, phosphine is one of the most widely used fumigants for stored products. The present paper describes a fully automatic online fumigation monitoring system for phosphine which eliminates the need of an operator to make periodic measurements during fumigation lasting over several days. The online monitoring system consists of i) A sampling line having a set of solenoid valves and manifold ii) a microprocessor based control unit housing the sample draw pump and sensor and iii) a GSM modem for data transmission.

The instrument is programmed in the beginning by setting all the relevant parameters such as sampling time, purge time, sleep time between two cycles and the total number of cycles of measurement. Once it is programmed and the fumigation started, the instrument sequentially draws sample gas to the sensor from four different locations of a silo and the measured concentration data is stored on the control unit. After one cycle of operation, the sensor is purged with fresh air and the instrument goes into sleep mode till the next cycle starts after the preset period. The measurement cycle repeats till the end of fumigation. The instrument has data-logging facility which stores data with ID-tag. The stored data can be transferred to a computer via Bluetooth or to a GSM modem through RS-232 communication. GSM modem can send the data to a pre-configured mobile as an SMS. It can also send this data to pre-configured email IDs or can upload this data on a chosen FTP server. This server data can further be accessed by a user through web-application.

Key words: Fumigation, Fumigant, Phosphine, Aluminium Phosphide, Silo, Infestation Control.

INTRODUCTION

Phosphine remains the fumigant of choice for all the bulk storage operators and grain growers because of its effectiveness and environmental suitability. It also scores over other fumigants on many aspects including the ease of application, low cost, less residue problem and least effects on treated food commodities. Fumigation is generally carried out by covering the commodities with a leak proof enclosure and applying phosphine gas from phosphine cylinders or using appropriate amounts of ALP as a source of phosphine. In either case,

phosphine concentration of 500-2000 ppm is used. However to keep a watch on the concentration- time profile as a quality control procedure during fumigation, phosphine concentration has to be measured periodically during the entire period of fumigation lasting several days.

Ideally an online monitoring system should be the right choice. But because of the high fumigant concentration involved in the fumigation process, the sensors, particularly for phosphine, do not last long. The present online monitoring system described below and meant for phosphine monitoring overcomes the problem of sensor mortality by exposing the sensor to phosphine only during measurement and keeping it in ambient air during idle time. The smart sensor used in this instrument can be easily replaced when it fails by another smart sensor already calibrated at the factory.

DESCRIPTION

The automatic online fumigation monitoring system for phosphine described in this paper mainly consists of two parts - (i) a central processing module and (ii) a sampling line. Fig.1 shows the block diagram of the complete automatic fumigation monitoring system. The central processor module has the microcontroller which forms the heart of the entire monitoring system. It also has a signal conditioning unit, a digital display, a keyboard, a power supply and RS 232 serial communication port for data communication to PC.
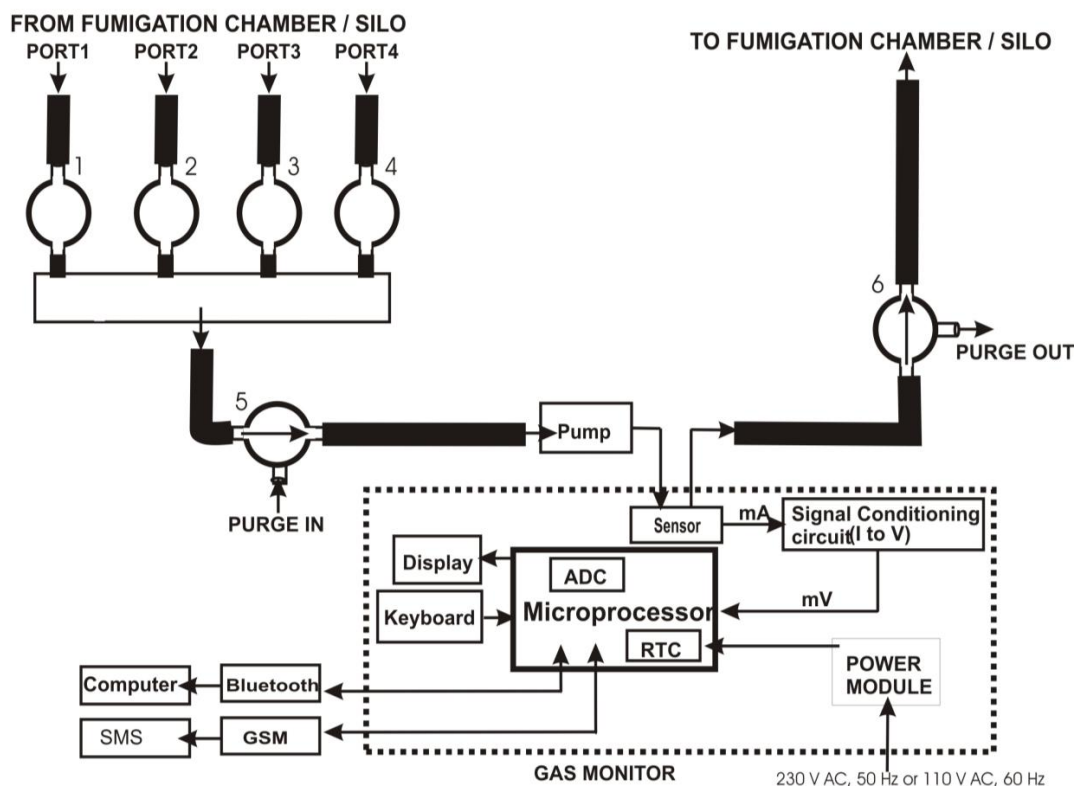
Fig. 1- Block diagram of automatic online fumigation system.

The sampling line consists of a set of solenoid valves, a manifold and sample draw pump connected together as shown in Fig.1. As per the set of instructions from microcontroller, the sampling line can draw air sample from four different locations and bring the sample air to the smart sensor. The smart sensor is designed in such a way that, if the sensor fails, it can be easily replaced at the fumigation site by plugging in another smart sensor which is factory calibrated. The pluggable smart sensor module has an electrochemical sensor for the detection of phosphine in the range of 0-2000 ppm.

Being a microprocessor based unit, the instrument has data-logging facility which stores data with ID-tag. The stored data can be transferred to a computer via Bluetooth or it can be transferred to a GSM modem through RS-232 communication. GSM modem can send the data to a pre-configured mobile as a text message (SMS). It can also send this data to any pre-configured email IDs or can upload on a chosen FTP server. The data on the server can further be accessed by a user, through web-application.

OPERATION & CALIBRATION

The instrument has several modes of operation such as sampling mode, parameter setting mode, calibration mode etc. Navigation between one mode to another and setting the required parameters can be done using four membrane keys provided on the monitor. For making measurement, the relevant parameters like sampling time, purge time, interval between two cycles etc. are to be set by entering into parameter setting mode. Once the required parameters are set, measurement can be started by entering into the sampling mode. The instrument has six solenoid valves out of which, four (1-4) are two way valves and the remaining two are 3 way valves (5&6). When the sampling starts, the instrument is first purged with air for 30/60 sec during which all the 2 way valves (1-4) will be closed and valves 5&6 will remain open to free air. After purging, the sample gas from port 1 is drawn for measurement which passes over the sensor and is sent back into the silo. During this time the valves 1, 5 and 6 are all open to allow the gas to flow from the silo to the sensor and back to the silo. The sampling from port 1 continues for the specified time and changes over to the next port automatically by closing valve 1 and opening the second valve. The gas concentration is read and stored at the end of sampling time and displayed on the monitor. Once sampling from all the four ports is completed, the instrument again enters the purging mode. During this operation, valve 5 is open to air and valve 6 is kept open to the silo for 20 seconds and then to free air to purge the sensor fully with fresh air. This completes one measurement cycle and the instrument goes into sleep mode automatically. The second and subsequent cycles starts at pre determined times.

The calibration mode can be accessed by pressing a single key in the main menu. The calibration of the instrument involves two steps- (i) Zeroing the instrument in clean air and (ii) Setting the span gas value and applying the gas of known concentration (equal to the set span gas value). If there is a mismatch between the set span gas value and the calibration gas concentration, the gain is to be adjusted such that the reading on the monitor matches with the calibration gas concentration.

CONCLUSION

During phosphine fumigation in closed enclosures, monitoring of phosphine concentration over the entire period of fumigation is necessary. Ideally, a fully automatic online monitoring system which can periodically monitor phosphine concentration, store the measured data in its

memory and also transmit it to any remote location is desirable. The present instrument fully meets that requirement and should be very useful to the fumigators.

SESSION 6

POSTERS

PHOSPHINE RECIRCULATION SYSTEM IN A METAL BIN

Gennady Zakladnoy[1]*, Marat Abdiushev[2], Mikhail Soskin[2]

[1] GNU VNIIZ Rosselhosakademii, Russia
[2] OAO Melinvest, Russia
*Corresponding author's e-mail: *vlaza@list.ru*

ABSTRACT

Distribution of phosphine generating tablets requires grain transfer from one bin to another one and also leaves undecomposed aluminium phosphide in grain mass. Phosphine recirculation systems (PRS) avoid the negative consequences of direct addition to grain. PRS consists of a special constructed phosphine generator, fan, pipework and groups of ventilation channels in the bin floor with perforated covers. One pipe connects outlet of fan to the inlet of the channels of ventilation system. The second pipe joins inlet of phosphine generator with the grain headspace. A metal bin was equipped with PRS. Rice (paddy, 1500 tonnes) was stored in the bin. Aluminum phosphide tablets equivalent to a dosage of 1.37 g phosphine per m3 were put into the phosphine generator. The recirculation fan was operated for 66 h, with another 2 h to vent the phosphine out of the grain mass. Tests showed the very uniform distribution of phosphine in different parts of the grain mass. Almost all adults of *Sitophilus granarius* L. (96.1%) and also preimaginal stages as hidden infestation in the grain (99.7%) were dead after fumigation.

Key words: phosphine, recirculation system, grain, metal bin, beetle.

INTRODUCTION

If grain is to be fumigated directly with phosphine-generating tablets, the grain must be moved from one bin in another one and tablets must be added into the grain stream. After such fumigation the grain mass contains remnants of the decomposition of tablets in which partly undecomposed aluminum phosphide is often present. It is not safe to work with such grain. The recirculation of phosphine through the grain mass avoids the costs for the movement of grain as well as eliminating hazardous residues of decomposition of tablets in the grain. In this demonstration of the technique, we decided to use a metal bin, a type that have recently been built in Russia in large numbers to store grain.

MATERIALS AND METHODS

Tests were carried out in a metal bin: volume 2919 m^3, diameter 13 m, height of wall 21 m and of the roof cone 3 m. The volume occupied by paddy rice, 1500 t, was 2653 m^3 and the volume of air between kernels was about 1592 m^3, giving a total air volume in the loaded bin of about 1858 m^3.

The metal bin was equipped with a special recirculation system which included: a specially constructed phosphine generator, fan, pipework and a group of ventilation channels in bin floor with perforated covers (see Fig. 1). A duct connected the outlet of the fan and inlet of the ventilation system. A second pipe joined the inlet of the phosphine generator with the bin headspace.

During the tests the temperature of the ambient air ranged from 1 - 6ºC during the daytime to –2 - 4ºC at night. The temperature of the grain ranged in different parts from 14 to 25ºC, average. approximately 19.3ºC. During the recirculation, the warm air from grain was moved through the phosphine generator, providing the temperature of the air inside the generator at the level of 12-13ºC.

Fig. 1- Metal bin equipped with phosphine recirculation system.

Tablets based on aluminum phosphide active ingredient were used as a source of phosphine. At the beginning the generator was charged with tablets at a dosage of 2.74 g m^{-3} (0.91 g m^{-3} phosphine). After 24 hours of exposure we added additional 1.37 g m^{-3} tablets (0.46 g m^{-3} phosphine), bringing the total dosage of tablets to 4.11 g m^{-3} (1.37 g m^{-3} phosphine).

Parameters of gas flows in the recirculation system were as follows: air flow 5758 m^3 h^{-1}, equivalent to 3.1 air changes per hour. The face velocity of air in the silo was 1.2 cm s^{-1}. After 66 h exposure, the remaining phosphine was vented over 2 h.

The assessment of the fumigation of grain was carried out on the distribution of phosphine gas in the recirculation system as well as on the mortality of adult and preimaginal stages of *Sitophilus granarius* L. in bioassays. Grain with preimaginal stages was incubated at 25 ºC and the emerged adults were assessed every week over 7 weeks.

RESULTS AND DISCUSSIONS

From the data shown in Table 1, it is evident that the recirculation system provided the ideal distribution of phosphine to the different parts of grain mass. The low phosphine concentrations after 18 h compared with the dosage applied (applied 0.91, observed 0.14 g m^{-3}) may be explained by high absorption by the paddy rice as well as leakage. Addition of further phosphine by the generator after 24 h maintained the gas concentration at the same level (0.14 g m^{-3}) during next 24 h.

After fumigation almost all adults of *S. granarius* (99.1%) were killed (see Table 2).

Table 1. The distribution of phosphine in a recirculation system

Measuring point	Phosphine concentration (mg m^{-3}) after							
	18 h	21 h	24 h	27 h	32 h	43 h	48 h	66 h
Grain, depth of 0.1 m, from the bin wall: 0.1 m	42	140	140	140	140	140	140	42
3.2 m	42	140	140	140	140	140	140	42
6.5 m	42	140	140	140	140	140	140	42
Grain, depth of 2.0 m, from the bin wall: 0.1 m	42	140	140	140	140	140	140	42
3.2 m	42	140	140	140	140	140	140	42
6.5 m	42	140	140	140	140	140	140	42
Inside the pipe in front of the generator	42	140	140	140	140	140	140	42

Table 2. The effectiveness of the fumigation of *S. granarius* adults

Measuring point	Mortality, %	
	Control	Fumigated
Grain, depth of 0.1 m, from the bin wall: 0.1 m	0	100
3.2 m	0	100
6.5 m	0	100
Grain, depth of 2.0 m, from the bin wall: 0.1 m	0	96.7
3.2 m	0	100
6.5 m	0	100
Near the roof of the bin	0	96.7
Average	0	96.1

The data on preimaginal stages as hidden infestation of the grain are given in Table 3. On average 452 beetles emerged from the kernels in the control bioassays over 92 days of the observations. A range of 0 to a maximum 4 beetles (average 1.6) hatched from the bioassays which were distributed in different parts of the grain mass under fumigation. This corresponds to a mortality of the preimaginal stages of 99.7%.

The test described here have shown that use of the method of recirculation of phosphine through the grain mass for the purpose of its disinfestation in metal bins is an effective method with good prospects.

Table 3. The effectiveness of the fumigation of preimaginal stages of *S. granarius*

Measuring point	The number of emerged adults		Mortality, %
	Control	Fumigated	
Grain, depth of 0.1 m, from the bin wall: 0.1 m	452	1	99.8
3.2 m	452	0	100
6.5 m	452	4	99.1
Grain, depth of 2.0 m, from the bin wall: 0.1 m	452	0	100
3.2 m	452	2	99.6
6.5 m	452	2	99.6
Near the roof of the bin	452	2	99.6
Average	452	1.6	99.7

SESSION 7

Resistance to fumigants and pest management strategies

Chairpersons:
Barry Bridgeman, Australia
Francis Fleurat-Lessard, France
Kutad Kurdoglu, Turkey

MANAGING RESISTANCE TO PHOSPHINE IN STORAGE PESTS: CHALLENGES AND OPPORTUNITIES.

Manoj K Nayak*

[1]Department of Agriculture, Fisheries and Forestry, EcoSciences Precinct, GPO Box 267, Brisbane, QLD 4001, Australia
[2]Cooperative Research Centre for National Plant Biosecurity, LPO Box 5012, Bruce, ACT 2617, Australia
*Corresponding author's e-mail:*manoj.nayak@daff.qld.gov.au*

ABSTRACT

Over several decades, two fumigants (methyl bromide and phosphine) have been used extensively to disinfest stored commodities across the world. In 1992, the landmark decision of the Montreal Agreement to phase out the fumigant methyl bromide by 2015 triggered research into development of alternative fumigants. During this period, industries have become highly dependent on phosphine, a unique fumigant with several positive attributes including its cheap price, versatility and ease in application and most importantly, its broad acceptance as a residue-free treatment. Over reliance on phosphine, however, has resulted in development of resistance in a range of stored product pest species. Several alternatives that have been developed so far (e.g. ethyl formate, ethanedinitrile, carbonyl sulphide and sulfuryl fluoride) cannot match the benefits offered by phosphine.

For these reasons, the focus has shifted to managing resistance to phosphine to ensure its future sustainability. This paper will present a critical appraisal of the challenges associated with resistance to phosphine and recent research advances in its management. An insight into the opportunities offered by the alternative fumigants and other pest management tactics towards alleviating phosphine resistance will also be discussed. The key components of a national phosphine resistance management program that has been adopted in Australia to combat resistance problems will be presented as a case study. This will include research highlights from the characterisation of strong-level of phosphine resistance in key pest species and rapid diagnosis for their detection; development of new phosphine fumigation protocols and the potential for using alternative fumigants such as sulfuryl fluoride as a 'phosphine resistance breaker'.

Key words: Fumigants, phosphine, stored product pests, resistance management, sulfuryl fluoride, eradication strategies.

1. INTRODUCTION

Disinfesting stored commodities including grain, dried fruits, nuts, cocoa, coffee, processed food and food products using fumigants is a practice spanning over a 100 years. According to Bond (1984) fumigants are chemicals which, at a required temperature and pressure, can exist in the gaseous state in sufficient concentration to be lethal to a given pest organism. Moreover, for its broad acceptance, apart from being economically affordable, an ideal

fumigant should also have minimal effect on environmental aspects, health of human beings, non-target organisms and structures. These particular properties required of fumigants have meant that among several chemicals developed over the years for fumigation purposes, relatively few chemicals have ever been considered suitable. Among them, methyl bromide and phosphine are the ones with the longest history of use with a high degree of effectiveness against a broad range of pests. Due to the steady increase in global concern about the effects of fumigants and chemicals on the environment, human and animal health, scrutiny of their use is becoming stricter. Unfortunately, industry is on the verge of losing methyl bromide due to its ozone-depleting nature and phosphine is threatened by the development of strong levels of resistance in key pest species. This paper will present a critical appraisal of the challenges and opportunities that have arisen from the major issue of resistance to phosphine in key pest species in view of the complete phase-out of methyl bromide in 2015. While examples will be provided from various parts of the world, the core examples on resistance management will be from Australia.

2. CHALLENGES

2.1. PHASE-OUT OF METHYL BROMIDE

Methyl bromide has been used as a fumigant for the 80 years since its insecticidal properties were first reported by Le Goupil (1932). The uniqueness of this fumigant has been its effective use against a broad range of pests across many fields of application including soil, durable and perishable commodities and structures (mills and buildings). Compared to other fumigants, methyl bromide delivers disinfestation quite rapidly, which enabled the industry to use it effectively in short-term fumigations, where a successful disinfestation is achieved within 24 h including the clearance of the gas. This aspect of methyl bromide has made it very attractive for industry, particularly for use in large storages in export terminals and shipping containers; where long-term fumigations are considered very expensive due to high demurrage costs involved in delaying of shipping. Successful control of stored product pests by methyl bromide is well documented (Estes, 1965; Howe and Hole, 1966; Bell and Glanville, 1973).

In the 1990s, several reports (SORG, 1990; WMO, 1990, 1992) have drawn attention to the ozone depleting properties of methyl bromide, and environmental organizations around the world, particularly in the U.S.A., have called for a rapid phasing out of the fumigant (Friends of the Earth, 1992). This movement culminated in the landmark decision under the Montreal Protocol on Substances that Deplete the Ozone Layer, which led to the gradual phase-out of methyl bromide from 1995 with exemptions for use for quarantine and pre-shipment purposes and for developing countries. While the developed nations have completely banned this fumigant in 2005 (with the exception of quarantine use), the worldwide phase-out of methyl bromide is scheduled to be completed in 2015 (Taylor, 1994); which will leave a major hole in the fumigant armoury. Moreover, it will put extra pressure on phosphine, which will be left as the only major fumigant for use by industry in disinfesting stored products across the globe.

2.2. INCREASE IN STRENGTH OF RESISTANCE TO PHOSPHINE

Like methyl bromide, phosphine as a fumigant has a history of nearly 80 years and its use across the globe in disinfestation of durable commodities has been well established (Taylor, 1989; Rajendran and Narasimhan, 1994; Zettler et al., 1989; Collins et al., 2005; Lorini et al., 2007). As expected with the use of any chemical treatment, development of resistance to

phosphine in several stored product pests has occurred over time and was first highlighted during the global survey for susceptibility to pesticides undertaken by the Food and Agriculture Organisation of the United Nations (Champ and Dyte, 1976). This survey detected resistance in stored product pests in 33 out of 82 countries and after this first survey; Chaudhry (2000) listed roughly eleven species of major stored product pests that had developed resistance to phosphine by 2000. In the last decade, however, new pests such as psocids have also been reported to have developed a high level of resistance to this fumigant (Nayak et al., 2003b). Overall, the situation has worsened during the last two decades in terms of both frequency and strength of resistance (Srivastava, 1980; Mills, 1983; Taylor, 1989; Zettler, 1997; Nayak et al., 2003a, 2003b; Collins et al., 2005; Lorini et al., 2007). In most of the cases, the development of resistance is presumed to be related to inadequate fumigation practices involving poorly sealed structures and repeated fumigations.

Recent studies in Australia and Brazil have reported the strongest level of resistance detected so far in any stored product pests for this fumigant in these countries (Collins et al., 2005; Nayak et al., 2003b; Lorini et al., 2007; Nayak et al., in press). As registered rates of phosphine have failed to control these resistant pests, new fumigation protocols are being developed to manage them. The increase in strength in resistance means that the traditional FAO method (FAO, 1975) to detect resistance is inadequate for diagnosis of such strongly resistant insects and new methods need to be developed (see section 2.1).

2.3. RESISTANCE TRENDS NOT KNOWN IN MOST COUNTRIES

A key to the successful management of resistance to phosphine is its early detection and proper characterisation. Australia is probably unique in that it has had a national resistance monitoring program that has been operating successfully since the 1980s. Apart from providing early warning to the industry on new resistance developments, this monitoring program also provides the annual trend in resistance frequency across Australia (Emery et al., 2011). The program runs concurrently in three laboratories across the country representing three grain growing regions (northern, southern and western), where thousands of insect samples representing a range of stored grain pests are tested for resistance to phosphine using a nationally agreed statistically robust monitoring protocol. Throughout the year trained staff collect samples from farms and central storages through random and targeted sampling apart from samples being sent directly to the laboratories from storage operators for resistance testing. All resistance data are being stored in an integrated database (Australian Grain Insect Resistance Database) for future reference on trends and frequencies of resistance. The program has been accredited for providing early warning of strong resistances in the lesser grain borer *Rhyzopertha dominica* (F.) (Collins et al., 2005), the psocid *Liposcelis bostrychophila* Badonnel (Nayak et al., 2003b) and most recently the flat grain beetle *Cryptolestes ferrugineus* (Stephens) (Nayak et al., in press). Moreover, research has established two levels of resistance to phosphine ('weak' and 'strong') and it has been suggested that once the frequency of 'weak' resistance reaches 80% in populations of a particular pest species across a particular region, there is a strong possibility of development of strong resistance in that species (Collins and Emery, 2002). This was proven to be the case in the development of strong resistance in *Tribolium castaneum* (Herbst) in Western Australia in 2010 (Emery et al., 2011).

The only other comprehensive survey of resistance published since the FAO survey (Champ and Dyte, 1976) was undertaken by Benhilima et al. (2002) across several grain storages in Morocco and reported very high frequency of phosphine resistance in *T. castaneum*, *R. dominica* and *Sitophilus oryzae* (L.). In another small-scale survey, the

frequency of strong resistance was found to be alarming in central grain storages in Brazil, where 14 of the 19 populations of *R. dominica* collected were detected with strong resistance (Lorini et al., 2007). Due to the lack of a country-wide resistance survey, however, the exact frequency of this resistance in Brazil over time and space is not known.

It is important that countries using phosphine to disinfest stored commodities need to monitor for resistance in key pest species. Late detection of 'strong' resistance doesn't help the case for sustainability of phosphine as early detection would help in eradication of the resistant populations as well as allow time to develop new fumigation protocols to manage them (see section 2.1.2).

2.4. LIMITATIONS OF ALTERNATIVE FUMIGANTS

In view of the development of resistance to phosphine in key pest species and imminent phase out of methyl bromide, there has been significant research undertaken in recent years to explore the potential of other fumigants as alternatives. Unfortunately, almost all of them fail to match the combined advantages that have been offered by phosphine. The limitations of some of the alternatives are discussed here briefly and detailed information on their effectiveness against stored product pests is deliberately omitted.

Among several alternatives, sulfuryl fluoride (SF), a broad spectrum fumigant commercialised by DowAgroSciences seems to be the most promising. It is currently registered in Australia to fumigate flour mills, food factories, dried fruits and stored grain. With the limited published data available, this fumigant has been shown to have poor efficacy against the egg stage of storage pests (Drinkall et al., 1996; Bell, 2000). Moreover, the potential of SF as a greenhouse gas and its fluoride residue on treated food materials are becoming major concerns, which may jeopardise its future use. Another fumigant, carbon dioxide (CO_2), has been shown to have excellent potential for rapid disinfestation only at high pressure and has limitations due to logistical constraints such as high construction and operating costs of pressure chambers (Prozell et al., 1997).

Carbonyl sulphide (COS), although found to be highly effective against a range of stored product pests (Desmarchelier, 1994) fails to control the rice weevil *S. oryzae* (Rajendran, 2001). In addition, fumigation with COS can affect germination of a range of cereals, can leave off odours in walnuts and milled rice and can cause discolouration of soybeans (Navarro, 2006). Ethyl formate (EF) can be effective against a range of pests but only when combined with CO_2 (Haritos et al., 2006). When used independently, it failed against several of these pests. The other major drawback with this fumigant is that it is highly volatile and flammable at normal ambient temperature, which raises serious safety concerns.

Hydrogen cyanide (HCN), a very old and highly toxic fumigant, has limited use on grain due to its high sorptive nature (Navarro, 2006). Ethyl dinitrile (EDN) (also called as cyanogen), a broad spectrum fumigant, has the major drawback of being phytotoxic, which affects seed germination ((Ducom, 2006; Navarro, 2006).

Although not considered as a fumigant, modified atmospheres (MA) involving elevated CO_2, lowered oxygen or an atmosphere with nitrogen generated from cylinders or by separation of air on site has shown excellent effects against range of stored product pests (Adler et al., 2000). However, these methods are not cost effective in large scale operations and compared to fumigants, MA has the serious limitation of needing a very long time to achieve required levels of disinfestation (Donahaye et al., 1994).

3. OPPORTUNITIES

3.1. PHOSPHINE REMAINS VIABLE IF RESISTANCE IS MANAGED

As discussed earlier, the development of high levels of resistance to phosphine has been documented in several major insect pests of stored products. With the imminent complete phase out of methyl bromide by 2015, and market reluctance to accept chemical residues; it is inevitable that phosphine will continue to play an important role in stored product disinfestation for the foreseeable future. The following are ways that will help in maintaining the viability of this unique fumigant in stored product protection.

3.1.1. CHARACTERISATION OF STRONG RESISTANCE AND DEVELOPMENT OF NEW FUMIGATION PROTOCOLS

One key feature of phosphine is that concentration and exposure period can both be altered to maximise its efficacy (Daglish et al., 2002). Research has also clearly demonstrated the influence of temperature on phosphine efficacy (Bell, 1992; Nayak and Collins, 2008). These aspects have direct implications for management of strong level of resistance to phosphine in key pest species. For example, with the detection of strong resistance to phosphine in *R. dominica* and *L. bostrychophila* in the 1990s in Australia, extensive research was undertaken (Collins et al., 2005; Nayak et al., 2003b) to characterise these resistances and development of new fumigation protocols to manage them. After their field validation, these protocols are being incorporated into the phosphine label in Australia (APVMA, 2012) and are used by bulk storage operators in successful control of strongly resistant pest populations. However, compared to these resistances, a much stronger resistance was detected in *C. ferrugineus* in 2006, which was not been controlled by the current registered rates of phosphine (Nayak et al., 2010; Nayak et al., in press). Like previous resistances, this new resistance is now being characterised and new fumigation protocols are being developed to manage it (Nayak et al., 2010). This means that the phosphine label in Australia will need to be modified again to incorporate these new protocols. In a similar study, Lorini et al. (2007) characterised the strong resistance in *R. dominica* from Brazil and determined effective rates of phosphine for its control.

On another front, a population of *S. oryzae* from China was imported under quarantine permits to Australia for resistance research that was characterised to be almost 10% more resistant to phosphine than the local resistant population of this pest (Daglish et al., 2002). Several fumigation protocols have been developed against this resistant strain in an attempt to prepare the Australian industry for combating this resistance in the future if it develops locally (Nayak et al., 2003a).

3.1.2. RAPID DIAGNOSIS FOR STRONG RESISTANCE

The FAO method (FAO, 1975) is a standard procedure that is followed by researchers around the world to detect resistance to phosphine in a particular pest species by exposing a field population to a fixed concentration of phosphine over a 20-h period and assessing mortality after another 14 days. In addition, some researchers have shown that a longer exposure period (e.g. 48 h) is needed for some types of resistance (Collins et al., 2002; Daglish et al., 2002). Recent research by Nayak et al. (in press), however, characterised the strong resistance in *C. ferrugineus* and established a rapid method that can discriminate between susceptible, weak and strongly resistant individuals in a population. This method provides industry with advice on resistance status of samples of *C. ferrugineus* within 7 h of its receival in the laboratory, including the preparation and processing of insects. The same day advice on strong resistant

populations enables the storage operators to make quick decisions on implementing an eradication plan.

There is enormous opportunity to develop rapid tests to diagnose strong resistance to phosphine in other key pest species including *R. dominica* and *T. castaneum* for establishing a more robust early warning system for their detection and timely management.

3.1.3. IMPROVED UNDERSTANDING OF MOLECULAR GENETICS AND ECOLOGY OF RESISTANCE

Understanding of the genetic basis of development of resistance provides valuable information towards development of any pest management strategy. Our understanding of the molecular aspects of phosphine resistance has grown significantly in recent years. It has been established now that at least two major genes control high level phosphine resistance in *R. dominica* (Collins et al., 2002) and *T. castaneum* (Jagadeesan et al., 2012). More comprehensive genetic and molecular analysis of *R. dominica* (Schlipalius et al., 2008) revealed the presence of two loci, *rph1* and *rph2*, responsible for phosphine resistance in this insect. *Rph1* controls the "weak" resistance phenotype providing moderate resistance to phosphine, whereas *rph2* conferring only very low level of resistance by itself. *Rph2* was not discovered in the field until *rph1* had become common and when both are combined in the same individual, they synergise to produce a much higher level of resistance known as the 'strong' resistance phenotype. These findings have led to a series of genetic complementation experiments to establish whether populations of *R. dominica* across widely separated geographic locations in Australia share the same genes for development of strong resistance (Mau et al., 2012a, b). The findings from this research have concluded that resistance in each of the three populations under investigation was derived independently from others despite genetic analysis being consistent with two major genes being responsible for resistance in each case. Research in this area is in progress involving pest populations from Australia and India to confirm whether resistance mechanisms in *R. dominica* and *T castaneum* across continents are the same. The outcomes of this research will be significant in that resistance management strategies developed in one country will be relevant to the other country.

Research is also in progress in Australia on stored grain pest ecology for better understanding of movement of resistance populations and their implications on the grain biosecurity. This research is combining trapping with population genetics analysis to investigate insect dispersal and gene flow (Ridley et al., 2011). Basic information on these aspects would contribute to more effective grain hygiene practices leading to lower infestation threat and reduced need for chemical treatment. Data on how ecological processes contribute to the development and spread of phosphine resistance will be incorporated into a strategy for reversion to susceptibility under reduced selection pressure.

3.2. DEVELOPMENT OF ERADICATION STRATEGIES

In 2006, when the strong level of phosphine resistance was detected in several populations of *C. ferrugineus* in bulk storages in Australia, it was established in laboratory research that registered rates of phosphine failed to control *C. ferrugineus* with resistance of this type. To maintain its 'nil tolerance' principle for live insects in export grain, the industry urgently developed an action plan to combat this resistance problem. This plan was developed collaboratively by the bulk handling companies and researchers aimed at eradicating infestations of phosphine resistant *C. ferrugineus* populations from bulk storages and preventing their spread (Nayak et al., 2010, Nayak et al., in press). The key components of this plan include use of an alternative fumigant such as SF (see section 2.3), strategic

application of grain protectants (chlorpyrifos-methyl, fenitrothion) and adoption of an intensive hygiene program, monitoring of insect populations and resistance testing.

This strategy has been instrumental in the eradication of strongly resistant *C. ferrugineus* populations in at least 60 bulk storages since the implementation of the plan in 2009 (MK Nayak, unpubl. data).

3.3. STRATEGIC USE OF ALTERNATIVE FUMIGANTS

Industry around the world should take advantage of the available alternative fumigants and use them strategically to overcome the phosphine resistance and associated problems. Reliance on a single treatment should be avoided and the strength of each alternative fumigant should be manipulated to fit it to an integrated pest management program. A recent example of successful implementation of this approach has been the use of SF as an alternative to phosphine for managing strongly phosphine resistant *C. ferrugineus* populations in bulk storages in Australia, after this pest's emergence as a major problem in the bulk storage system in recent years. The number of incidences of strongly phosphine resistant populations in these storages has been halved within a year after exclusive use of SF in 2010. The current registered rate of a ct-product (concentration x exposure period) of 1500 mg.h.L^{-1} of SF was found to be quite effective in controlling strongly phosphine resistant flat grain beetle populations for a minimum of 3 months in large-scale bunker (pad) storages (MK Nayak unpubl. data).

After the initial success of SF, research and consultations are in progress in Australia to maximise the potential of this fumigant as an alternative to relieve the ever growing pressure on phosphine. It is suggested that this fumigant should only be used as a 'phosphine resistant breaker' and be used exclusively where phosphine fails to control infestations and that the number of fumigations should be limited in a calendar year to delay the development of resistance. Moreover, with the increasing use of SF by the industry, it is important that a resistance monitoring protocol be established now to prepare industry for detection of resistance as and when they emerge in the future.

4. CONCLUSION

To protect phosphine and extend its usefulness into the future, a strategy including several approaches as described above needs to be in place. This has been well demonstrated by the Australian grain industry. A National Phosphine Resistance Management Strategy was developed over several years of consultation between researchers, key bulk grain storage operators, farmers, extension specialists and other end-users and policy makers of key state and federal government agencies that are relevant to the registration and use of phosphine in Australia (Collins, 2009). Under the umbrella of the National Working Party on Grain Protection, these stakeholders unanimously agreed to adopt and implement this strategy that provides a foundation to achieve the goal of ensuring the long-term sustainability of phosphine. The key components of this strategy are: a national resistance monitoring program, identifying the factors responsible for development of resistance, reducing selection (eg. limit number of fumigations), destroying resistant populations through use of alternative methods and implementation of eradication plans and adherence to recommended use of phosphine by all industry users. This holistic approach to manage phosphine resistance has already shown encouraging results as reflected in the effective management of strongly resistant *C. ferrugineus* populations in bulk storages through use of SF as an alternative fumigant and implementation of an eradication plan.

To conclude, industry around the world should look at the case of Australia and develop similar programs to help protect phosphine and maintain its viability in the future.

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IMPROVING THE EFFICACY OF PHOSPHINE FUMIGATION IN ON FARM STORAGE IN SOUTH EASTERN AUSTRALIA

Peter Botta[1*], Catherine Botta[2], Chris Warrick[3]

[1] PCB Consulting Pty Ltd 44 Porters Rd Benalla Victoria 3672
[2] PCB Consulting Pty Ltd 44 Porters Rd Benalla Victoria 3672
[3] Proadvice 194 High St Belmont Victoria 3216
*Corresponding author's e-mail: *pbotta@bigpond.com*

ABSTRACT

Phosphine fumigation is widely used to kill grain insect pests in on farm storages in SE Australia. On farm storages are predominately unsealed silos or silos which may have a level of sealing but do not meet a standard pressure test and are not considered to be gas tight. Fumigants and controlled atmosphere are the most cost effective ways to kill insects in silos, however, to work effectively, silos need to be sealable to the gas tight standard. This has resulted in poor results for farmers storing grain including insect attack, infestation, and poor grain quality for markets. Phosphine use in unsealed storages has seen a steady increase in resistance which has the potential to adversely affect the Australian Grain Industry's ability to control insects as phosphine is the primary treatment used.

Farmer practice is not always best practice for chemical use and for OHS issues. Contact treatments are not working on the lesser grain borer. Dichlorvos will be withdrawn from on-farm use in March 2013. Markets are increasingly asking for pesticide residue free grain.

This project involves a multi approach to improve on farm practice including:

- Introduction of the Australian standard AS 2628-2010 Sealed Silos
- Whole of industry extension program
- Information packages and booklets
- Label changes to -phosphine
- Phosphine use farmer training in South Australia and Victoria
- You (U) Tube media
- Media

Preliminary results are encouraging.

Growers are now actively asking for Australian Standard Compliant sealed silos when purchasing on-farm storage. Stakeholders across the grains value chain are asking for and disseminating best practise information through their networks and the many grower workshops and field days conducted through the national extension project and industry forums.

Key Words: Gas-tight, fumigation, national extension, Australian Standard, on-farm storage, pesticide residue free.

INTRODUCTION

This paper reports on a Grains Research and Development (GRDC) sponsored project in extension for on-farm grain storage. The project was a national extension project, this paper focuses on the south eastern grain growing region of Australia, where the author is based and on-farm grain storage has increased significantly as a consequence of the deregulation of the domestic and export markets.

Phosphine fumigation is widely used to kill insect infestations in on-farm storages. When applied in unsealed storages poor results are typically attained which can lead to rejection at receival sites due to poor insect control and or detection of phosphine in the delivered grain. Use of phosphine in unsealed storages is a risk to the occupational health and safety of the user and increases resistance to phosphine.

The project involved a multi approach to improve on-farm storage practises to improve the efficacy of fumigation and the overall management of on-farm grain storage. The project consulted and worked with key industry stakeholders including state-based departments of agriculture, research institutions, regulators, silo and machinery manufacturers, training bodies, private agronomists and consultants, peak farmer representative bodies and farmer growing system groups.

Key elements of the project were delivery of best practise grain storage management and phosphine fumigation workshops and field days aligned to adult learning principles, information packages, media releases and communication, phosphine label changes and the introduction of an Australian Standard for gas-tight sealable silos.

MATERIALS AND METHODS

The project involved using multiple approaches to improve on farm grain storage management and phosphine fumigation practises including the following aspects;

Workshops and field days were conducted for farmers, agribusiness and advisers on the principles of best management grain storage and fumigation practises. Grower, industry, silo and agricultural machinery networks were used to set up events in local areas. Events were advertised through these networks and local media and local grain storage issues were assessed to ensure the events were relevant to the immediate needs of participants as well as delivering the key messages around best practise fumigation and grain storage management.

Workshops and field days were developed and conducted using adult learning principles to create a positive learning environment for participants, build capacity in the industry to further support farmers and enable farmers and industry to implement ongoing changes in their grain storage systems. Typically the event would be for 3 to 4 hours depending on discussion and either started or finished with a meal and socialising to foster further discussion and knowledge transfer.

The events used a variety of delivery and training techniques, including auditory, sensory, visual and practical learning examples to cater for the different learning styles of the participants. Learning materials were supplied during the event and contact and further information details were given for participants to follow up on any questions.

Sessions included presentations of best management practises combined with demonstrations and practical examples either at an on-farm grain storage or local grain storage site. Group discussion and knowledge sharing was an integral component of each session, fostering learning and skills development for the participants.

A certified phosphine training course, "Responsible, Safe and Effective Use of Phosphine Generating Formulations on Farms" was developed with a chemical training provider (AusChem Training Inc) for on-farm users. This course is available for training on-farm users of phosphine throughout Australia.

Best practise fumigation and grain storage management presentations were delivered at a variety of industry forums including GRDC (Grains Research and Development Corporation) Research and Grower updates, agribusiness and industry field days and seminars.

To support the training activities a variety of media articles were written and disseminated throughout local newspaper, journal and newsletter outlets. Articles were written in response to arising issues and as general information for growers and to promote best practise phosphine fumigation and management. A DVD was recorded on gas-tight sealed silos and phosphine use and distributed via the GRDC to all registered grain growers in Australia and uploaded on to YouTube and GRDC television. Radio interviews and discussions were also used to disseminate information and advice.

As an integral part of the nationwide project information packages and materials were developed and distributed through a variety of industry channels and were available at workshops and field days. Two written specifically for fumigant use were a GRDC factsheet called "Pressure Testing Sealable Silos" and a booklet called "Fumigating with Phosphine, other Fumigants and Controlled Atmospheres. Do it Right – Do it Once".

A website www.storedgrain.com was developed to further provide a source for growers and industry to look for and download information. This website has been widely accessed by farmers and industry for information and signposting to further information and service providers.

Label changes to phosphine are currently being written to improve overall management of the product, safe use and to improve resistance management. These changes will assist regulators to enforce breaches of the label, particularly in regards to what constitutes a suitably gas-tight sealed structure.

In conjunction with SAI Global (formerly Standards Australia) the author convened a committee to write an Australian Standard for sealed silos. Prior to the Australian Standard (AS 2628-2010), there was no industry benchmark for sealed silos which growers could use to determine whether a silo they were purchasing was actually sealed and gas-tight when purchased. The committee was made up of representatives from State Department of Agriculture research scientists, the CSIRO, State and national farmer peak industry bodies and silo manufacturers. The committee consulted widely with industry, chemical registrants, the AVPMA (Australian Pesticides and Veterinary Medicines Association), grower bodies and representatives.

All of these approaches were used to deliver a whole of industry extension program promoting best practise on-farm grain storage management and phosphine fumigation.

RESULTS AND DISCUSSION

Personal communication and anecdotal evidence in communication with the grains industry and value chain has demonstrated that on-farm grain storage management and the awareness and implementation of best practise phosphine fumigation has increased. Feedback from silo manufacturers has shown that growers are actively asking for Australian Standard compliant sealed silos when comparing and purchasing sealed silos. The Australian standard enables growers to purchase a sealed silo which meets a standard gas-tight pressure test, enabling

them to have the correct system to fumigate. Prior to this standard being enacted growers found it difficult to benchmark silos in the marketplace, where a number of silo manufacturers claimed their silos were sealed but the silos did not in fact meet the standard pressure test.

The Australian standard alone does not ensure that efficacious phosphine fumigations can be administered; however it is the first step in ensuring a grower has the correct system in which to undertake an efficacious and safe fumigation.

Stakeholders across the grains value chain are asking for and disseminating best practise information through their networks and the many grower workshops and field days conducted through the national extension project and industry forums. Development of the various information packages covering best management practises for on-farm storage and fumigation provides a mechanism for growers and industry to support the training and knowledge development they have undertaken. The phosphine booklet "Fumigating with phosphine, other fumigants and controlled atmospheres" was a comprehensive and farmer friendly publication covering sealed storage management, silo testing and best practise fumigation. The Australian standard provided growers with the tools to select sealed storage, the extension program and information packages have built on and supported best practise fumigation and grain storage.

At workshops and field days growers are taught the theory behind a successful fumigation and with practical demonstrations shown the features of a gas-tight sealed silo, how to maintain and replace seals and how to perform a standard pressure test to establish whether a silo is gas-tight.

Using mediums such as the stored grain website and the media allowed specific and timely information to be brought to the attention of growers and industry and to promote key messages when necessary. An example of this was a major media campaign using rural media and industry networks promoting and discussing the Australian standard for sealed silos, which was a great success and very quickly converted to growers actively asking whether silos being considered and or purchased met the standard.

Newspaper and newsletter articles and radio interviews are regularly released to promote best practise fumigation and grain storage practises when the information is timely and can be used to assist farmers in their storage management.

The accredited phosphine training module has had a minor uptake to date, largely due to growers still not being required to undertake training specific for the use of phosphine in states other than New South Wales. Currently New South Wales farmers are required to undertake phosphine training as a Work Cover (Occupational Health and Safety regulator) requirement. State regulators of chemical use are currently considering mandatory training for phosphine use, particularly in Victoria. The introduction of training is being considered as part of a response by regulators to the potential label changes for phosphine being proposed to the APVMA (Australian Pesticides and Veterinary Medicines Authority).

Overall the extension project has had a positive impact on improving the efficacy of phosphine fumigation in on farm storage. Growers are actively asking for Australian Standard Compliant sealed silos. Growers, industry and agribusiness are asking for and disseminating best practise information through their networks, and there has been a continuing demand for workshops and field days, and addresses at industry forums. With the funding of a new extension project starting in July 2012, the successes of this current project can be built on and incorporate changes made to the phosphine label and any new requirements of chemical regulators to ensure correct and effective fumigations can be conducted in on-farm storage.

PHOSPHINE FUMIGATION PROTOCOLS USING ECO$_2$FUME® FOR COMPLETE CONTROL OF STRONGLY RESISTANT CIGARETTE BEETLE, *LASIODERMA SERRICORNE,* IN INDONESIA

Justin Tumambing[1], Remil Mallari[1], S Widayanti[2], Idham S. Hararap[2] and Gabriel Salazar[3]

[1]CYTEC Australia Holdings Pty Ltd, P O Box 7125, Baulkham Hills, NSW 2153 Australia
[2]SEAMEO BIOTROP, Jl. Raya Tajur Km 6, Bogor, Indonesia
[3]PT Sterix Indonesia, Epicentrum Walk, Lantai 6, Suite A-623, Kuningan, Jakarta Selatan, Indonesia
*Corresponding author's e-mail: *Justin.Tumambing@cytec.com*

ABSTRACT

Efficacy trials were conducted at BIOTROP to establish phosphine fumigation protocols using ECO$_2$FUME® achieving complete mortality of all stages of strongly resistant cigarette beetle, *Lasioderma serricorne*, infesting dry tobacco leaves in Indonesia. Tobacco leaves in Indonesia have been fumigated with metal phosphides such as aluminum phosphide tablets and magnesium phosphide plates for a long time. Over time, metal phosphides have proved unable to achieve 100% control of all stages of cigarette beetle and with repeated failed fumigations, phosphine resistance developed with strains of insects exhibiting strong and weak levels of resistance to the current protocols. Experimental treatments with combinations of three phosphine concentrations (1000 ppm, 700 ppm and 350 ppm) and three exposure times (5 days, 8 days and 12 days), at average temperatures of 28°C or higher were used to determine the conditions achieving 100% mortality of all stages of both the strongly and weakly resistant cigarette beetle strains.

ECO$_2$FUME® (2% phosphine, 98% CO$_2$ by weight) is a cylinderized gas formulation of phosphine which offers the advantages of absence of fire risk, enhanced worker safety, effective and rapid distribution of gas, easy application and control, no waste generation or disposal thus being environmentally friendly, and cost effectiveness.

Results of the study suggest potential fumigation protocols achieving 100% efficacy for all stages of strongly resistant cigarette beetle in Indonesia are 1000 ppm for 5 days, 700 ppm for 8 days and 350 ppm for 12 days at average temperatures of 28°C or higher. The minimum recommended phosphine concentration should be maintained through regular phosphine concentration monitoring and top up throughout the required exposure period. The larva of the strongly resistant strain appeared to be the most tolerant stage.

Key words: phosphine gas, ECO$_2$FUME®, *Lasioderma serricorne*, tobacco fumigation, resistance.

INTRODUCTION

Tobacco is one of the most important crops in Indonesia as its leaves are used in the production of cigarettes and cigars for domestic and international markets. Tobacco leaves just like any other plant commodity are subject to insect pest infestation during storage which leads to both significant qualitative and quantitative losses. The major insect pests of tobacco leaves are the cigarette beetle, Lasioderma serricorne (F.) and tobacco moth, Ephestia elutella (Hubner). Of the two insect species, cigarette beetle is the most damaging and more tolerant to treatment.

The general treatment for insects infesting tobacco leaves is with the use of phosphine from metal phosphides, solid formulations of magnesium phosphide or aluminum phosphide. Metal phosphide had been used for decades in fumigating tobacco leaves against cigarette beetle and over time resistance has developed. Phosphine resistance of cigarette beetle has progressed substantially in Indonesia so that it is now very difficult to achieve 100% mortality of all developmental stages (adults, pupae, larvae and eggs) using the current protocols developed and recommended by CORESTA (Cooperation Centre for Scientific Research Relative to Tobacco). CORESTA is an international association of companies, research institutes and independent laboratories with R & D interest in the production, manufacture and use of tobacco. CORESTA has separate recommended phosphine fumigation protocols for susceptible and resistant strains of cigarette beetle. The recommended protocols for susceptible cigarette beetle are 300 ppm for 6 days at 16 – 20°C and 200 ppm for 4 days at 20°C or higher. For resistant cigarette beetle, the recommended protocols are 700 ppm for 10 days at 20 – 25°C and 600 ppm for 6 days at >25°C.

Many factors enhance the development of phosphine resistance in insects such as: 1) use of sub-lethal doses, 2) not maintaining the minimum recommended phosphine concentration over the required exposure time, 3) lack of regular monitoring of phosphine concentration 4) repeated fumigation with phosphine and 5) no monitoring or action taken regarding insect survival that later can give rise to resistant strains.

This paper describes the efficacy trials conducted to establish fumigation protocols using ECO2FUME® phosphine fumigant that will achieve 100% efficacy against strongly resistant cigarette beetle that infests dried tobacco leaves in Indonesia. ECO2FUME® is a non-flammable and ready-to-use cylinderized formulation of 2% phosphine (PH3) and 98% carbon dioxide (CO2) by weight. It is packaged in high pressure steel cylinders with net content of 31 kg of PH3/CO2 mixture and equivalent phosphine amount of 620 g. ECO2FUME® (formerly known as Phosfume) was first commercially applied in Australia in 1988 by BOC Gases Australia who produced and patented the phosphine/CO2 blend and developed special dispensing equipment for fumigating grains and oilseeds in unsealed and good sealed silos and horizontal sheds (Cavasin et al., 2000). From then on, ECO2FUME® has been used globally for treatment of other commodities such as cut flowers, nuts, tobacco, and wheat flour and non-food applications such as structural fumigation.

The use of ECO2FUME® has the advantages over solid metal phosphide phosphine formulations of 1) safer operation by application outside the structure, 2) quick dispensing and attainment of target concentration throughout the structure and possibility of maintaining the concentration by measured top-ups, 3) independence of humidity and temperature for reaction, 4) lower phosphine dosages due to maintenance of phosphine concentration close to the target concentration, 5) eliminating dust or solid waste generated from using solid

formulations, 6) no waste deactivation and disposal, and 7) no ammonia emissions that require additional scrubbing.

The objectives of the study were 1) to test the effectiveness of ECO2FUME® against strains of cigarette beetle showing weak and strong resistance to phosphine in dried tobacco leaves in Indonesia and 2) to define parameters and propose phosphine fumigation protocols for complete control of all stages of these cigarette beetle strains.

MATERIALS AND METHODS

The fumigation trials were conducted during the period 12 - 24 February 2012 with a further two weeks for the bioassay efficacy assessment. The trials took place at the postharvest warehouse of BIOTROP (Tropical Biology Institute) in Bogor, Indonesia. Preparation of test insects and bioassay was performed at the BIOTROP entomology laboratory. The climatic conditions during the trials were 23 – 33°C ambient temperature and 44 - 89% relative humidity.

The materials used in the trials were as below:

1. Tobacco bales, 36 boxes (approx.1 m^3 each)
2. Wooden pallets, one under each bale
3. Polypropylene tarpaulin sheet (150 micron)
4. Sand snakes for sealing tarp covered stock
5. ECO$_2$FUME$^®$ cylinder and dispensing hose with special gun injector for small doses
6. SILOCHEK$^®$ phosphine monitor (0 – 2000 ppm)
7. Digital weighing scale (100 kg with 1 g precision)
8. ¼ inch plastic tubing for gas sampling
9. Industrial fan to control exposure levels
10. Full face gas masks with phosphine canisters

The efficacy trials were designed in Completely Randomized Design (CRD), with times and dosages as factors in the experiment.   The four level of dosages tested were; 0, 25, 50, and 70 g/m3 of ECO2FUME® and 3 levels of exposure time tested; 5, 8 and 12 days. Each treatment combination consisted of 3 replications. The tobacco bale boxes were arranged in a fully randomized fashion.

The test insects used in these trials were all life stages of the cigarette beetle, L. serricorne (eggs, larvae, pupae and adults). There were two sets of test insects collected. The strongly resistant test insects were collected from a leading cigarette company and the weaker resistant test insects were from a leading cigar company. Each stage was assigned a plastic tube with dry tobacco leaves as a culture media. The plastic tubes covered with gauze which allowed the fumigant to penetrate into the tube through it. There were about 40 individuals of each stage of both strains of cigarette beetles. The plastic tubes were then placed into the center of tobacco bales and buried with dry tobacco leaves. Gas sampling hoses were installed into the space between pallets and tobacco bales and into the center of tobacco bales, for monitoring the phosphine concentration during fumigation.

Each tobacco bale with test insects was then covered with PVC fumigation sheets (thickness 0.15 mm = 150 µm) to make fumigation enclosures. The edges of the top covering sheet and the under liner sheet were folded together into interlocking folds and pressed down

with sand snakes for sealing. The folded plastic sheets were also secured with Teflon tape on to the concrete floor for extra sealing.

The equivalent amount of phosphine from ECO$_2$FUME$^®$ was injected inside the tarp using a braided stainless steel hose and gun type gas injector with gas flow rate of as low as 1 g/sec. The exact amount of ECO$_2$FUME$^®$ dispensed was determined by the weight change of the cylinder on top of a digital weighing scale accurate to 0.001 kg or 1 g. Fumigation was terminated at 5, 8 and 12 days of exposure time and followed by aeration of the slightly opened enclosure until the phosphine concentration reached the threshold limit value (TLV) of 0.3 ppm or lower. The plastic cover sheets were completely removed afterwards.

Phosphine gas concentrations were monitored using two lengths of ¼ inch diameter plastic tubing as gas sampling lines, one located at the core of the bale and one below the bale within the frame of the wooden pallet. Monitoring was conducted at each of the following times; 1) 6, 12, 24, 48, 72, 96, 120 h for 5 days exposure time, 2) same as item 1 plus 144, 168, 192 h for 8 days exposure time and 3) same as item 2 plus 216, 240, 264, 288 h for 12 days exposure time. Phosphine concentration readings were made with a calibrated SILOCHEK$^®$ phosphine monitor (0–2000 ppm).

When the phosphine concentration fell below the target concentration, top up of ECO$_2$FUME$^®$ dosing was conducted to bring the concentration back to or above the target concentration. The top up procedure was conducted in the same way as the initial gas dispensing, the exact amount calculated based on the difference between the target concentration and the actual reading.

Mortality of adult stage test insects was evaluated shortly after the fumigation period was completed (after aeration). To ensure accurate assessment of mortality of larvae, pupae and eggs stages, tubes were examined 5 – 7 days after fumigation. The eggs mortality was further assessed within two weeks of the first mortality assessment if any larvae emerged from eggs that survived.

RESULTS AND DISCUSSION

1. Five Days Exposure Time

Mortality of the test insects exposed to 5 days fumigation showed that the test insects from the cigarette company appeared to be of stronger resistance than the test insects from the cigar company. As shown in Table 1, dosages of 350 ppm and 700 ppm did not achieve 100% mortality for all stages of insects. The larval stage also appeared to be more tolerant than the inactive egg and pupal stages at dosages of 350 ppm and 700 ppm. A dose of 1000 ppm had two replicates (R2 and R3) achieving 100% mortality for all stages of insects. Replicate 1 (R1) achieved 100% mortality for the adults, pupae and eggs but only 82.4% for the larvae.

Based on the phosphine concentration profile of the three replicates as shown in Fig. 1, it can be seen that R1 which showed <100% mortality for the larvae had two days (day 4 and day 5) with phosphine concentration way below the target concentration of 1000 ppm. The other two replicates (R2 and R3) had phosphine concentration below 1000 ppm at day 3 but a top up of phosphine was conducted to bring the phosphine concentration back to the target of 1000 ppm. The operator missed the top up of R1 which resulted in a further decline in

phosphine concentration. This is a good learning experience on the importance of regular monitoring of gas concentration and the topping up of phosphine to ensure that the target lethal concentration is maintained. From the results, it can be deduced that maintaining the target concentration for the whole fumigation period will ensure the achievement of minimum concentration-time (ct) product for 100% mortality of all stages of insects. If the target phosphine concentration of 1000 ppm had been maintained in R1 then 100% mortality of larvae could have been achieved. In this trial, top up of phosphine with ECO_2FUME was done safely and quickly using a stainless steel quick dispensing hose with gun type gas injector.

Table 1. Mortality of the different stages of cigarette beetle at the cigarette company at four levels of phosphine concentration (0, 350, 700 and 1000 ppm) for the three replicates (R1, R2, R3) at 5 days of exposure time

Treatment	Adult % Mortality	Pupae % Mortality	Larvae % Mortality	Eggs % Mortality
Control R1	0.0	0.0	0.0	0.0
Control R2	0.0	0.0	0.0	0.0
Control R3	0.0	0.0	0.0	0.0
350 ppm R1	97.5	100.0	94.1	80.0
350 ppm R2	100.0	60.0	88.2	85.0
350 ppm R3	92.5	60.0	88.2	90.0
700 ppm R1	100.0	60.0	88.2	100.0
700 ppm R2	95.0	100.0	94.1	100.0
700 ppm R3	100.0	100.0	94.1	100.0
1000 ppm R1	100.0	100.0	82.4	100.0
1000 ppm R2	100.0	100.0	100.0	100.0
1000 ppm R3	100.0	100.0	100.0	100.0
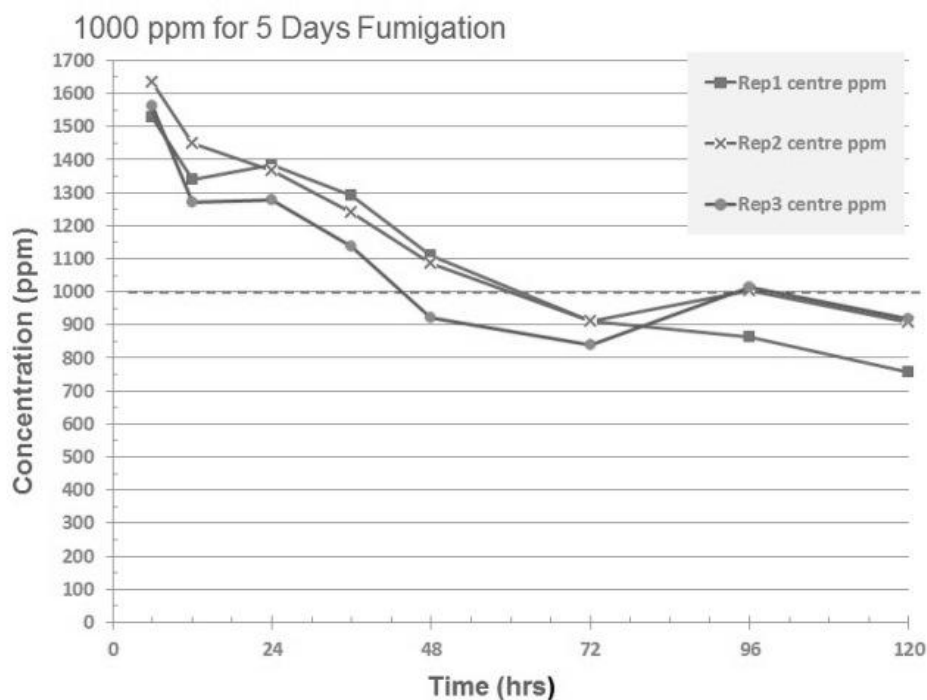
Fig. 1- Phosphine concentration at five days fumigation for the three replicates.

In Fig. 1, the initial phosphine concentration for the three replicates was much higher than the target concentration of 1000 ppm since the phosphine gas was occupying only the empty air space around the tobacco bale and in between tobacco leaves and not the whole volume of stock. The phosphine dosage was based on the total volume of the stock such that a higher concentration of 1550 – 1650 ppm was reached initially. As fumigation progressed, there were gas losses due to gas leakage and phosphine sorption into the tobacco leaves.

The test insects from the cigar company were less resistant with almost all of the replicates of the 700 ppm and 1000 ppm treatments achieving 100% mortality at 5 days exposure time (Table 2). The 350 ppm treatment achieved <100% mortality for the four stages of insects.

2. Eight Days Exposure Time

Table 3 shows that mortality of the four stages of test insects of the strongly resistant (SR) strain at four levels of phosphine concentration (0, 350, 700 and 1000 ppm) for the three replicates (R1, R2, R3) at 8 days of exposure time. The 350 ppm dose was not sufficient to control stages other than eggs. In the treatment of 700 ppm, replicate 2 achieved only 76.5% mortality of larvae as compared to 100% mortality for replicates 1 and 3. This can be explained from the phosphine concentration profile of replicate 2 which showed that the concentration fell below the target concentration for 5 out of 8 days and thus had a much lower ct-product than replicates 1 and 3. In contrast the three treatments of 350 ppm, 700 ppm and 1000 ppm for 8 days achieved 100% mortality for all stages of the weaker resistant (WR) test insects from the cigar company.

Table 2. Insect mortality of the four stages of test insects at the cigar company at four levels of phosphine concentration (0, 350, 700 and 1000 ppm) for the three replicates (R1, R2, R3) at 5 days of exposure time

Treatment	Adult % Mortality	Pupae % Mortality	Larvae % Mortality	Eggs % Mortality
Control R1	0.0	0.0	0.0	0.0
Control R2	0.0	0.0	0.0	0.0
Control R3	0.0	0.0	0.0	0.0
350 ppm R1	100.0	100.0	100.0	100.0
350 ppm R2	100.0	80.0	100.0	100.0
350 ppm R3	95.0	80.0	80.0	90.0
700 ppm R1	100.0	100.0	100.0	100.0
700 ppm R2	100.0	100.0	100.0	100.0
700 ppm R3	100.0	100.0	100.0	100.0
1000 ppm R1	100.0	100.0	100.0	100.0
1000 ppm R2	100.0	100.0	100.0	100.0
1000 ppm R3	100.0	100.0	100.0	100.0

Table 3. Insect mortality of the four stages of test insects at the cigarette company at four levels of phosphine concentration (0, 350, 700 and 1000 ppm) for the three replicates (R1, R2, R3) at 8 days of exposure time

Treatment	Adult % Mortality	Pupae % Mortality	Larvae % Mortality	Eggs % Mortality
Control R1	0.0	0.0	0.0	0.0
Control R2	0.0	0.0	0.0	0.0
Control R3	0.0	0.0	0.0	0.0
350 ppm R1	97.5	60.0	100.0	100.0
350 ppm R2	100.0	100.0	88.2	100.0
350 ppm R3	92.5	80.0	76.5	100.0
700 ppm R1	100.0	100.0	100.0	100.0
700 ppm R2	100.0	100.0	76.5	100.0
700 ppm R3	100.0	100.0	100.0	100.0
1000 ppm R1	100.0	100.0	100.0	100.0
1000 ppm R2	100.0	100.0	94.1	100.0
1000 ppm R3	100.0	100.0	100.0	100.0

3. Twelve Days Exposure Time

The three treatments of 350 ppm, 700 ppm and 1000 ppm achieved 100% mortality for both resistant strains except for one replicate (R2) which showed only 94.1% mortality of larvae of the SR strain from the cigarette company at 350 ppm. The lower than 100% mortality of test insects in R2 was again due to the phosphine concentration falling way below the target phosphine concentration of 350 ppm in 6 out of 12 days.

CONCLUSIONS AND RECOMMENDATIONS

1. Effective control of all stages of both SR and WR strain insects could be achieved in 5 to 12 days.
2. The larvae stage of SR cigarette beetle is the most tolerant stage.
3. Based on the phosphine concentration profile analysis the proposed fumigation protocols are:
   a. 1000 ppm for 5 days at 28°C average temperature or higher for strong resistant strain
   b. 700 ppm for 5 days at 28°C average temperature or higher for weak resistant strain
   c. 700 ppm for 8 days 28°C average temperature or higher for strong resistant strain
   d. 350 ppm or 500 ppm for 8 days 28°C average temperature or higher for weak resistant strain
   e. 350 ppm for 12 days 28°C average temperature or higher for strong resistant strain
   f. 350 ppm or 200 ppm at 28°C average temperature or higher for weak resistant strain
4. These protocols are suitable for tropical climates in Indonesia and other SE Asian countries with similar phosphine resistance issue.
5. The proposed protocols can be effectively obtained using cylinderized phosphine gas such as $ECO_2FUME^®$.
6. Validate the proposed fumigation protocols in larger scale trials before adoption in Indonesia and other tropical countries.
7. Optimize the dose for the weakly resistant strains of cigarette beetle.
8. Replicate these trials to cover lower temperature ranges common in temperate climates where a similar issue of strong phosphine resistance is encountered.

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RESISTANCE MANAGEMENT IN STORED PRODUCTS FUMIGATION

Barry Bridgeman[1]* and Pat Collins[2]

[1]Forsure Pty Ltd, Toowoomba, Australia
[2]DEEDI, Brisbane Qld. Australia
*Corresponding author's e-mail: *barry.bridgeman@bigpond.com*

ABSTRACT

Various resistance management strategies were successfully implemented across the Qld and NNSW Grain Growing areas during 2009 to 2011 seasons, to minimise the effects of high resistance to phosphine in target species, specifically *Cryptolestes ferrugineus*. The resistance levels studied determined this strain was able to survive fumigation at current label rates with a dose of 700 ppm phosphine for 20 days at 20°C being required to achieve control (Nayak and Collins 2011). This strain Fumigation, phosphine, resistance, grain, bunker, rotation, management, *Cryptolestes*, PRF, selection was detected predominantly in the large bunker storages of the area where achieving this C/T is not practical with currently available processes.

Several of the issues which may have provided a selection pressure resulting in the manifestation of these control failures and development of this resistance are discussed.

This paper outlines several of the strategies tested in a PRF "Pesticide Residue Free" Storage system and the circumstances in which they were employed. The common theme is a focus on the basics, such as, management rather than control. Specific issues discussed include the use of chemical/fumigant rotations /substitutions, which were critical in the ultimate control of this strain in particular, as well as the effect of hygiene, monitoring, storage sealing and fumigation basics on the potential development of this resistance. The resistance development minimisation strategies utilised to mitigate development of resistance in any storage situation and how that may relate to operations of PRF storage facilities is also discussed.

Key words: Fumigation, phosphine, resistance, grain, bunker, rotation, management, *Cryptolestes*, PRF, selection

INTRODUCTION

The development of the phosphine resistant *Cryptolestes ferrugineus* strains in grain storages throughout the eastern grain growing areas of Australia, has resulted in numerous control failures within that area. The resistant populations were detected predominantly in the larger bunker storages, which are commonly used in the area.

Initially bunkers were designed for temporary storage. Bunkers provide a harvest receival buffer system, which enables ballooning of storage capacity at specific sites. The strategy is a cost effective alternative to construction expensive permanent storage in areas where they may not be cost justified. These bunker storages have continued to evolve,

becoming the preferred long term storage type in Australia generally. The low cost construction and ability to store large and variable volumes economically, has relieved the need for extensive construction of more permanent storage facilities. The well built bunker presents as a very well sealed/gastight enclosure, which can be fumigated very cost effectively. Insect control is achieved through an IPM plan heavily reliant on phosphine fumigation. The bunker storage system has provided the industry with the ability to provide PRF storage facilities which is a preference in an increasing number of markets.

Customer bias against chemical residues on food commodities has driven the focus for Pesticide Residue Free (PRF) grain storage. Bunkers are used extensively to provide residue free storage. Phosphine fumigation is used to maintain insect free status on out-turn. The use of contact pesticides at anytime within the grain path in a PRF facility is not an option.

The resistant stains were and still are under study by various Australian Departments of Agriculture and Qld DEET is particular. The studies have determined that the level of resistance exhibited within this stain enabled the individuals to survive label rates of phosphine. The control of this strain was found to require dose rates of 700 ppm for 20 days at $20^{o}C$ (Nayak and Collins 2011).

The research has also developed an opinion on how the resistance is selected. It is the considered opinion that this selection process required repeat, sub lethal phosphine doses on a single population. Data indicates a approximately 4-7 repeat selection fumigations would be required (Collins 2009).

The questions must be asked. Has this resistance developed because existing bunker storage insect control has been ineffective over a long period? or Is resistance development within a population inevitable when a single chemical is used over a long period? What can be done to minimise resistance development?

THE ISSUES

The storage system
The development of this resistant strain must be considered a wakeup call to the grain industry in Australia, particularly in the PRF sites. It has highlighted the vulnerability to any strategy which is dependent on a single chemical for successful control of insects. The industry has been forced to review all procedures and compliance with basic IPM principles. All previously uncontested assumptions on Bunker sealing and fumigation practice in bunkers are being tested.

It is however difficult to guarantee that all bunkers are effectively sealed for fumigation. Bunkers also present several grain husbandry issues, which may have contributed to the development of this resistant strain in particular, as well as add significantly to the complexity of the fumigation. Two of these anomalies are the degree of moisture migration within the bunker and the effect of wind currents on the distribution of fumigant within the storage.

The significance of the presence of a wet area on the crest of bunkers is well documented. The peak or crest is commonly affected by wet, mouldy crust of spoiled commodity due to the presence of this wet area, which is due to the moisture migration from within the bulk. The presence of this wet area in the context of understanding the development resistance within this species is an obvious concern. The resistant insect populations have been detected primarily in this area and have most likely developed by a continuous selection process, which has been facilitated by this wet area. Areas of wet commodity are either not permeated by the fumigant at all or receive sub lethal concentrations.

Studies have detailed the significance of the wind on the bunker and the distribution of fumigant within the bunker. It is apparent that the effect of wind on the bunker fumigant distribution is arguably the single most critical element ensuring the success of fumigations within bunkers. Subsequent and current studies into the significance of the wind on distribution within bunkers and the effect of fumigation effectiveness suggest that it is not providing the selection pressures exhibited by the wet areas on the crest of the bunkers. The effect of wind on the fumigation of these storages is not discussed here.

Operations and logistics

It is evident that some operational processes and logistical realism of the grain industry also affects the resistance selection processes. The most perplexing of these operational realities is the preference for prophylactic scheduled (3 monthly), routine fumigation with phosphine.

This practice is usually a directive from the grain exporters who are looking to manage the risk of insect detection on the way to or at the market place. The market has realised that the risk of detecting insects increases with time since last fumigation. After 3 months the risk is considered unacceptable and fumigation prior to out-turn is demanded. This is often despite the fact the grain may well be fumigated at Port in any case, as a Phyto-sanitary request from the importing country. With this fact in mind the grain storage management will schedule fumigation to ensure out-turn program predictability and control of the fumigation timing.

The practice results in each bulk of grain that is being kept for 12 months will be fumigated 3 – 4 times with phosphine fumigant (depending on location). If the grain is to be held over for the next season, it is possible the bulk could be fumigated a further 3 -4 times with phosphine. Some of the bulks exhibiting large populations of resistant insects had been held for 3-4 years. At 3-4 fumigations per bunker, per year, that is between 9 and 16 fumigations with the same phosphine fumigant on the single population. This selection pressure on this population is enhanced when there is moisture present in the bulk.

Another operational issue is the reality of commodity being held as carry over from last season. Ideally under an IPM the storage and complete site would be emptied for some time prior to harvest to allow thorough hygiene and disinfestations of residual insects from the previous season. The unfortunate reality as that this practise needs to continue and as such needs to be carefully managed.

The other issue worthy of mention with logistics and operations in regards to resistance development risk is the practice of cutting fumigations short. This happens more often than it should and becomes an annoyance when only a single train load is taken from the bunker. So the bunker with a compromised fumigation must be then resealed and scheduled for re-fumigation. Again, apart from just managing the practice, there is little else to be done when the client demands out-turn of their own grain regardless of the forward scheduling of fumigation agreed or otherwise.

Operationally, bunker covers are reused from season to season. The assumption is that a visual inspection of the bunker cover provides a satisfactory measure of its integrity. Such assumptions need to be tested and a standard provided for the industry. An empirical test could be developed but results would need to be collated over a period.

Commodity issues

Moisture content of commodity stored within a bunker is the most critical quality consideration in regards to the risk of resistance development in target species. During harvest (spring to summer) moisture migration issues are fairly minimal however in the autumn, the cooler evenings will drive the condensation on the underside of the tarps

particularly along the peaks. Once the commodity is wet, the risk of fumigation failure and resistance selection is a very real possibility. Fumigants will not penetrate these wet areas and the survivors will remain protected from fumigation to fumigation. It is probable that those insects existing in the wetter areas, at the edge of the area where fumigant is at a lethal concentration, such as the _Cryptolestes_ sps are going to provide the population most likely to develop selection to phosphine resistance in this scenario.

Fumigation issues
Reliance on a single chemical to achieve control has always been highlighted as a risk factor to the Australian grain Industries continued use of phosphine fumigation. The repeated use of this fumigant must be considered as a risk factor in selection of resistance regardless of how well the fumigation is conducted. Management of the pest is really a strategy to prolong its use as long as possible.

The effectiveness of the storage for fumigation, the skill of the fumigator and the efficacy of the dose rate being applied are the critical fumigation success factors.   The development of resistance has caused these factors to be considered more closely.

A fumigation is only as good as its weakest point. All fumigations must be conducted in effectively sealed enclosures and be accurately monitored to ensure success. Poor results must be attended to during the fumigation process.

At the time the resistance was discovered and in current general practice, the bunker storage, if constructed correctly and checked for holes and proper closing, was considered to be effectively sealed. The data collected since then indicates this assumption is a stretch at best and often the seal is hopelessly inadequate. It is clear that a standardised test for assessing the integrity of the bunker seal prior to fumigation is a priority. While a test can be devised the data collected will need to be assessed to develop a standard measure of gas tightness with in a bunker storage.

This discussion has highlighted that current practice has increased selection pressure for the development of this resistant strain. It therefore follows that if current practice is modified the strain may be controlled.

STRATEGIES USED TO MANAGE RESISTANCE

In PRF facilities, the primary strategy has been to refocus on the basics of hygiene, sealing and fumigation practice. The use of contact pesticides is not an option in these facilities although it has been used successfully implemented in non-PRF sites. Due to the high level of phosphine required to control these resistant insects, successful fumigation at some sites became impossible. These sites required the use of an alternative fumigation or not residual strategy. The use of sulphuryl fluoride was having some success as a methyl bromide substitute and some studies on longer term fumigations had indicated although more expensive than phosphine it may provide a viable substitute in areas where phosphine was not achieving control. Trials using sulphuryl fluoride at a variety of locations and application rates were carried out and the results indicated efficacy could be achieved using much lower concentrations if the time of the fumigation could be extended to 10-12 days.

At the sites where insects had developed resistance to phosphine, sulphuryl fluoride was substituted completely for a season. No phosphine was used at any of these sites for this period. The sites were locked down and all areas disinfested using residual chemicals in non contact areas and diatomaceous earths in the grain path. Covers were replaced where they were damaged and the areas of wet grain along the peak were removed. All tarps and grain handling equipment was routinely cleaned and disinfested.   All areas of refuge were

eliminated or treated. The data collected on populations detected at these sites indicated that although the strategy had controlled the insects during the season the resistance was still present in most sites. Some sites did not detect insects for six nine months post fumigation.

As well as targeting the sites where resistance was known, the sulphuryl fluoride was substituted for phosphine at all sites for the fumigation of carry over grain and as such providing a break from phosphine in the selection cycle.

Apart from the significant increase in cost of this fumigant (in comparison to phosphine), several issues were highlighted from these trials. The fact that a significant increase fluoride levels were detected on the fumigated commodity is one such issue. Although the levels were low, the levels after repeat fumigations exceeded background levels. As such this is an area to be further investigated. Another issue was the significance of sorption onto the commodity was under estimated and varied significantly between products and quality.

A major observation from the substitution exercise was that the issues discussed above, which were thought to contribute to the phosphine resistance development, were significant in any control failure with sulphuryl fluoride fumigation.

The concluding comment from this observation must be that regardless of the chemical being used the basics are critical to successful control and resistance management.

CONCLUSION

The success of PRF facilities in delivering insect free and pesticide residue free grain is only possible if pest management programs are seriously implemented and monitored.

Fumigation rotation/substitution presents as a solution to current control failure due to resistance and resistance management or phosphine resistance in the future. However if the failures of the storage system and fumigation practice are not changed the risks of resistance selection will remain to challenge all fumigants utilised.

The use of rotation of fumigants can only be successful if all industry participants agree to be involved in a coordinated effort.

The discussion has highlighted several issues which still need attention.

The suitability of reused bunker covers for next season needs to be assessed against a standard in a uniform methodology.

The use of routine fumigations as a scheduling issue rather than a pest management strategy is questionable and needs to be reassessed.

The bunker as a sealed storage needs to be assessed with a pressure test or similar, prior to fumigation using a uniform comparable standard.

Systems to prevent the presence of moisture in the fumigation enclosure need to be developed urgently. Should bunkers be aerated, have vents in them or be regularly uncovered to highlight problem areas.

Fumigants may need to be recirculated within the bunker to ensure even distribution of chemical in the commodity.

The industry needs to agree to a coordinated approach to any fumigant rotation strategy.

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AN INDIVIDUAL-BASED TWO-LOCUS MODELLING OF PEST CONTROL IN A SPATIAL HETEROGENEOUS STORAGE FACILITY WITH PEST IMMIGRATION

Mingren Shi[1*], Michael Renton[2]

[1]M084, School of Plant Biology, FNAS, The University of Western Australia
35, Stirling Highway, Crawley, WA, 6009
[2]Cooperative Research Centre for National Plant Biosecurity, Canberra, Australia
[*]Corresponding author's e-mail: *shi.mingren@gmail.com*

ABSTRACT

The grain industries world-wide are confronted with a severe problem of pest control. Computer simulation models can provide a relatively fast, safe and inexpensive way to weigh the merits of various management options for pest control. We constructed an individual-based two-locus stochastic model to investigate the impact of two important issues on controlling a very serious cosmopolitan pest of stored grain, lesser grain borer (*Rhyzopertha dominica*). One issue is the consistency of phosphine dose achieved within a spatially heterogeneous storage facility and the other is the immigration rate of the adult pest. To test how consistency of dose affects the pest infestation, we assumed that each beetle actually experiences a different dose to every other beetle. This is generated individually according to a power law distribution. These different doses represent various factors in the storage facility including varying amounts of phosphine circulation, physical and chemical reactions etc. We also considered different immigration rates. These represent the movement of pests from outside to inside a storage facility, the amount of grain hygiene used around the facility, and the degree of proper sealing of the facility, etc. Based on the available data, we use a two-locus model, with two alleles at each locus, giving nine possible genotypes in total. We set up the initial resistance allele frequencies for the beetles so that the equilibrium frequency for the strongly resistant genotype was 0.1. The simulation results showed that when the dose achieved within the silo is very inconsistent, there will always be a problem for population control, especially if immigration rate is high.

Key words: Individual-based, two-locus, pest control, lesser grain borer, phosphine dose consistency, immigration rate.

1. INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica,* is a very destructive primary pest of stored grains. There is a world-wide need for the development of sustainable management strategies to avoid the evolution of resistance and to control pest infestation. Computer simulation models can provide a relatively fast, safe and inexpensive means to weigh the merits of various management options.

In this study we extend our individual-based two-locus model to include spatial variability in dose and immigration of adult insects. This enables us to address two main management questions:

- Q1: How does the consistency of dose achieved within the storage facility affect the evolution of resistance to phosphine and population numbers?
- Q2: What is the impact of immigration rate of adult beetles on the evolution of resistance to phosphine and population numbers?

2. METHODS

2.1. Overview of the model

The overall model dynamics for an individual are illustrated in Fig. 1. Our models run on a daily time step. A number of processes within the simulation are determined stochastically. Full details of the processes and simulation steps have been provided previously (Shi et al, 2012a, 2012b).
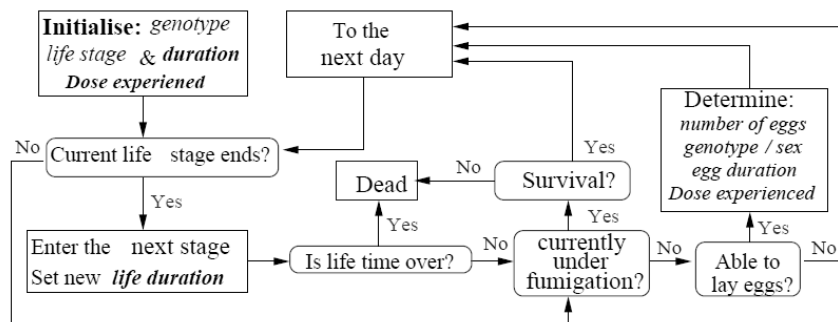
Fig. 1- The simulated dynamics for individual beetles at each daily time step during the simulation.

2.2. Two loci and nine genotypes

In our two-locus model, there are two possible alleles on each of two loci, meaning nine genotypes in total (Table 1, Shi and Renton, 2011).

2.3. Parameters

The model parameters take the same values as those in our previous papers (Shi *et al*, 2011, 2012a, 2012b; Shi and Renton, 2011). We set the number of starting female (a 1:1 sex ratio was assumed) beetles to be 100,000 (100K) and the initial frequencies for the nine genotypes are as follows:

$$\begin{matrix} ss & hs & rs & sh & hh & rh & sr & hr & rr \\ [0.21860, & 0.20219, & 0.04675, & 0.20219, & 0.18702, & 0.04325, & 0.04675, & 0.04325, & 0.01] \end{matrix} \tag{1}$$

Table 1. The identifiers of nine genotypes (*ss*, *sh*, …, *rr*), in the two-locus model: *s* – homozygous ("homo") susceptible ("suscept"); *r* – homozygous resistant (resist); *h* – heterozygous

2nd gene / 1st gene	s homo suscept	h heterozygous	r homo resist
s homo suscept	**ss** Both homo suscept	**sh** 1st homo suscept & 2nd heterozygous	**sr** 1st homo suscept & 2nd homo resist
h heterozygous	**hs** 1st heterozygous & 2nd homo suscept	**hh** Both heterozygous	**hr** 1st heterozygous & 2nd homo resist
r homo resist	**rs** 1st homo resist & 2nd homo suscept	**rh** 1st homo resist & 2nd heterozygous	**rr** Both homo resist

2.4. Addressing Q1 regarding the impact of dose consistency

In this study, the starting target fumigation dose is selected as $C_0 = 0.15$ mg/l for 14 days. The total survival rates for this treatment are 3.114×10^{-5} for *rr*, 3.341×10^{-12} for *rh* and $< 5.14 \times 10^{-29}$ for the other seven genotypes. However, each beetle actually experiences a different dose to every other beetle, due to spatial heterogeneity within the silo. To model this variability, the dose that each beetle is exposed to is generated individually according to a power law distribution defined by a parameter k. This parameter k depends on the maximum or nominal target dose (d_{max}) and the median dose (d_m) in the following way:

$$k = \log(0.5)/\log(d_m/d_{max})$$
(2)

We generate a uniformly distributed random number p for each individual and then the dose d experienced by the individual is yielded from:

$$d = d_{max} p^{1/k}$$
(3)

This ensures that the expected median dose over many individuals is indeed d_m. We used three median doses ($d_m = 0.14, 0.11, 0.08$ mg l^{-1}) to test how different amounts of variability in dose affects the evolution of resistance and population increase. These represent real factors in a spatially heterogeneous storage facility including varying PH_3 circulations, leakage of PH3 from silo, degree of physical and chemical reactions such as uptake or release of gas from or into grain (sorption-desorption) (Banks, 1989), and so on.

2.5. Addressing Q2 regarding the impact of immigration rate

Immigration was represented by simply adding a number of adult beetles into the population each day of the simulation. We considered four different immigration rates: 0 (no immigration), 20, 100 and 500 (adult beetles per day) respectively. The factors represented by these different rates include hygiene conditions, the degree of proper seal of the facility, and the movement of pests from dirty places outside to inside a storage facility.

2.6. Simulations

All (12) combinations of median doses (3 levels) and immigration numbers (4 levels) were simulated (Table 2).

Table 2. The short-hand identifiers for the 12 combinations of median doses and immigration rates considered in the study, including three cases without immigration and nine cases with immigration (Maximum dose = 0.15 mg l^{-1})

Median dose (mg l^{-1})	No immigration	With immigration (number of immigration per day)		
		20	100	500
0.14 (D14)	D14N0	D14N20	D14N100	D14N500
0.11 (D11)	D11N0	D11N20	D11N100	D11N500
0.08 (D08)	D08N0	D08N20	D08N100	D08N500

3. RESULTS

We ran all of the 12 simulations six times to check for stochastic variation, and found the results were very close to one another each time. The important patterns in the results for the 12 cases when the initial frequency of the *rr*: *f*(*rr*) = 0.01 are summarized in Table 3. These show that:

- In the four cases with a very consistent high dose (D14) population numbers are zero or close to zero.
- In the case with medium consistent dose (D11)
  - if the immigration is high (D11N500), population numbers are increasing
  - for other immigration rates or no immigration (D11N0, D11N20 and D11N100) population numbers are decreasing
  - In the four cases with a very inconsistent dose (D08), population numbers are all increasing.

There is an important transition point at the case D11N100 where the pattern changes from decreasing when the immigration rates are not more than 100 per day to increasing when the rate is higher than 100 per day.

On the other hand, the corresponding proportions of the *rr* beetles (Prrs) at the end of each of eight fumigations are as follows

- In the four cases with a very consistent high dose (D14) the *rr* proportions are zero or in a stable interval.
- In the case with medium consistent dose (D11)
  - if there is no immigration (D11N0) the *rr* proportions are increasing up to 100% and then decreasing down to zero.
  - if there are immigrations (D11N20, D11N100 and D11N500), the *rr* proportions are decreasing and then stable varying in a small interval.
- In the four cases with a very inconsistent dose (D08), the *rr* proportions are all increasing.

Table 3. Patterns of total (local minimal) population numbers (TPNs) and corresponding *rr* proportions (Prrs) at the end of fumigations listed. The following notation is used:

- $_{N1}{\uparrow}^{N2}$: TPNs or Prrs increasing over time with each fumigation from N1 up to N2
- $^{N1}{\downarrow}_{N2}$: TPNs or Prrs decreasing over time with each fumigation from N1 down to N2
- ~[N1, N2]: TPNs or Prrs remain relatively stable within a small interval [N1, N2]
- #0: TPNs or Prrs are all zeros at the end of each fumigation

Median dose (mg l^{-1})	Immigration number (per day)			
	N0	N20	N100	N500
TPN				
0.14 (D14)	#0	#0	#0	~[8,12]
0.11 (D11)	$_{84}{\downarrow}\,\#0$	$_{81}{\downarrow}\,{\sim}[8,14]$	${\sim}[94,98]{\downarrow}\,{\sim}[47,53]$	$_{114}{\uparrow}\,{\sim}[270,293]$
0.08 (D08)	$_{960}{\uparrow}^{4500}$	${\sim}[752,1017]$ $_{}{\uparrow}^{1800}$	$_{942}{\uparrow}^{2749}$	$_{1181}{\uparrow}^{8825}$
Prr				
0.14 (D14)	#0	#0	#0	~[0.08, 0.4]
0.11 (D11)	$_{0.66}{\uparrow}^{1.0}{\downarrow}\,\#0$	${\sim}[0.65,0.69]{\downarrow}_{0.38}$	${\sim}[0.57,0.59]{\downarrow}_{0.45}$	~[0.49,0.61]
0.14 (D08)	$_{0.23}{\uparrow}^{0.99}$	$_{0.23}{\uparrow}^{0.79}$	$_{0.22}{\uparrow}^{0.54}$	$_{0.22}{\uparrow}^{0.44}$

4. CONCLUSION

It can be seen clearly from the above pattern analyses that phosphine dose consistency is the key factor in avoiding evolution of resistance to phosphine and population increase in *R. dominica*. When a high and consistent dose is achieved, there is no problem with population numbers or evolution of resistance, regardless of immigration rate. When the dose achieved is very inconsistent, there is always a problem with population numbers or evolution of resistance, more so as immigration rate increases.

ACKNOWLEDGEMENT

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ERADICATION OF A STRAIN OF A STRONG PHOSPHINE RESISTANT INSECT POPULATION FROM STEEL FARM SILOS IN WESTERN AUSTRALIA

Christopher R Newman[1,2], Robert N Emery[1,3], Michelle Chami[3]

[1]Department of Agriculture and Food Western Australia
[2]Grains Research and Development Corporation
[3]Cooperative Research Centre for National Plant Biosecurity
*Corresponding author's e-mail: *chris.newman@agric.wa.gov.au*

ABSTRACT

Phosphine has been readily available to Australian farmers for control of grain insects since the late 1940s. The original product label stated that it could be used in unsealed grain storages by admixture with the grain stream. Repeated use in these unsealed storages created selection pressure on local grain insect populations resulting in escalating phosphine resistance frequency and resistance factors.

Western Australia (WA) exports over 80 percent of the annual grain crop into discerning international markets and, after insecticides were banned in the mid 1980s, the state has relied on phosphine at export to meet the mandated nil-tolerance standards for exported grain. Phosphine fumigation is also the main insect control on farms in WA and to protect the export market it is imperative to limit further development of resistance and to prolong the useful life of this important grain treatment.

A phosphine resistance monitoring, mapping and management program was established in WA during the early 1980s to collect samples of grain insects from farms, merchants and central storages for phosphine resistance testing. This program has studied over 19,000 insect strains using a combination of quick screening assays with follow-up confirmatory international standard FAO tests allowing phosphine resistance to be traced to individual premises. In 1996 WA joined an industry-funded national grain insect resistance monitoring program and now shares data and techniques with laboratories in Queensland and New South Wales.

Weak phosphine resistance frequency continues to increase in WA at the rate of about five percent of strains tested each year. However it was the first detection of strong phosphine resistance on a farm in eastern wheatbelt of WA in June, 2008 that initiated a management program to assess the practicality of eliminating the strain of rust red flour beetle, *Tribolium castaneum* (Herbst).

Surrounding farms were initially surveyed for strong resistance to provide confidence that an eradication program on the property would be worthwhile. No additional strong resistance was found nearby and the eradication project commenced in late 2009 and was considered a success when follow up insect samples taken from the farm through 2011 were tested using the FAO protocol at the Department of Agriculture and Food WA (DAFWA) and determined to be no longer strongly resistant.

Key words: resistant grain insects, grain storage, phosphine, monitoring, eradication, fumigation, sealed silos.

INTRODUCTION

Since aluminium phosphide (AlP) was developed and released for stored grain protection worldwide it has been misused in many storages that are not compatible with good fumigation practice. For example early product labels in Australia indicated that the AlP could be used in unsealed storages; this meant that phosphine was well-received by grain storage managers because there were few, if any, gas tight storages used in Australia at that time and phosphine provided an apparently quick fix for an intractable grain insect problem.

Doubtless there were survivors from each of these poor fumigations and, where the grain needed to be treated multiple times due to reappearance of grain insects, selection of insects with a genetic tolerance to phosphine quickly occurred. Over the last 40 years this continued selection pressure has resulted in elevated incidence of phosphine resistance. Over the last 10 years in association with elevated use of phosphine as exporters moved away from protectants, some phenotypes of grain insects have progressed to very strong resistance. Collins (2006) reported that from 1997 there appeared a quantitative change in resistance levels in four of the five major species and he predicted that strong resistance will become a major problem in Australia unless appropriate management is enacted.

A critical prerequisite to resistance management is anticipation of resistance before control measures actually fail (Brattsten, 1986) and with this in mind the Western Australian Department of Agriculture initiated monitoring of both farms and central storages for phosphine resistance in 1984 (Emery, 1994). Resistance monitoring should serve a purpose whether it is a contribution to a resistance management strategy or to support eradication/containment. Where initial resistance is localised, early warning allows eradication to be implemented before the infested bulk is moved or placed into the market. In addition resistance monitoring programs can provide useful information for a grain storage extension program by highlighting situations and practices that may be selecting for resistance and provide localised advice to remediate the practice.

In Western Australia (WA) the development of phosphine resistance has been slower than the other states. This is likely due to several factors:

- the WA central storage system moved to gas tight storage in the 1980s;
- farm silo manufacturers commenced producing gas-tight silos for growers at the same time as the bulk handlers and the majority of farms now have one or more sealable silos (Newman 1996);
- there is less grain stored on WA farms than in other states;
- a strong extension campaign promoted effective fumigation practice and improved stored grain management by farmers through education;
- high intensity grain insect sampling from farms for resistance testing kept the need for sealed storage at the forefront.

A broad extension campaign has been underway since the early 1980s in WA (Newman 2010) which focused on the need to improve management of grain stored on farms, principally to receive the maximum economic benefit from the treatment, but also to slow the escalation of phosphine resistance.

Phosphine is vital to WA export grain and has been exclusively used by CBH Group (the major bulk grain handling company in WA) since 1990. The difficulty WA faces is that this product is also freely available for use by grain growers who store grain for sale, feed or seed in their farm silos. There is concern across the industry that strong resistance that develops on farms through ongoing inadequate treatments, may inadvertently be transported

to the central handling system, merchants and exporters thereby increasing the cost of controlling the pests.

Phosphine resistance monitoring of farms and central storages in Western Australia since 1985 has shown a steady rise in the frequency of weak resistance status across all species and Fig. 1- highlights the increase in weak resistance in *Tribolium castaneum* from under 20 percent in 1985 to more than 75 percent in 2012. Research and experience in other states has shown that when the frequency of weak phosphine resistance in a grain insect population reaches ~80 percent it can be expected the population will soon become strongly resistant (Collins, 2006b). Genetic analysis revealed that weak resistance is controlled by one major gene and that it was this gene plus the selection of a second resistance gene (that has little effect on its own) that produced the strong resistance phenotype (Schlipalius et al., 2002).
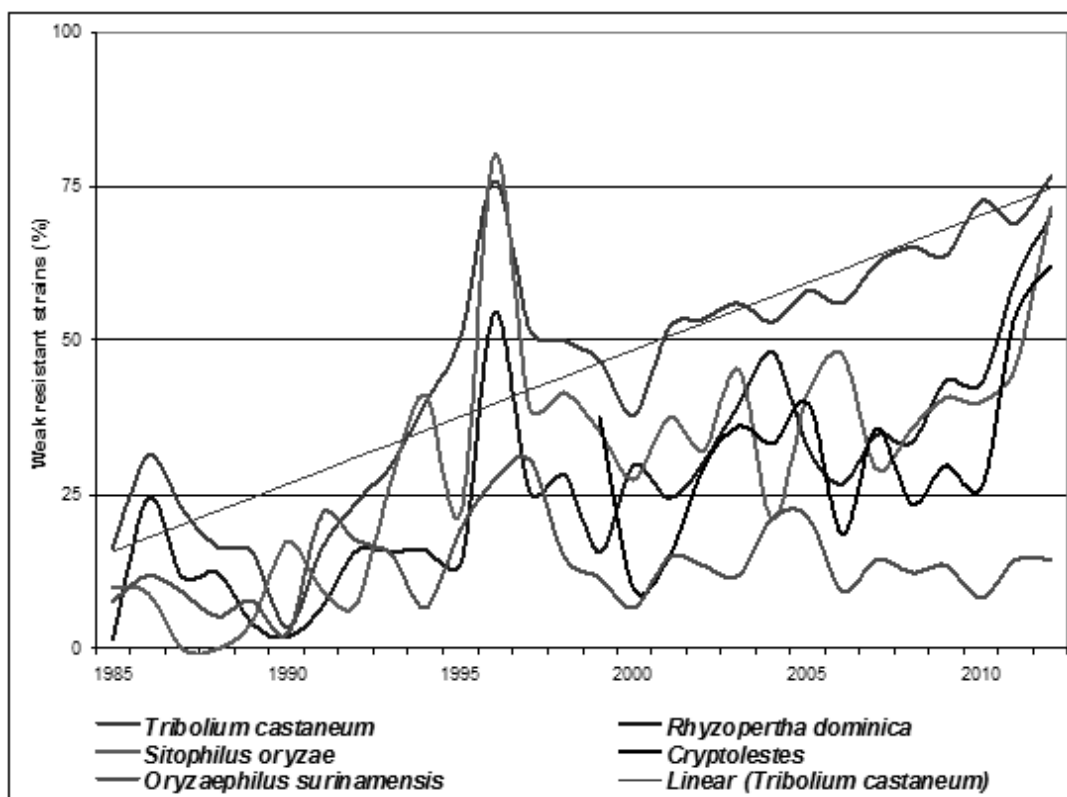
Fig. 1- Frequency of phosphine resistance in major stored grain insect species in Western Australia (Note: 1996 peaks are attributed to defective test gas source).

By 2008 some farms had resident populations of *T. castaneum* that were 100% weak resistant and the overall frequency of weak resistance in WA was approaching 70 percent of strains tested; strong resistance was expected. In June, 2008 there was a detection of strong resistance in *T. castaneum* at Dalwallinu (220km north-east of Perth, Western Australia, 30.2833° S, 116.6667° E).

The strong resistance was initially detected through a random survey that collected seven *T. castaneum* strains all of which tested positive for weak resistance with the rapid screening test (518 individuals, mean weak resistance 73%). There were sufficient numbers of one strain to permit an FAO 20 hour discriminating dose test for weak resistance (80 individuals, 93% weak resistant).

Three strains were immediately tested for strong resistance and all were positive (240 individuals, mean strong resistance 3.3%). These three strains were cultured in the laboratory for confirmatory strong resistance assays but interestingly the resistance was no longer present in any of the cultured strains. The farm was sampled for fresh field insects between July and October 2009 and three additional *T. castaneum* strains were tested with the rapid test and proved to have an average resistance of 57 percent. Two of these strains showed strong resistance at a frequency of one percent.

Laboratory cultured strains were retested for strong resistance in February and August 2010 and again in July 2011 with 33 additional assays. Strong resistance again could not be confirmed despite 3,610 laboratory reared individuals being assayed.

Sub-cultures of the two confirmed strongly resistant strains were sent to DAFWA's sister laboratory at Department of Agriculture, Forestry and Fisheries, Queensland, in north-eastern Australia, however their laboratory testing was also unable to replicate positive tests for strong resistance on these cultured strains.

As the property was relatively isolated and field strains exhibited strong resistance in repeated tests, eradication through correct management principles was deemed to be the most responsible action to protect the Western Australian grain industry.

MATERIALS AND METHODS

In Western Australia a network of about 40 field Biosecurity Officers conduct random and targeted inspections of farms to collect grain insect strains for resistance monitoring. Targeted samples are taken from sites with a history of poor storage practice or resistance while data from random inspections can be used to compare resistance frequencies between storage methods and regions. Collected strains are sent to the DAFWA South Perth Entomology laboratory under bio-secure packaging (Glock and Hall, 2010) for resistance testing.

Follow-up visits to the infested Dalwallinu property and grower interviews determined that AlP in tablet formulation had been applied annually for approximately 11 years using the ineffective and dangerous method of punching holes in the product container and hanging it in the silo headspace (Fig. 2). The gas evolves very slowly from the container due to limited air moisture penetration and is most likely to be lost from the silo quickly through poor seals on the top lids. This practice provided ideal conditions for selection of resistance in the storage.

T. castaneum strains collected in this study underwent a preliminary phosphine resistance screening rapid test following the method described by Reichmuth (1992) which exposes insects to 1mg/L gas for a period of 30 minutes. The test is scored as 'knock-down' for any insect that is incapable of co-ordinated movement. Insects not knocked down in this test were considered to have some level of resistance and, if sufficient insects were present in the field sample, an additional test using the United Nations Food and Agriculture Organisation (FAO) prescribed procedure of 0.05mg/L phosphine for an exposure time of 20 hours followed by 14 days holding time to confirm resistance (Anon, 1975).

Strong resistance was determined using an extension of the FAO method outlined by Daglish and Collins (1999). For *T. castaneum* this discriminating dose test is 0.25mg/L

phosphine for an exposure time of 20 hours, followed by seven days holding time. Survivors of this test that behave normally are assessed as strong resistant.

Confirmation of the emergence of strong resistance was a major concern for WA, so only FAO strong discriminating dose tests were conducted on subsequent strains in order to maximise numbers of fresh field insects available for testing. Where possible test gas concentrations were verified using a Varian gas chromatograph to measure correct assay dosages.

Fig. 2- Silos fumigated by punching holes in AlP containers and hanging in the headspace.

Properties contiguous with the target property were inspected and insects collected to determine their resistance status, none were found to have strong resistance indicating an eradication program would have some chance of success.

The infested Dalwallinu property had nine 40-70 tonne silos of which eight were found to be sealable, but had various faults due to ongoing lack of maintenance, and one unsealable silo. Remedial work was undertaken on the sealable silos by replacing rubber seals in the inlet and outlet ports and refilling the pressure relief valves with oil. The remaining older silo was not manufactured as a sealed unit and would need special treatment.

A hygiene program was enacted cleaning up spilt grain, disposing of derelict grain and removing dry grass around the silos. (Fig 3) The concrete silo pads were cleaned and silos were sprayed with Deltamethrin. Care was taken not to contaminate any grain because contact insecticides have been banned from farm use on farm-stored grain in Western Australia since 1993 (Dean 1994).

Preparation was made to eradicate by applying correct fumigation practice. Previous studies by Collins et.al. (2000) showed strong resistance in *Rhyzopertha dominica* (Fabricius) could be controlled provided the registered rates of 0.3 mgL^{-1} for 10 d <25°C and 1.0 mg L^{-1} for 7d >25°C were maintained.

Pressure testing of the sealable silos ranged between 5 and180 seconds. Previous fumigation studies by Newman et.al (2004) showed that silos exhibiting a pressure half-life of 180 seconds were needed for an effective fumigation and therefore the lower figure was not acceptable for effective fumigation. However, the eradication fumigations were conducted under these conditions because the cost and time required for full retro sealing of the poor silos was restrictive and it was reasoned that this fumigation soon after harvest would quickly eliminate any adult insects that were present.

Fig. 3- Clean away of spilt grain.

Fumigation of all silos commenced mid December 2009 using tablet formulation AlP at 1.5g /m³ on warm grain that was over 30°C. The gas concentration readings were taken on day 7 with a Canary Company SiloChek electronic monitor at the base of the silos. Three silos showed zero phosphine and they were recharged with 1.5g/m³ of AlP. The other 6 silos returned gas concentrations between 7 and 1738 ppm with the higher concentration found in the best sealed silo that achieved a half-life pressure test of 180 seconds.

Commercially available Storgard® pitfall traps and in-house developed pitfall traps made by; drilling 1 mm holes in pvc tubes and open drink cans covered in 1 mm flywire mesh were inserted into the headspace of all silos two months post-fumigation. It was hoped that these traps would verify the effectiveness of the treatment.

Seven of the silos were found to be free of insects however, some T *castaneum* were trapped in the unsealed silo as well as one of the poorer silos. Both silos were re-fumigated and the grain out-loaded for seed. A tub (Fig. 4) containing approximately 25 kg of whole grain was installed under the silos, to attract and trap any insects that may have flown onto the property. Smaller perforated containers holding flour and rolled oats were buried in the whole grain contained in the tub.

There was some uncertainty the strong resistant strain had been eliminated in the first year because of survival in the unsealable silo and the presence of insects in the tub trap under the silos. Accordingly, two months prior to harvest 2010 a follow-up hygiene procedure was conducted on the silo area in October. All empty silos were dusted inside with ~300g of Dryacide® according to the recommended practice, (GRDC 2010). Follow up fumigations were conducted soon after loading in December 2010 using bag chain formulation of AlP at ~1.5g/m³. The unsealable silo was not fumigated with phosphine based on the experience of the first year when insects survived. Monitoring of the fumigations was not conducted but pitfall traps were installed in February 2011. Insects were found in one silo in April it was re-fumigated with a bag chain of AlP at 1.5g/m³.

Fig. 4- External insect trap.

The older unsealable silo contained some insects which indicated that a residual population of potentially strong resistant insects remained on the farm and likely to move into other silos. This is a dilemma when attempting to eliminate a potentially damaging population of resistant insects or quarantine pests. We were aware that Ethyl Formate had been used with some success in other unsealed silo situations (Ren, 2006) to control insect pests so an experimental treatment was set up to use Ethyl Formate in this silo in May 2011 to eradicate the last of the insects. (Newman 2012 – these Proceedings)

Insect samples were removed from the external tub trap under the silos twice and in October 2011 all grain was removed and all insects submitted to the DAFWA grain insect resistance testing laboratory for strong resistance analysis. No strong resistance was detected and the eradication was considered complete and the response team stood down.

CONCLUSION

The property was declared free of strong resistance after 18 months of follow-up inspections, sampling and resistance testing and we concluded that this strain had been created on-farm through poor fumigations in unsealed silos over many years.

The steady development of phosphine continues to be a major threat to the Australian stored grain industry. The national resistance monitoring work currently conducted through the Cooperative Research Centre for National Plant Biosecurity and supported by Australian farmers through the Grains Research and Development Corporation is unique in the world and provides a detailed resistance picture. This information provides the opportunity for strategic planning and tactical response.

The Dalwallinu eradication demonstrated that an effective insect resistance testing program can provide early warning of strong resistance infestations and that application of standard label rate of AlP and recommended grain management principles can eliminate strongly resistant strains of *T. castaneum*. The farm will continue to be visited under the ongoing DAFWA grain insect resistance monitoring program.

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WEB BASED FUMIGATION MONITORING FOR QUARANTINE FUMIGATIONS PART IV

D .Glennon[1*], A. Caravello[2], A. Volpicelli [3]

[1] Spectros Instruments, Inc., 1643 Warwick Ave., PMB127, Warwick, RI, 02889 USA
[2]Root Seven Technologies, 216 Vernon St, Norwood, MA 02062 USA
[3]Diversified Measurement & Control, 17D Airport Road, Hopedale, MA, 10747 USA
*Corresponding author's e-mail: *dglennon@spectrosinstruments.com*

ABSTRACT

The predominant measurement technologies for fumigation gases over the past 60 years include colorimetric tubes, photoionization detectors and thermoconductivity detectors. Their limitations and inaccuracies are well known and documented. In the past fifteen years advances in non-dispersive infrared monitors (NDIR) have increased their adoption as a valuable fumigation tool. Any compliant fumigation monitor must be accurate, reliable and affordable. Stored Product Protection has additional requirements in remote regions such as Central China and Western Australia. In these cases the value of real time access via the internet to fumigation data collected with NDIR Technology from a remote location adds heretofore unknown benefits. Allocation of manpower and materials resources are optimized by access to information about fumigant gas levels in grain storages via the internet. Data is automatically transferred to a central database that can be accessed in real-time from any location with internet access. Intelligent monitors with built-in diagnostics tracking barometric pressure, temperature, sample flows and detector voltages are described. This data collection, data warehousing and reporting platform maintains measurement traceability to certified compliance with secure, encrypted electronic notebook format. Knowing REAL phosphine concentrations allows informed decisions to be made to achieve required CxT and avoid situations leading to target pest phosphine resistance.

Key words: NDIR, non-dispersive infrared, fumigation gas monitors, phosphine, web, internet, remote access

INTRODUCTION

Stored Product Protection requires a compliant fumigant to be applied as a gas and achieve penetration within the grain mass. Control of insect populations necessitates precise phosphine fumigation control and accurate gas concentration measurements. Phosphine has achieved premier status as the fumigant most used worldwide. It is inexpensive, offers good results when used correctly and leaves no residues but also has unique requirements for accurate measurement.

At the present time stored grain is heavily reliant on phosphine to eradicate infestations. The warmer climates have increased likelihood of more widespread insect occurrence stored grains. Countries such as Australia have used phosphine since the 1950s. As the need for low

chemical residues on grains was mandated on international markets through the 1980s; phosphine became the viable solution and its use increased significantly through the 1990s. World-wide, some estimate that phosphine is used over 80% of the time in grain storage/pest control applications.

<div align="center">PHOSPHINE RESISTANCE</div>

Along with the increased use of phosphine there has been a well-documented increase in the frequency of global resistances of major target pests. This resistance to phosphine is a major challenge to the worldwide grain market. Insect resistance to phosphine occurs because of improper application of the product usually applied as aluminum phosphide tablets under various trade names. In grain storages these react with moisture in the air to release phosphine gas. This can take only a day at high temperatures, or as long as four days at low temperatures. The gas moves around by diffusion and in air currents inside the silo. Phosphine leaks in non-gas tight silos are quite common as shown in Figure 1.
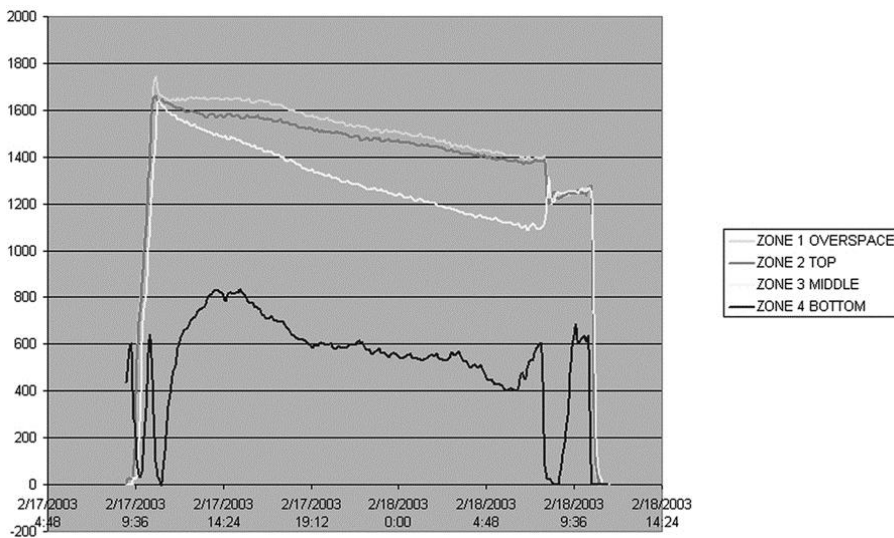
Fig. 1- Four-Position phosphine fumigation at a grain processing facility. Each line represents one sampling point of gas concentration vs. time and shows compromise (loss of gas) over time at one of sampling positions (Zone 4). Courtesy of Fosfoquim SA.

The widespread use of phosphine gas fumigation in unsealed silos in farm, merchant, and bulk handling facilities has significantly contributed to insect resistance to phosphine fumigations. Also, frequent exposure of insect populations to sub-lethal dosages allows some insects with a new resistance gene to survive treatment and continue breeding, passing on their resistance. Repeat fumigations favour the insects that carry the resistance gene by allowing them to survive, but killing normal, susceptible insects.

When strongly resistant insects are present, phosphine fumigation in an unsealed silo will have virtually no effect on the insects. One key to success is the ability of a silo to pass a pressure test. Compliant Silos that can be sealed well enough will hold the required

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concentration of phosphine for long enough to kill all stages of the insects, including resistant insects.

In addition there is a cylinder delivery system (Horn Diluphos System) which applies pure phosphine avoiding the pyrophoric characteristics of the gas.

MONITORING PHOSPHINE FUMIGATION GAS CONCENTRATIONS

Accurate measurements of phosphine gas concentrations will increase the likelihood of successful fumigations. A precise dose level is desired. Situations are avoided where either too little or too much gas is used. Dissemination of measured physical parameters in a timely manner will aide in informed decisions to make this all happen.

Spectros Instruments has shown and proven infrared monitoring to be a superior analytical tool for the practical measurement of fumigation gases as shown in Table 1.

Table1. History of Fumigation Gases Monitored by Spectros Instruments

Year	Fumigation Gas	Development Partners
1996	Ethylene Oxide	Johnson & Johnson
1998	Phosphine	Lorillard; RJR
2004	Sulfuryl Fluoride	Dow Chemical
2005	Methyl Bromide	USDA APPROVED
2009	Ethanedinitrile	Linde

Infrared Spectroscopy measures absolute physical constants and allows monitor immunity to changes in temperature; barometric pressure; relative humidity as well as other interfering gases. Goals of increased efficiency, secured electronic records, compliance proof (traceability) and financial savings have been realized. Confidence in target CxT is achieved. Spectros Instruments has implemented its' latest real world solution for phosphine fumigation monitoring, the PM400 with Fumigation Hub as shown in Figure 2.

Fig. 2- Multi-position fumigation monitor for phosphine with integrated internet/cellular /ethernet communication capability.

Precise measurements of phosphine are now coupled with commonly available communication protocols and data transfer options as shown in Figure 3.
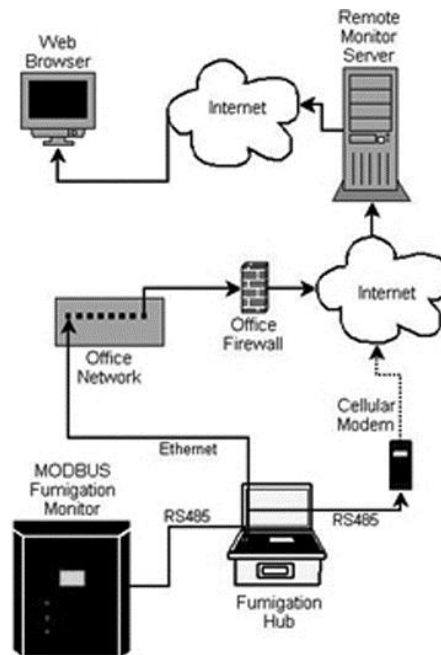
Fig. 3- Multi-position fumigation monitor communication architecture for secure communication of fumigation data to the ethernet, internet, and cellular options.

The Spectros Instruments PM400 Monitor with Fumigation Hub provides communication for remote collection, organization, and reporting of fumigation data that the phosphine monitor collects as well as any alerts generated. There are three ways to communicate with The Fumigation Hub as the LCD Interface, Built in Web Server and Spectros Portal to the Internet. The LCD interface is for local access when no network is available. The Web Server exposes more functionality when a network is available but is not connected to the internet. The Portal is the most functional but requires internet connectivity to show GUI's (Graphical User Interfaces).

LCD INTERFACE

The Fumigation Hub has a built in LCD interface with a keypad for navigation and a set of LED's for notification of operation status.

Moving thru the menu tree presents the Home Screen and Main Menu from which fumigation event jobs may be created, viewed and managed. It is possible to confirm your access to the internet and to get the information for accessing the onboard web application.

WEB SERVER

The Fumigation Hub has a built in Web Server. This web server exposes configuration and diagnostic information beyond what is available via the LCD interface. It also provides a

better method of managing events when an internet connection is not available. This feature is especially helpful for fumigation jobs in remote locations where an internet connection is not available and more information is needed.

FUMINATOR-IR: DESK TOP-PC APPLICATION

The Spectros FuminatIR Software Package is a desktop application that communicates with your Spectros MODBUS monitor and presents the data collected in a variety of formats. It can poll latest readings from an active fumigation, or download historic information from a completed job. It can post information to the Spectros Portal for Remote Access as well as provide real-time multi-position phosphine concentration trends. The console and graphical output are shown in Figure 4.
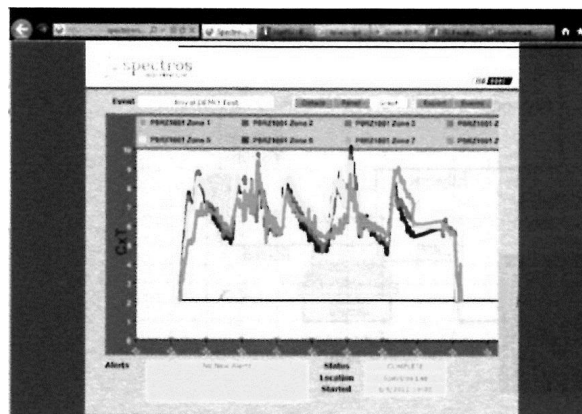
Fig. 4- Multi-position phosphine fumigation monitor showing gas concentration over time. Data is recorded, archived, and transmitted to web host and reviewed remotely.

CONCLUSIONS

Accurate, traceable and accessible phosphine concentration monitoring technology offers a residue free fumigation solution with enhanced safeguards to minimize potential insect resistance. These fundamental advantages will allow an expanding global market to reasonably rely on a compliant, uninterrupted supply chain for stored grain stuffs. Data accuracy and integrity of collection and transfer is key for informed decisions.

PHOSPHINE RESISTANCE AND SOLUTIONS

Pushpaksen Asher*

United Phosphorous Limited Mumbai, India
*Corresponding author's e-mail: *asherpp@uniphos.com*

ABSTRACT

Fumigation managers are concerned about insect resistance to phosphinesince it is a serious problem of insect pests on stored commodity. Phosphine plays a vital role in fumigations based control strategies an insect-free status is the objective to achieve. Many years of experience indicates that weak resistance frequency has increased to the current rate. The presentation demonstrates how improving fumigation practices, can contribute to achieve the desired results and discusses the preventive steps needed to correct the problem. Important feature includes effective sealing of structure to retain the gas long enough exposure time to eliminate all life stages. . Another important step over the course of the fumigation is gas concentration monitoring conducted using a phosphine monitor. The most difficult point for gas to penetrate in the structure / commodity should be monitored. New structures / silos need to be designed to retain gas. There are several reasons why fumigation is not successful. There is no doubt that good fumigation practices also prevent insect survival, which is assumed as preventing further insect resistance. Sampling and laboratory test methods are available to check, if the insect species is resistant. Such test results inform the fumigator what measures need to be taken to increase phosphine fumigation effectiveness. By following correct fumigation practices, it is possible to avoid failures and enhance the life of phosphine as a fumigant. The paper presents results of fumigation using QuickPHlo-R phosphine generator resistant insects with nominal dose of 1 and 1.5 gms/ cub meter.

Key words: Phosphine resistance, storage insects, fumigation practices, phosphine generator

PHOSPHINE RESISTANCE IN MAJOR STORED GRAIN INSECTS IN THE USA

TW Phillips,*[1] GP Opit,[2] MJ Aikins,[1] and MM Hasan[1],[3]

[1]Dept. of Entomology, Kansas State University, 123 West Waters Hall, Manhattan, Kansas 66506-4004, USA
[2]Dept. of Entomology and Plant Pathology, Oklahoma State University, 127 Noble Research Center, Stillwater, Oklahoma 74078-3033, USA
[3]Dept. of Zoology, Rajshahi University, Rajshahi-6205, Bangladesh
*Corresponding author's e-mail: *twp1@ksu.edu*

ABSTRACT

Phosphine gas, or hydrogen phosphide (PH_3), is the most common insecticide applied to durable stored products worldwide and is routinely used in U.S. for treatment of bulk-stored cereal grains and other durable stored products. Research from the late 1980s revealed low frequencies of resistance to various residual grain protectant insecticides and to phosphine in grain insect species collected in Oklahoma. The present work, which employed the same previously established discriminating dose bioassays for phosphine toxicity as in the earlier study, evaluated adults of nine different populations of red flour beetle, *Tribolium castaneum*, and five populations of lesser grain borer, *Rhyzopertha dominica*, collected from broad geographic locations in Oklahoma. One additional population for each species was a laboratory susceptible strain. Discriminating dose assays determined eight out of the nine *T. castaneum* populations, and all five populations of *R. dominica*, contained phosphine-resistant individuals, and maximum resistance frequencies were 94% and 98%, respectively. Dose-response bioassays and logit analyses determined that LC_{99} values were approximately 3 ppm for susceptible and 377 ppm for resistant *T. castaneum*, and approximately 2 ppm for susceptible and 3,430 ppm for resistant *R. dominica*. The most resistant *T. castaneum* population was 119-fold more resistant than the susceptible strain and the most resistant *R. dominica* population was over 1,500-fold more resistant. Results suggest a substantial increase in phosphine resistance in these major stored-wheat pests in the past 20 yr, and these levels of resistance to phosphine approach those reported for other stored-grain pest species in other countries.

SESSION 7

POSTERS

AN EXPERIMENTAL PROCEDURE TO ERADICATE STRONG PHOSPHINE RESISTANT GRAIN INSECTS FROM UNSEALED STEEL SILOS USING ETHYL FORMATE

Chris Newman[1,2,3*], James Newman[1, 2], Hui Cheng[1, 2], Yonglin Ren[1, 2, 3]

[1] Cooperative Research Centre for National Plant Biosecurity.
[2] Post-harvest Biosecurity Laboratory, School of Biological Sciences, Murdoch University, Western Australia.
[3] Department of Agriculture and Food, Western Australia
*Corresponding author's e-mail: *chris.newman@agric.wa.gov.au*

ABSTRACT

Western Australia (WA) relies on phosphine at export to meet the regulated nil tolerance standards for exported grain. Phosphine fumigation is also the main insect control method used on farms in WA. To protect the export market it is important to limit the development of phosphine resistance. To create a gas tight enclosure for a successful fumigation of unsealed silos involves covering them with gas proof sheeting. To reduce the cost of sheeted fumigation a technique of recirculating ethyl formate was used effectively in an unsealed silo on a farm in the mid northern wheat belt of WA to control a strong resistant strain of *Tribolium castaneum*. The technique and bioassay results are discussed.

Key words: *Tribolium castaneum, Rhyzopertha dominica, Sitophilus oryzae, Trogoderma variable*, grain fumigation

INTRODUCTION

Phosphine resistance has become a major problem in many parts of the world. In Western Australia (WA) a phosphine resistance monitoring and management program on across farm and central storage enables collection of samples of grain insects for testing to determine resistance status. When a strong resistance is detected on a farm an eradication plan is activated and for this to be successful all parcels of grain must be treated. Eradication can be achieved in sealable silos which enable efficient control, holding phosphine at a higher dose for a longer exposure period, but unsealed silos on many farms are unable to retain the gas long enough to control all life stages of the insect. To create a gas tight enclosure for a successful fumigation involves covering the unsealed silo with gas proof sheeting. The farm selected for this experimental treatment contained a strong resistant strain of *Tribolium castaneum* (Herbst) created by many years of poor quality fumigations in unsealed silos.

MATERIALS AND METHODS

The 51.5 m³ capacity unsealed steel silo selected for the fumigation trial with Ethyl Formate (EF) was prepared by installing a 90 mm PVC flange on the roof and taping a steel plate with a 90 mm steel flange to the lower grain outlet. A 90 mm i.d. PVC drainage hose was connected from the lower outlet to a fan and then to the headspace (Fig. 1). Bioassays of laboratory reared cultures containing all life stages of *Rhyzopertha dominica* (Fabricius)*, Sitophilus oryzae* (Linnaeus)*, Tribolium castaneum* and *Trogoderma variable* (Ballion) larvae were probed 0.5m into grain in the headspace and placed in the lower silo outlet. Liquid EF at a dose rate of 160g per t was poured onto the top of 50 t of wheat in the silo (Fig. 2). Grain temperature was 25-28ºC. The fan was activated drawing the air/EF mixture from the bottom of silo and blowing it into the headspace at a rate of 122 m³/h for 2 h providing 2.4 internal air exchanges/h.

A control group of mixed age insect cultures were established under the same incubation conditions with the treatment group before fumigation and seven-week post-fumigation. The bioassay comparison of weekly counting of insect adults between the treatments and the controls provided the confidence of fumigation efficacy for all life stages.

Fig. 1- Fan connected to base and headspace of silo by a 90mm i.d. flexible pipe.

RESULTS

After recirculation the concentration of EF as measured on site with a gas chromatograph was evenly distributed through the silo and the recirculation fan was turned off. The concentration × time (*Ct*) product is shown in Table 1 and the inter-granular concentration decay in Fig. 3. The bioassays were retrieved from the base of the silo after 24 h and from the headspace after seven days.

All insects retrieved from the base of the silo were found to be dead. The bioassays retrieved from 0.5 m from the grain peak showed 100% mortality of all life stages of *R. dominica,* and *S. oryzae,* and 100% mortality of *T. castaneum* adults and *T. variable* larvae. Three *T. castaneum* adults emerged at week 6 after fumigation, indicating some egg survival.

Fig. 2- Pouring liquid Ethyl Formate into the top of the silo.

The control bioassay on *R. dominica*, *S. oryzae*, and *T. castaneum* mixed age cultures in seven-week incubation after fumigation gave the reference population sizes in all stages to the fumigated cultures. This is demonstrated by the existing adult numbers (n) at week 0, and the total new emerging adults numbers from week 1 to 6: (123) 689 *R. dominica*, (893) 2047 *S. oryzae* and (356)185 *T. castaneum* adults. 217 *T.variable* larvae were removed from the control in week 0 and no further larvae developed from week 1 to week 6.
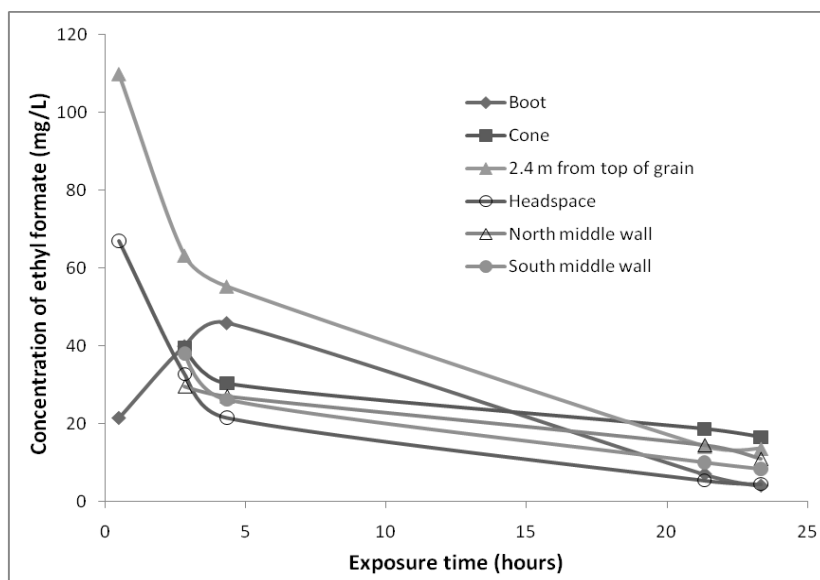
Fig. 3- Inter-granular concentrations of ethyl formate during the fumigation period of 24 hours.

Table 1. Concentration × time (Ct) products achieved at different locations within silo

Location of gas sampling ports	Concentration × time (Ct) products (g h m^{-3})*
Boot	597.1
Cone	550.1
2.4 m from top of grain	909.1
Headspace	396.0
North middle wall	455.5
South middle wall	418.1

*. $Ct = \sum (C_i + C_{i+1})(t_i - t_{i-1})/2$

Where: C is fumigant concentration (g m^{-3})
t is time of exposure (hours)
i is the order of measurement
Ct is concentration × time products (g h m^{-3})

DISCUSSION AND SUGGESTION

The experiment demonstrated liquid EF can be used as an emergency treatment in unsealed silos to control grain insects. The technique of pouring the liquid into the headspace was undertaken in consideration of the flammability of this product, allowing the EF time to vaporise and be partly absorbed as it passed though ~5 m of wheat before reaching the fan.

Emergence of *T. castaneum* after incubation is due to concentrations in the headspace below a Ct=450 g h m^{-3}, shown as a reference level from the previous studies to eliminate all internal stages of the insects. (Ni et al., 2008).

It is suggested that the recirculation should be operated for a period longer than 2 h after full distribution of the vapour to ensure higher concentrations of EF are maintained through the unsealed grain bulk.

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SESSION 8

Quarantine and regulatory issues

Chairpersons:
Thomas W. PHILLIPS, USA
Paul G. Fields, Canada
İlker Ozer, Turkey

GAS PROCESSES, CA AND FUMIGATION, FOR QUARANTINE AND BIOSECURITY

Jonathan Banks*

10 Beltana Rd, Pialligo, ACT 2609 Australia
*Corresponding author's e-mail: *apples3@bigpond.com*

ABSTRACT

Many countries rely on fumigation to eliminate insect pests of quarantine/biosecurity significance from durable goods in trade (import and exports). These goods include grains, wood and wooden materials and derived products. Biosecurity measures, giving an appropriate level of protection, are recognised as legitimate constraints to trade under the WTO SPS Agreement. Fumigation is also used to control pests that damage commodity quality in durable foodstuffs in trade, such as export grains. Some countries and importers may specify fumigation as a requirement for importation.

There is an urgent need to develop and deploy new gas processes (CAF, CA and fumigation) to replace the main fumigants, phosphine and methyl bromide, currently in use. Methyl bromide, though currently still permitted for Quarantine and Preshipment (QPS) uses, is a recognized ozone-depleting chemical. There is a stated policy to replace this fumigant where possible. Phosphine is under threat from development of resistance and the long exposure times required for full effectiveness are logistically inconvenient and costly. Potential alternatives for several major QPS uses include sulphuryl fluoride, cyanogen ('EDN'), carbonyl sulphide, ethyl formate and CAs (this Conference). Development of these alternatives is inhibited by many considerations including small market size, stringent requirements on effectiveness, lack of familiarity with their properties and application.

In postharvest treatment of durables, there is a particular need for locating and proving alternatives to methyl bromide for export grains and pulses against *Trogoderma granarium* Everts and for timber and solid wood packing materials against many pests but including longicorn beetles and pinewood nematodes. Methyl bromide treatments against *T. granarium* is an entrenched use. Its basis is not as firm as would be required of new treatments. Several treatments are under continued consideration for alternatives to methyl bromide (and heat) for ISPM 15.

Use of fumigants on exports requires a working procedure that renders the atmosphere within the treated enclosure safe to entry by personnel, including customs and quarantine officers. There have been several incidents with workers affected by residual fumigant in freight containers and surveys have detected excessive levels in a surprising number of containers in export trade. Several customs and quarantine authorities have adopted ventilation procedures and gas scrubbers that remove gas to safe levels before entry of fumigated containers.

Key words: biosecurity, quarantine, fumigation, methyl bromide alternatives, *Trogoderma*, ISPM 15, scrubbers, recapture, safety procedures.

INTRODUCTION

Many countries rely on fumigation to eliminate insect pests of quarantine/biosecurity significance from durable goods in trade (import and exports). These goods include grains, wood and wooden materials and derived products. The insect pests of concern may be directly infesting the durable goods or may be carried by other materials in particular consignments that are directly infestible. Some pests may be able to complete their life cycles on the goods (e.g., *Trogoderma granarium* Everts on cereal grains and *Cryptotermes brevis* (Walker) on some wooden materials) while others, such as many of the forest pests of biosecurity concern, cannot reproduce and multiply on the goods.

Application of biosecurity measures, such a fumigation of goods against pests of quarantine concern, are inconvenient, costly and are an interuption to free trade. They are subject to numerous constraints, not only to ensure they are used effectively and safely, but also to ensure they are not used excessively.

Biosecurity measures form an internationally recognised and permitted technical barrier to trade under World Trade Organisation (WTO) Agreements on the Application of Sanitary and Phytosanitary Measures (SPS) and Technical Barriers to Trade (TBT) agreements (WTO, 1995). These agreements, particularly the SPS agreement, impose important constraints on how and when quarantine measures can be applied to international trade. International biosecurity measures relating to plants and plant products, including durable commodities, are regulated in detail under the IPPC, with International Standard Phytosanitary Measures (ISPM). Two particular items, important to discussion below, follow as a result of these agreements. Individual countries must develop lists of pests of biosecurity concern and control measures applied against these pest must be set so they achieve an appropriate level of management of risk against establishment of the pest in the importing area.

The importance of fumigation, particularly with methyl bromide, for QPS (Quarantine and Preshipment) purposes is underlined by the exemption of methyl bromide from phaseout under agreed control measures under the Montreal Protocol. This continues, despite the high ozone depleting potential of methyl bromide and continuing discussion on its appropriateness. It is said that the specific exemption from phaseout for methyl bromide for QPS purposes was agreed, in 1992 (UNEP, 1992) and was because at that time it was argued that there were no technically available alternatives to methyl bromide where it was used for QPS purposes. This exemption is unique under the Protocol.

The exemption for QPS purposes under the control measures of the Montreal Protocol does come with some limitations. The International Plant Protection Convention (IPPC) has a stated policy (CPM, 2008) for member states on quarantine measures to reduce and avoid use of methyl bromide. Decisions VI/11 and VII/5 of the Montreal Protocol urge Parties to avoid using methyl bromide and to reduce emissions of methyl bromide where technically and economically feasible.

This paper discusses the potential alternative gas processes for quarantine and particularly to methyl bromide for QPS purposes, describes in detail the use of methyl bromide and alternatives against a key quarantine pest of durables (khapra beetle, *T. granarium*) and against forest pests potentially conveyed in wooden packaging material and the like as managed by ISPM 15, and finally looks at managing one of the problems that arise in use of toxic fumigants for QPS purposes. This is presence of residual gases in treated freight containers in international trade.

ALTERNATIVE FUMIGANTS FOR QUARANTINE AND BIOSECURITY

There is an urgent need to develop and deploy new or renewed gas processes (CAF, CA and fumigation) to replace the main fumigants, phosphine and methyl bromide, currently in use for QPS purposes on durables.

Methyl bromide, though currently still permitted for QPS uses, is a recognized ozone-depleting chemical and international policy requires its replacement if technically and economically feasible. Additionally, though it has a reputation for effectiveness against insect pests, it is not always very effective. If it were a new chemical fumigant and subject to the same rigorous assessment as these new fumigants, it may well not be found acceptable.

Phosphine is under threat from development of resistance and the long exposure times required for full effectiveness are logistically inconvenient and costly. Its very widespread use against pests of durable commodities such as grains is not only its strength but also a weakness. Introduction of highly phosphine-resistant strains of pests into areas at present having only easily manageable resistant strains would represent a major biosecurity breach.

The list of potential candidate gas treatments for QPS purposes is now quite long, as a result of programs to develop new gas processes for pest control. This contrasts with the situation a decade or so ago when fumigants were being lost to use at an alarming rate. This is not a criticism of the reasons for their loss, but an observation that the recent interest in development of new fumigants and revival of disused ones has been productive.

Development of a new process for a quarantine process from concept to approval for use is a long and complex process. Aside from requiring proof of efficacy to a high and reliable level, it also may require registration by regulatory authorities including national pesticide registration authorities and may often involve protracted negotiation between importing countries requiring appropriate biosecurity and exporters wishing to establish or continue a particular trade. In many situations, there is insufficient profit in the particular trade to support the work necessary to establish a new process.

Table 1 gives a listing of potential fumigants for durable commodities likely to be able to produce treatments on their own, or possibly in combination with helpers such as CO_2, heat or other fumigants. Some of these fumigants are still transiting the pesticide registration process, an uncertain, thorough and expensive process. The wide registration enjoyed by methyl bromide and phosphine seems unlikely to be achieved by the newcomers to the process.

It is interesting to compare the list in Table 1 with the fumigants in Table 2 described in Bond (1984).

Many of the papers at this conference describe developments in application of new and renewed fumigants, including some that relate to potential biosecurity treatments.

FUMIGATION AGAINST THE KHAPRA BEETLE, *Trogoderma granarium*

METHYL BROMIDE

The special biology of *T. granarium* poses particular problems with regard to quarantine treatments for this pest. As a larva, the pest may enter a very long lived diapause. In this form the pest seeks out crevices and other harbourage and can be very difficult to find by inspection or access by many normal control measures. Cast skins (exuviae) and trapping may give an indication of its presence, but in general the habits of the pest are such that

fumigation treatments are carried out because of the risk of presence of the pest, rather than because of detection.

Table 1. Fumigants available in some countries or at an advanced but incomplete stage of registration

Fumigant	Comments
Carbon disulphide	Very flammable, out of patent
Carbonyl sulphide	Longer exposures than methyl bromide
Cyanogen ('EDN')	Water soluble, flammable
Ethyl formate	Very rapid action, poor penetration
Ethylene oxide	Carcinogenic, flammable, requires chambers
Hydrogen cyanide	Poor penetration particularly in wet commodities, out of patent
Methyl bromide	Wide registration, ozone depletor, permitted for QPS
Methyl iodide	Registrant withdrawing support from some applications
Methyl isothiocyanate	Poor penetration, may require other fumigant in combination
Ozone	Action needs further definition
Phosphine	Requires long exposure periods
Propylene oxide	Flammable, requires chambers
Sulphuryl fluoride	Poorly effective against insect eggs, leaves fluoride residues

Worldwide the fumigant of choice for quarantine treatment against *T. granarium* is methyl bromide (MB). This is despite its known unusually high level of tolerance to the fumigant, particularly when in diapause. Practical problems with fumigating some oily, high risk commodities, e.g., expeller cake, with methyl bromide further compounds the difficulties. With the very high dosages required for complete kill, there is a risk that residue limits for bromide ion may be exceeded in some treated commodities.

Table 2.'Bond's List.' Fumigants listed in Bond (1984) with threats, real or alleged, to continued use in 1984. From Banks (1994)

Fumigant	Threat
Acrylonitrile	Suspect carcinogen, residues
Carbon disulphide	Lack of interest
Carbon tetrachloride	Ozone depletor, residues
Chloropicrin	almost forgotten
Dichlorvos	Residues, alleged carcinogen
Ethylene dibromide	Environmental contaminastion, fertility effects, alleged carcinogen
Ethylene dichloride	Not very effective, alleged carcinogen
Ethylene oxide	Suspect carcinogen, residues
Ethyl formate	Almost forgotten
Hydrogen cyanide	Lapsed Codex Alimentarius registration
Methallyl chloride	No food registration
Methyl bromide	Ozone depletor, alleged carcinogen
Methyl formate	Almost forgotton
Phosphine	Resistance
Sulphuryl fluoride	No food registration
Trichlorethylene	Not very effective, residues

Historically, dosage recommendation for control of khapra beetle have been based on 'double the normal dosage' for typical stored product pest control (e.g., Bond, 1984 ; p.238-239). The latter are often aimed at $ct = 200$ g h m^{-3} at 20°C, implying $ct = 400$ g h m^{-3} at 20°C for *T. granarium*. This is close to the value of 480 g h m^{-3} for 100% kill at 20°C given by Bell et al. (1985), but less than the Russian quarantine dosage implied by Mordkovitch and Sokolov (1992) of 600 g h m^{-3}. Bogs (1976) give a dosage of 600 g h m^{-3} for >15°C, which may be the origin of this recommendation.

Modern 'khapra' dosages to meet quarantine requirements are typically very high. Australian quarantine requirements are for 80 g m^{-3} for 48 h at >21°C (Dept. of Agriculture, Fisheries and Food, 2009), corresponding to a *ct*-product (CTP) of about 1700 g h m^{-3} on basis of exponential decay of fumigant concentration and end point retention of 20% of applied dosage. The USDA Treatment Manual (T302-c-1, USDA, 2012) specifies a dosage of 96 g m^{-3} with a retention of 30 g m-3 at 12 h at 15-21°C, corresponding to a CTP of about 530 g h m^{-3}. It is of interest to see what level of mortality this actually corresponds to on the basis of published studies. There are no recent published studies as a check on current susceptibilities, so this analysis relies on studies from 20 years ago or more.

During the apparently successful khapra beetle eradication campaign in the 1950's in USA, the dosage recommendations finally adopted correspond to a minimum CTP of about 1200 g h m^{-3} at unspecified temperatures (initial dosage 80 g m^{-3}, 32 g m^{-3} remaining after 24 h) (calculated from Armitage (1958)).

Bell et al. (1985) provide the most modern laboratory assessment of the effect of methyl bromide on *T. granarium* at low temperatures. Exposures used by Bell et al. (1985) are not stated but appear to have been 24-48 h with a few hundred larvae used per exposure and '100% kill' estimated by extrapolation to an undefined level of kill (>99%).

El-Lakwah (1977a) tested the effect of rearing of *T. granarium* at 25 and 28°C and various low temperature conditions on susceptibility of larvae to methyl bromide. Overall, his data shows that there is a range of susceptibility which can be induced by particular rearing temperatures and subsequent handling. Larvae allowed to enter diapause by holding at 25°C appear the most tolerant of the forms he investigated. From the point of view of quarantine control, the dosage should presumably be targeted at the most tolerant form that has a significant risk of occurrence at point of treatment.

El-Lakwah's (1977a) observations at 15°C correspond closely with those of Bell et al. (1985) (*ct*-products for the most tolerant material for 99% kill was 396 and 450 g h m^{-3} respectively) giving confidence that the two data sets can be treated as a single series. El-Lakwah's data also shows that material at least partly in diapause, i.e., held at 25°C or below for at least 8 days, showed increased tolerance to MB compared with material reared at 28°C and cooled quickly (over less than a few days) (e.g., reared at 28°C and fumigated at 12°C required 263 g h m^{-3}, or reared at 28°C, held at 25°C for one month and then fumigated at 12°C requires 420 g h m^{-3} for 99% kill.).

Figure 1 gives data points from various studies on *T. granarium* and methyl bromide, including those requiring highest dosage for kill under particular conditions. The envelope enclosing points of least susceptibility gives a estimate of *ct*-product for control of these forms. Test material at around 10°C appears anomalously susceptible, but larvae not fully in diapause may have been tested. At 21°C the trend line shows a CTP of about 400 g h m^{-3} is required for 99% mortality. This supports modern quarantine dosage requirements where a much higher kill is required and CTPs of 1700 or more are set for 21°C.

At this time no field resistance of *T. granarium* to methyl bromide has been recorded. However, slightly increased levels of tolerance to MB can be selected for in the laboratory

with levels of 2x resistance achieved after many selections (Mordkovich and Sokolov, 1992). While this resistance level is low, it would jeopardise control with standard, already high dosages used currently with MB for quarantine purposes.
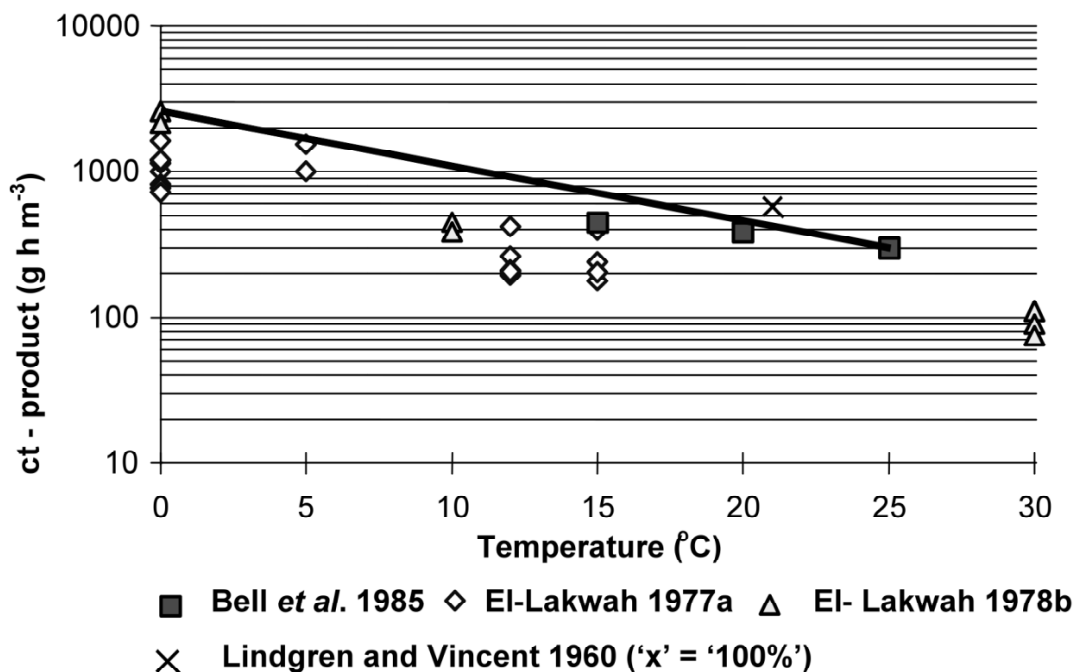

Fig. 1- *Ct*-products of methyl bromide (logarithmic scale) giving estimated 99% kill of *T. granarium* larvae, including those in diapause, for different temperatures. Line indicates envelope of maximum dosage required to give 99% kill of diapause larvae.

PHOSPHINE

Phosphine fumigation is widely used in countries where khapra beetle is common for its control. It is not listed as a quarantine measure against *T. granarium*. Reasons for this appear to be historical - with suitable precautions to prevent leakage and exposure times of 12 or more days, depending on conditions, data available for phosphine action on *T. granarium*, up until the early 1990s, supported consideration of its use as an alternative to MB for this pest. However, as noted below, resistance development may now have rendered this former option inappropriate.

Susceptible strains of *T. granarium*, even as a larva in diapause, is quite sensitive to phosphine. Typical long exposure dosage rates for control of *Sitophilus* species (e.g., 1.5 g m^3 for 7 days) are sufficient to control normally-susceptible *T. granarium* at >20°C, and appear to be so too for some resistant *T. granarium*.

The best studies on action of phosphine against *T. granarium* are those of Bell et al. (1983, 1985) and Hole et al. (1976). There are numerous other studies (e.g., Lindgren et al., 1958; El-Lakwah et al., 1989; Punj and Girish, 1969; Dhaliwal and Lal, 1973), but as these are carried out under conditions where diapause is absent or not adequately proven, they are, at best, indicative only of the relative susceptibility of *T. granarium* to phosphine.

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Bogs (1975) testing (apparently) diapause larvae of *T. granarium* found LT_{99} at 0, 5, 10 and 15°C of 16, 9, 8 and 5 days, respectively at 0.7 g m^{-3} PH$_3$. El-Lakwah (1978a) using larvae reared at 28° (not in diapause) found LT_{99} at 29°C at 80 ppm (0.1 g m^{-3}) after only 34 h (86 h at 20 ppm). From apparently the same test stock (El-Lakwah, 1978b) the LT_{99} at 25°C at 50 ppm was 40 h, 12°C and 150 ppm was 140 h and at 0°C and 50 ppm was 565 h. He provides a variety of other data on this test sample involving effects of rising and falling concentration and other (higher) fixed dosages.

Bell et al. (1983) give a 6 day minimum exposure to give "100%" kill of diapause larvae at 20° using a CTP of 203 g h m^{-3} (dosage applied 1.5 g m^{-3}). They reinforce the conclusion of Hole et al. (1976) that extended exposure even at 30°C was required, with 0.4 g m^{-3} for 2 days giving only 99% control of freshly laid eggs.

The situation with phosphine against *T. granarium* has now changed. Various studies from the Indian subcontinent (Sharma and Kalra, 1998; Alam et al., 1999; Sarfraz et al., 2000; Ahmedani et al., 2007) are showing a high level of resistance to phosphine even in active, non diapause larvae. Sarfraz et al. (2007) studied 3 strains of *T. granarium* that required 14 days exposure at 800 ppm (c. 1 g m^{-3}) phosphine at 34°C/65% r.h. With the expectation that the pests will be more tolerant still at lower temperatures or in diapause, this level of resistance would require excessive exposures to achieve quarantine levels of security.

OTHER FUMIGANTS AND CA

Hydrogen cyanide, chloropicrin and carbon disulphide show some promise as rapid treatments against *T. granarium* including diapause larvae (e.g., Lindgren et al., 1955; Lindgren and Vincent, 1960), but have not been developed to modern quarantine standards. They all will suffer from poor penetration of commodities under some conditions.

Low oxygen CAs show some promise for elimination of *T. granarium* but carbon dioxide-based atmospheres (< 70% CO_2) are less effective against *T. granarium* than most other stored product pests, requiring much prolonged exposure for control of diapause larvae. Annis (1987) concluded that 16 days exposure at 80% CO_2 (20-30°C) was required to eliminate *T. granarium* (data of Spratt et al. (1985), Verma and Wadhi (1978) and Le Torc'h (1983)).

Low-oxygen atmospheres however appear to be quite effective against *T. granarium,* including eggs and diapause larvae (Verma and Wadhi, 1978; Le Torc'h, 1983), requiring the same exposures as other tolerant stored product insect pests. Annis (1987) suggests 0.1% oxygen at 20-29°C for more than 20 days.

CAs have not been tested to quarantine standards for required levels of security.

DISINFESTATION OF WOODEN PACKAGING MATERIAL (ISPM 15)

Disinfestation of wood and wood packaging material remains the largest user of methyl bromide globally, despite widespread use of heat in place of methyl bromide for ISPM 15 treatment of wood packaging material in international trade. Work continues on the search for alternative fumigants to methyl bromide for ISPM 15. To date, no alternatives have been approved to a level that allows inclusion in ISPM 15 revisions.

Table 3 lists the fumigants that have been considered recently as alternatives to methyl bromide for ISPM 15.

Until recently there have been major impediments to approving alternatives to methyl bromide for ISPM 15, with methyl bromide itself not subject to the same level of scrutiny and

requirements. There are signs that this is now changing. TEAP (2012) summarized the changes within the technical committees of the IPPC as:

Table 3. Fumigants under condsideration as alternatives to methyl bromide for ISPM 15 treatment of wood packaging material in international trade

Sulphuryl fluoride
Methyl isothiocyanate / sulphuryl fluoride (Ecotwin)
Hydrogen cyanide
Methyl iodide

Source: CPM. 2011.

"The Standards Committee [of the IPPC] made several recommendations in 2011 that may enhance the prospects for additional ISPM-15 treatments being developed and accepted. Firstly, the Committee recommended that the treatment must be shown to be effective against *Bursaphelenchus xylophilus* (pinewood nematode, PWN) and *Anoplophora glabripennis* (Asian longhorned beetle, ALB). Secondly, the Committee recommended that the current list of pests should be narrowed further to individual species if possible and should also focus on organisms to be eliminated at the point of treatment, i.e., the issue of infestation after treatment should not be considered. Thirdly, any new treatment was recommended to be at least as efficacious as heat and MB that are already approved for ISPM-15. As the efficacy of these two treatments might not be known, the Committee recommended that an expert judgement of their efficacy may be sufficient if exact scientific data were not available, as these data are urgently needed for the approval of new treatments. Fourthly, the International Forestry Quarantine Research Group at its meeting in September 2011 (CPM, 2012) agreed that Probit-9 was impractical for many wood pests and proposed an alternative approach to treatment that did not prescribe an efficacy target."

Some of the proposed replacements for wood fumigation, including for ISPM 15 and unsawn timber, have better penetrant ability than methyl bromide. This potentially addresses a continuing concern that methyl bromide only penetrates large section of wood slowly and may lose efficacy as a result. Phosphine, carbonyl sulphide, cyanogen ('EDN') and sulphuryl fluoride all are more penetrant than methyl bromide, and also show less loss by reaction (Ren et al.*, 1997, 2011).

Treatment of export whole logs continues to be a major user of methyl bromide as a QPS treatment. Some alternative processes are in use for particular trades. The use of phosphine in transit on logs going to China from New Zealand (Glassey, 2005) is well established. A mixture of methyl isothiocyanate and sulphuryl fluoride has been shown to be effective against a number of wood pests, including pinewood nematode (Soma et al., 2001), and is available for use on imported timber in Japan.

RESIDUAL GAS IN FREIGHT CONTAINERS

Continuing use of methyl bromide for QPS purposes brings with it some continuing safety hazards. This is also true for phosphine and, no doubt, will be so for new persistant fumigants as they are adopted. One such hazard of direct concern to quarantine and those involved in quarantine is the presence of residual fumigant gas in treated frieght containers. These may or may not have been well ventilated at point of treatment, but residual fumigant (and other toxic

gases) may be commonly expected when the containers are opened by workers and inspectors including quarantine officials on import.

These are several studies that have been carried out to assess the frequency of presence of residual fumigant, particularly methyl bromide, in imported freight containers at levels dangerous to human health. In one such study, Baur et al. (2010) studied 2113 containers arriving at Hamburg port over a 10 week period in 2006 and found 294 of these with over 0.1 ppm methyl bromide with 23 of these with over 1 ppm methyl bromide. There was a similar incidence of high phosphine detections in other studies.

Some quarantine and customs agencies have deployed forced venting equipment, based on activated carbon recapture technology (Nordiko Quarantine Systems, pers. comm.) to assist in protecting their staff from undue exposure to residual fumigant.

It seems prudent to develop and deploy systems to avoid exposure of workers, inspector and bystanders to excessive fumigant concentrations. This is an important part of sustainable development of new fumigants for quarantine, as well as for continuing use of established ones.

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REMEDIATION OF RESIDUES ON STORED PRODUCT SURFACES USING OZONE-BASED FUMIGATIONS

Spencer S Walse*

USDA-ARS-SJVASC
*Corresponding author's e-mail: *spencer.walse@ars.usda.gov*

ABSTRACT

Evolving environmental and public health concerns surrounding non-target exposure of consumers to pesticide residues, regardless of concomitant toxicological evidence, necessitates the development of safe and effective methodologies for residue minimization. Ozone fumigation, alone and in combination with other gases, was explored as a means for degrading "undesirable" organic residues on stored products. Organic residues sorbed onto model abiotic glass surfaces or onto stored products were fumigated separately in a flow-through chamber at 150 - 900 ± 10 ppmv (μL L^{-1}) reagent gas. Heterogeneous rate constants of gaseous ozone, and its mixtures, reacting with a sorbed organic residue, k_{O3} (M^{-1} min^{-1}), were calculated for all surface types. The kinetics and mechanism of residue removal, supported by gas chromatography- and liquid chromatography-mass spectrometry product analyses, is discussed in the context of facilitating compliance with maximum residue level (MRL) tolerances for stored products. Results indicate that the extent of residue reduction varies with chemical structure; however, most residues can be rapidly degraded via decomposition radicals from ozonide intermediates and subsequent chain reactions involving (e.g., ·OH, ·OOR, etc.). This study provokes a myriad of research opportunities that can be consistent with the overall goal of maximizing degradation of pesticide residues while minimizing unintended toxicity produced by "disinfection/remediation" technologies. Future work will focus on developing ozone fumigation as a remediation technology that is a more universally applicable tool for industries that use chemicals for pest control.

Key words: Ozone fumigation; residue mitigation; residue degradation

INTRODUCTION

Fungicides are applied to control plant diseases that reduce commodity yield, deteriorate quality, and pose a food-borne health risk to consumers (Elad et al., 2004; Sipsas and Kontoyiannis, 2008; Tournas, 2005; Barkai-Golan, 2001). In an initial assessment of the practical feasibility of using gaseous ozone for fungicide residue removal/remediation, the degradative potential of fungicides was comparatively evaluated. Specifically, we report the extent that residue levels of boscalid, iprodione, fenhexamid, cyprodinil, and pyrimethanil decreased on grapes following fumigation with gaseous ozone. Using model glass systems to isolate abiotic contributions toward degradation, we probed the mechanistic role of ozone in fungicide destruction using kinetic profiles and byproduct identification with gas

chromatography- and liquid chromatography-mass spectrometry (GC-MS and LC-MS, respectively).

MATERIALS AND METHODS

Experimental procedures are as described in Walse and Karaca (2011).

RESULTS AND DISCUSSION

Strategies must be developed for the remediation of pesticide residues on agricultural commodity, including stored product, when tolerances of maximum residue levels (MRLs) may not be exceeded. This study provides the first example of gaseous ozone being used in this context and provokes a myriad of research opportunities that can be consistent with the overall goal of maximizing degradation of pesticide residues while minimizing unintended toxicity produced by "disinfection/remediation" technologies. Results indicate that the extent of residue reduction varies with chemical structure, with some residues expected to undergo negligible degradation. However, pesticides amenable to ozonolysis can be rapidly degraded via decomposition radicals from ozonide intermediates and subsequent chain reactions. Future work will focus on developing ozone fumigation as a remediation technology that is a more universally applicable tool for industries that use chemicals for pest control; forthcoming manuscripts will detail efforts to circumvent the structurally selective nature of ozone by developing mixtures, in both the gas and solid phases, to maximize production of structurally indiscriminate radicals (e.g., $\cdot$OH, OOR, etc.) that facilitate decomposition of surface-sorbed pesticides (Palm et al., 1997; Palm et al., 1999; Pflieger et al., 2009). When considering fungicide degradation on fresh produce, technical aspects of ozone fumigation need to be refined and dovetailed with the infrastructural capabilities and challenges of commercial production as well as synchronized with the trade and environmental requirements facing agriculture.
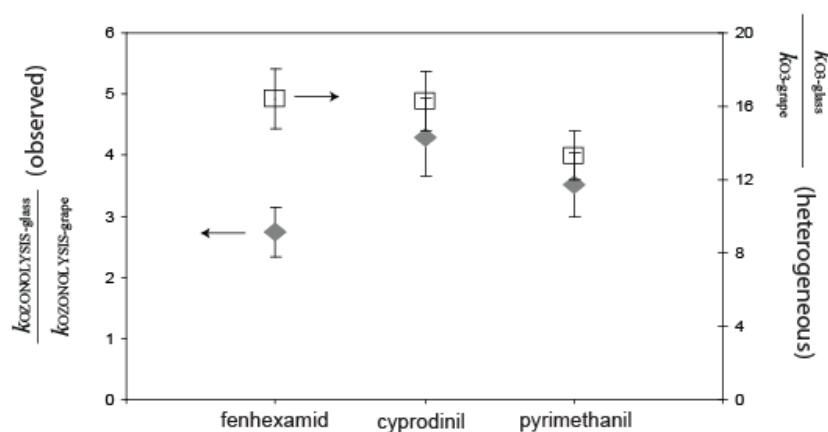
The glass to grape ratio of observable rate constants of ozonlysis, $k_{OZONOLYSIS}$, and heterogeneous rate constants of gaseous ozone reacting with a sorbed fungicide, k_{O3}, which is corrected for $[O_3]_g$. Residue degradation proceeded $\sim$ 15-fold slower on stored product sutrfaces relative to glass, possibly due to preferential reaction of ozone with the stored

product surface and/or inert ingrediants in the commercial formulations that limit acess of ozone to reactive groups of the fungicides.
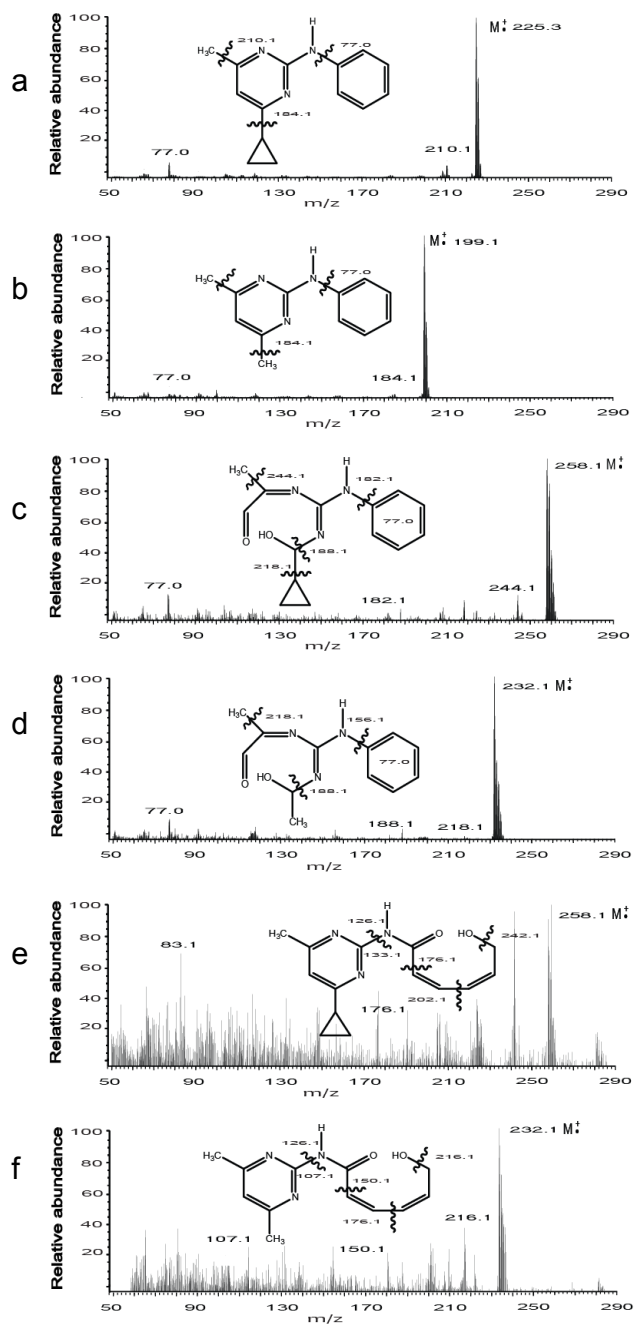
The 26 amu (C_2H_2) mass difference in between the structural analogs, cyprodinil (a, c, e) and pyrimethanil (b, d, f), was a convenient mass spectrometric diagnostic of reactivity for both the aniline and pyrimidine aromatic systems as shown above for GC-EIMS spectra.
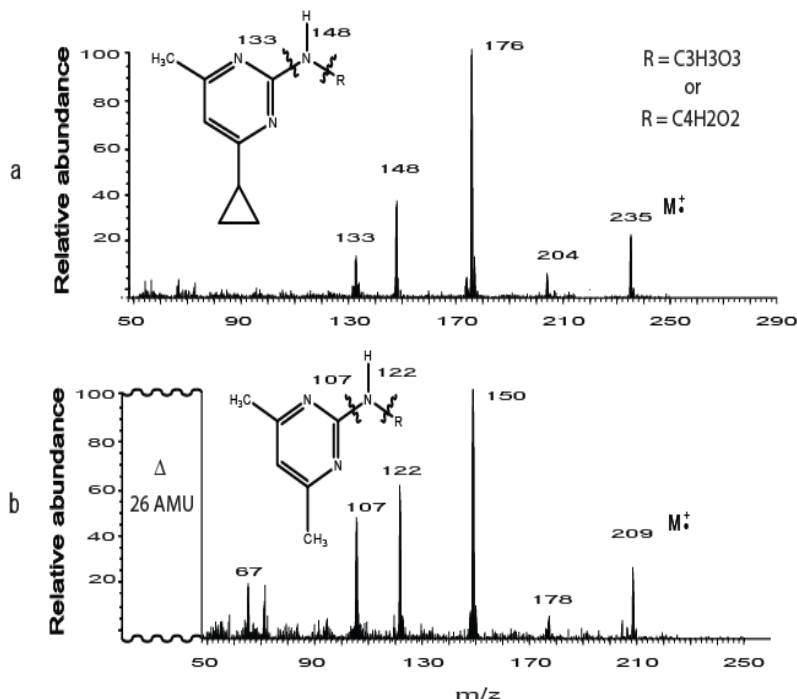
Mechanistic support for the involvement of radical oxidants in the heterogeneous ozonolysis of residues stems from GC-EIMS identification of trace oxidative products with MWs of 235 and 209 for cyprodinil (a) and pyrimethanil (b), respectively, as confirmed by CI GC-MS (not shown); either two (i.e., $C_4H_2O_2$) or three oxygen atoms (i.e., $C_3H_3O_3$) were added to the aniline group in a manner that is not consistent with dicarbonyl addition in either 1-2 (α) or 1-4 (γ) orientation.

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POSTHARVEST TREATMENT OF FRESH FRUIT FROM CALIFORNIA WITH METHYL BROMIDE FOR POSTHARVEST CONTROL OF LIGHT BROWN APPLE MOTH (LEPIDOPTERA: TORTRICIDAE)

Spencer S Walse*

United States Department of Agriculture, Agricultural Research Service
*Corresponding author's e-mail: *spencer.walse@usda.ars.gov*

ABSTRACT

Methyl bromide (MB) chamber fumigations were evaluated for postharvest control of light brown apple moth, *Epiphyas postvittana* (Walker), in fresh fruit exports from California USA. To simulate external feeding, larvae were contained in gas-permeable cages and distributed throughout loads of peaches, plums, nectarines, apples, raspberries, or grapes. Differential sorption of MB by fresh fruit types and between replicate fumigation trials of the same fruit type resulted in a range of exposures that were verified by gas-chromatographic quantification of headspace concentrations. Concentration x time products (CTPs) $\geq$ 60 and $\geq$ 72 mgL^{-1}h at 10.0 $\pm$ 0.5 and 15.6 $\pm$ 0.5 °C ($\bar{x}$ $\pm$ *s,* AVE $\pm$ STDEV), respectively, resulted in complete mortality of $\sim$ 6,200 larvae at each temperature. These confirmatory fumigations corroborate mortality data for the first time in relation to measured MB exposures and collectively contain the largest number of *E. postvittana* larvae tested to date. Exposures observed for each fumigation trial were used to develop a kinetic model of MB sorption for use as a tool to identify how the load factor and load geometry of different types of packaged fruit can be manipulated to ensure an applied dose results in exposures consistent with a desired insecticidal efficacy.

Key words: *Epiphyas postvittana*, postharvest fumigation, sorption kinetics

INTRODUCTION

The potential to spread *E. postvittana* through commercial distribution channels involving CA-grown fresh fruit is addressed by domestic and international quarantine. Postharvest chamber fumigation with methyl bromide (MB) has a long history of insecticidal effectiveness and is often the phytosanitary treatment that is selected to control insects on commodities affected by quarantine regulation. In fact, postharvest MB use in this quarantine capacity is exempted as outlined in the Montreal protocol of 1987 and is expected to continue, at least in the USA, until technically efficacious and economically feasible alternatives are developed and readily available (Johnson et al., 2012).

The purpose of this investigation was to establish minimum MB exposure thresholds, in the form of concentration × time cross products (CTs), for control of *E. postvittana* larvae in loads of fresh fruits packaged for exported from CA at fumigation temperatures frequently used by industry, 10.0 - 20.6°C. Moreover, a predictive kinetic model was developed from the exposure data of each efficacy trial as tool to better understand the processes underlying the sorption of MB by packaged produce; the need for, and benefits of, such a tool has been articulated previously (Banks, 1989). We report quantitative estimates of the relationship between applied dose, loads factors, and load geometries and discuss how these parameters can be modulated (i.e., tuned) to ensure an exposure of adequate insecticidal efficacy is attained when fumigating palletized-loads produce.

MATERIALS AND METHODS

Experimental procedures are as described in Walse et al. (2012).

RESULTS AND DISCUSSION

The percentage of adults that emerged from non-fumigated control larvae at 15.6 ± 0.5°C was respectively 87.9 (survivors/treated, 102/116), 97.5 (119/122), 95.7 (112/117), 94.8 (1101/1161), 96.6 (204/211), and 95.7% (203/212) for peach, plum, nectarine, apple, grape and raspberry trials. Similar adult emergence was observed for each fruit type at 10.0 ± 0.5 °C, respectively, 80.8 (101/125), 71.9 (82/114), 91.6 (110/120), 86.5 (873/1009), 99.5 (209/210), 97.2% (212/218).

Confirmatory chamber fumigations were conducted in the context of establishing efficacy of MB toward *E. postvittana* larvae over the range 10-20.6°C, which accommodates temperatures pertinent to most fresh fruit industries of CA, or at least those represented in this study. Differential sorption of MB across fruit types and between replicate trials of the same fruit type were used to generate a range of CTPs, centered on 60.0 and 71.8 mgL^{-1}h, for treatment regimes of 10.0-15.5 and 15.6-20.6°C, respectively (Tables 1 & 2, Figure 1). Minimum exposures of 60.0 mgL^{-1}h at $15.6 < T < 20.6$°C and 71.8 mgL^{-1}h at $10.0 < T < 15.5$°C are required for certification of the USDA T104-a-1 MB schedule, which is used to treat fresh fruit imports infested with "surface feeding caterpillars" on "variable hosts" (USDA, 2010). These minimum exposures were calculated by the method of Monro (1969) using the headspace concentrations that actual measurements must equal of supersede at each specified time interval as required for an APHIS certified fumigation: a 40 mgL^{-1} (2.5 lb/1000 ft^3) applied dose, 32 mgL^{-1} at 30 min, and 24 mgL^{-1} at 2 h for fumigations conducted ≥ 15.6 °C and a 48 mgL^{-1} applied dose, 38 mgL^{-1} at 30 min, and 29 mgL^{-1} at 2 h for fumigations conducted ≥ 10.0 °C.

Table 1. MB dose-mortality of *E. postvittana* larvae in various fruit loads at 15.6 ± 0.5°C ($\bar{x} \pm s$); exposures ≥ 60.0 mgL^{-1}h, which is consistent with the minimum requirement of the USDA T104-a-1 import schedule, resulted in complete mortality of 6,755 test specimens

Fruit 60 °F	Applied mgL^{-1}	Initial [MB]	1/2 hr [MB]	2 hr [MB]	% Sorp.	CxT (± 1.8)	n Obs	n Live	% Surv.
Peach	35	27.8	23.1	18.3	34.2	43.8	160	0	0.0
Plum	27	27.3	25.4	23.7	13.2	50.0	169	0	0.0
Plum	27	27.5	27.0	24.7	10.2	52.4	184	0	0.0
Plum	27	28.3	27.0	24.9	12.0	52.8	179	0	0.0
Peach	40	32.7	28.3	23.1	29.4	53.8	45	0	0.0
Nectarine	35	35.0	29.3	23.8	32.0	55.9	173	0	0.0
Nectarine	35	36.1	30.3	24.6	31.9	57.9	180	0	0.0
Apple	33	33.0	28.6	28.4	13.9	58.2	773	0	0.0
Apple	33	34.8	28.9	28.2	18.9	58.7	741	0	0.0
Nectarine	35	36.5	30.7	25.4	30.4	59.0	182	0	0.0
Apple	34	35.1	29.0	28.5	18.8	59.1	789	0	0.0
Grape	30	36.8	31.4	25.7	33.6	59.5	658	1	0.00152
Apple	34	35.8	29.2	29.0	19.1	59.9	784	0	0.0
						< 60.0	∑ 5017		
Minimum[1]	40	40.0	32.0	24.0	40.0	60.0	—	—	—
Apple	35	34.3	30.3	28.2	17.7	60.0	745	0	0.0
Peach	40	35.0	32.1	25.5	27.1	60.2	186	0	0.0
Plum	30	33.5	30.8	28.3	15.5	60.4	45	0	0.0
Peach	39	39.3	32.1	26.3	33.1	61.9	177	0	0.0
Plum	31	34.4	31.6	28.9	16.0	61.9	45	0	0.0
Raspberry	42	42.5	31.4	26.7	41.6	62.1	627	0	0.0
Peach	43	39.9	33.9	24.3	39.1	62.1	47	0	0.0
Raspberry	42	42.1	31.6	27.1	39.6	62.5	951	0	0.0
Grape	31	39.5	32.8	27.0	35.5	62.9	778	0	0.0
Apple	35	38.1	31.4	29.8	21.9	63.2	790	0	0.0
Grape	31	38.7	33.3	27.1	33.6	63.3	901	0	0.0
Raspberry	43	44.2	32.8	27.2	43.8	64.2	650	0	0.0
Nectarine	40	41.6	37.5	30.2	27.4	70.6	46	0	0.0
Peach	42	44.0	37.2	30.8	30.0	71.3	45	0	0.0
Nectarine	40	47.3	39.9	26.6	43.8	71.7	45	0	0.0
Nectarine	40	44.5	38.3	31.4	29.4	73.0	47	0	0.0
Plum	40	45.8	42.8	38.8	15.3	83.4	50	0	0.0
						≥ 60.0	∑ 6175		
						Total	∑ 11192		

[1] Minimum gas concentrations per USDA treatment schedule T104-a-1 (APHIS PPQ Manual)

Table 2. Observable rates (k_{OBS}) of MB loss, as well as, rates of MB sorption by packaged loads that were corrected for load factors and surface area to volume ratio of the loads (k_{SPT}); means not connected by the same letter are significantly different (Tukey-Kramer HSD, $P = 0.05$).

Methyl bromide fumigation kinetics

	Temp. (°C)	k_{OBS} (h^{-1}) ave	± stdev	means comparison	k_{SPT} (Lm^{-2}h^{-1}) ave	± stdev
raspberries	10.0	0.2275	0.0118	A	0.0334	0.0018
	15.6	0.2130	0.0118	A B	0.0318	0.0017
peaches	10.0	0.1984	0.0272	A B C	0.0324	0.0045
	15.6	0.1783	0.0163	B C	0.0290	0.0020
nectarines	10.0	0.1990	0.0182	A B C	0.0327	0.0031
	15.6	0.1741	0.0101	C	0.0286	0.0016
grapes	10.0	0.1198	0.0141	D	0.0132	0.0016
	15.6	0.1772	0.0043	B C	0.0196	0.0004
apples	10.0	0.0777	0.0159	E	0.0154	0.0032
	15.6	0.0832	0.0143	D E	0.0165	0.0028
plums	10.0	0.0769	0.0150	E	0.0163	0.0031
	15.6	0.0698	0.0109	E	0.0154	0.0018
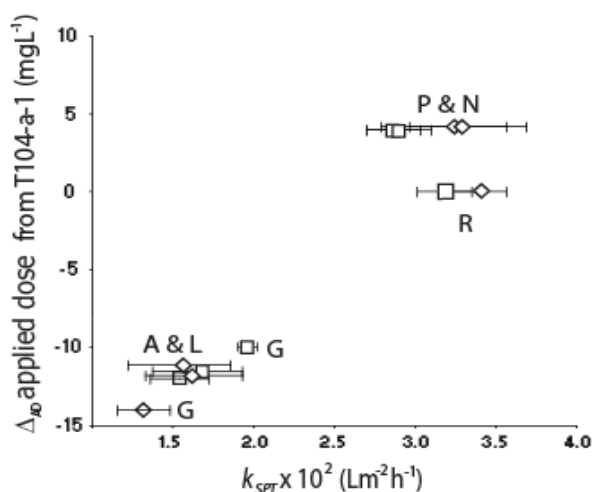
Fig. 1- Adjustments in the amount of applied MB, Δ_{AD}, from USDA T104-a-1 (40 mgL^{-1} at 15.6 < T < 20.6°C and 48 mgL^{-1} at 10.0 < T < 15.5°C) that is required at load factors of 0.5 to achieve exposures ± 5 mgL^{-1} h from those targeted for *E. postvittana* control, respectively 71.8 and 60.0 mgL^{-1}h at 10.0 < T < 15.5 and 15.6 < T < 20.6°C. Fruit and packaging recommended by industry were consistent with export scenarios from California: P, peach; N, nectarine; R, raspberry; A, Apple; L, plum; G, grape.

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HYDROGEN CYANIDE FOR INSECTICIDE PHYTOQUARANTINE TREATMENT OF PACKAGE WOOD

V. Stejskal[1*], J. Liskova[2], P. Ptacek [3], Z. Kucerova[1], R. Aulicky[1]

[1] Crop Research Institute, Drnovská 507, 161 06 Prague 6, Czech Republic
[2] Lucební závody Draslovka a.s. Kolín, Havlickova 605
280 99 Kolin IV, Czech Republic
[3] Timber Institute s. e. Na Florenci 7-9, 111 71 Praha 1, Czech Republic.
* Corresponding author's e-mail address: *stejskal@vurv.cz*

ABSTRACT

Currently hydrogen cyanide (HCN) is industrially produced in the Czech Republic (Lucebni zavody Draslovka Kolin) and is extensively used for Czech mill fumigation. Therefore we evaluated the potential of HCN fumigant for treatment of wooden transport packages (pallets). We tested the penetration of HCN trough wooden pallet materials using gas chromatography. The preliminary results on biological efficacy of HCN on eggs, larvae, pupae and adults of 2 test species (*Hylotrupes bajulus* and *Tribolium castaneum*)- placed in wooden chambers are presented. The results obtained can be used to establish alternative timber fumigation technologies and protocols to methyl bromide. The research was funded by a grant (QI111B065 - Ministry of Agriculture, Czech Republic.)

Key words: Fumigation, hydrogen cyanide, HCN, regulated pests, phyto-quarantine, timber, wood, pallets, *Hylotrupes bajulus*.

INTRODUCTION

There is a permanent threat of the introduction and spread of many pest organisms via infested wooden products and wood packages due to extensive international trade. The traditional regulated pests of processed wood include *Sirex*, spp. and *Urocerus* spp. However, European plant health organizations (EPPO and EFSA) have recognized several emerging wood infesting pests as a new threats, that have reached the status of regulated (phytoquarantine) organisms. They include dangerous cerambycids (*Anoplophora* sp.) and nematodes (*Bursaphelenchus* spp.). These species have been recorded in France, Germany, Austria, Italy and Portugal. However, the available phytoquarantine technology for wood treatment is very limited. The fumigant methyl bromide has been prohibited for wood phytoquarantine purposes due to its negative environmental impacts. Although the fumigant sulfuryl fluoride has been successfully tested (Buckley et al., 2010), it is not yet registered for wood phyto-quarantine . The only non-fumigant option is a heat treatment that requires chamber technology and is logistically and energy demanding. Hydrogen cyanide (HCN) is one of the remaining potential fumigation alternatives.

Hydrogen cyanide is a colorless liquid that evaporates immediately if in contact with air. Pure hydrogen cyanide with a concentration greater than 2 g.m^{-3} smells of bitter almonds. As an industrial insecticide HCN is a fast-acting fumigant (respiratory poison) with a broad range of pest-control applications. Hydrogen cyanide impairs the metabolism of the pests. Historically, HCN has been extensively used to control scale insects on citrus trees starting from 1866. The development of resistance to hydrocyanic acid has been described for scale insects (Quayle 1922, 1938). Later HCN fumigation structural technology has been developed for mill disinfestations. The first mill HCN-fumigation was performed in USA in 1889 and the first industrial mill fumigation was performed in Europe in 1917. HCN was also marketed in Great Britain and USA for the purpose of grain admixture fumigation. It was a solid formulation of calcium cyanide (e.g. Cyanogas G, Cyanogas A dust).

In Germany, according to Wikipedia, "Ferdinand Flury developed Zyklon A at Degesch in 1920 and Walter Heerdt was named the official inventor of Zyklon B in a Degesch patent application from 20 June 1922. Its development was a major advance over previous methods of delivering hydrocyanic acid for pest control because of its improved chemical stability and the presence of a warning odorant. The main invention in Zyklon B consisted of the absorption of liquid hydrocyanic acid into a highly porous adsorbent. Initially, heated diatomite (diatomaceous earth) was used as an adsorbent. Later, high-porosity gypsum pellets called Ercodice (described by eye witnesses as "crystals") as well as disks made from wood fiber were also used. The adsorbed hydrocyanic acid was very safe in handling and storage when placed in inexpensive airtight cans of various sizes. From 1929 onwards the U.S. used Zyklon B to disinfect the freight trains and clothes of Mexican immigrants entering the US."

Nowadays the only available HCN canned formulation for pest control fumigation is produced in the Czech Republic by Lucebni zavody Draslovka a.s. Kolin under the trade name URAGAN D2 (Stejskal and Adler, 1997). URAGAN D2 is stabilized, liquid hydrogen cyanide (HCN) fully soaked in porous material and sealed in gastight tins. It is stabilized with phosphoric acid (0.1%) and sulfur dioxide (0.9 – 1.1 %). URAGAN D2 is packaged in 0.40-0.45 mm thick tins. One tin contains 1.5 kg of hydrogen cyanide absorbed in cardboard paper reels with a diameter of 145 mm, with a central opening of 30 mm and a thickness of 4 mm.

In Europe, HCN has been traditionally used for structural fumigation. HCN was employed for insecticide fumigation of wooden parts of historical buildings, churches in particular. HCN fumigation method has been used to treat parts of the gallery as well as the structural woodwork, which had been infested by various wood pests. This method does not provide protection against pest reoccurrence. Despite the toxicity of the substance, no accidents have been reported for this type of structural fumigation (Germar, 2003). Cases of damage to the facilities themselves have also been very rare. The only exception was discoloration of some type of plaster. The highly reactive cyanide ion combines with iron ions to form, among other things, the complex salt known as Prussian Blue (Grosser and Roßmann 1974, Emerling 1995, Germar, 2003).

Because of HCN's good insecticidal and fumigation properties and commercial availability we evaluated the potential of HCN fumigant for treatment of wooden transport packages (pallets). We tested the penetration of HCN through wooden pallet materials using gas chromatography. The preliminary results on biological efficacy of HCN on eggs, larvae and adults of 2 test species placed in wooden chambers, *Hylotrupes bajulus* (L.) and *Tribolium castaneum* (Herbst), - - are presented.

MATERIAL AND METHODS

Fumigation chamber, HCN formulation and concentration estimation
All experiments were performed in the hermetic fumigation chamber (volume 650 L) with air circulation located at Lucebni zavody Draslovka a.s. Kolin, Czech Republic. Ambient temperatures were 20-25°C. HCN was used in a cooled (5°C) and liquid form (stabilized 0,01% H_2SO_4) and applied in a fumigation chamber by syringe via a rubber septum. Fumigation chamber HCN-in-air concentrations (inside / outside = headspace / wooden-spruce block) were estimated by GC (Shimadzu GC-17A, RT-QPLOT, 30m, ID 0,53mm, GC Software Clarity DataApex v. 2.6.6).

HCN wood penetration and desorption
Five spruce (*Picea alba*) wooden blocks (100 x 100 x 150 mm; Fig. 1) were placed in the 650-L experimental fumigation chamber. The required dose (20 g.m^{-3}) of HCN was injected into the fumigation chamber and the HCN rate of penetration into the central cavity of each spruce block during a 50-h exposure was assessed. Air samples were taken from the central spruce block cavity (see "extraction chamber" in Fig. 1) using a syringe via the block's rubber septum, as well from the fumigation chamber headspace. After the 50-h exposure the chamber was quickly ventilated and closed. Then we continually measured HCN concentrations (i.e. desorption from the spruce blocks) outside (headspace) and inside blocks.
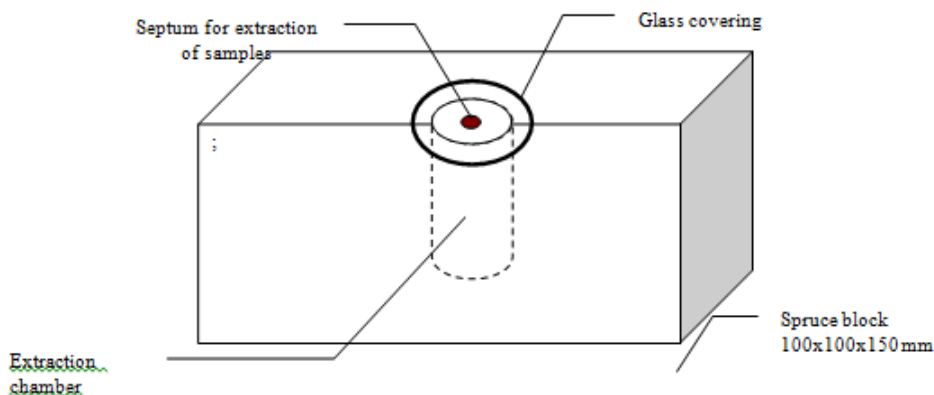
Fig. 1- Experimental spruce block (100 x 100 x 150 mm). It is equipped with an extraction chamber and rubber septum enabling continual air sampling for HCN concentration from the central part of the wooden block.

Efficacy of HCN on *Tribolium castaneum* (Tenebrionidae)
Experimental individuals of *Tribolium castaneum* were taken from insecticide susceptible RICP strain (Crop Research Institute, Drnovska 507, 161 06 Prague 6, CZ). We estimated the effect of exposure period (15, 60, 120 and 180 min) on mortality of eggs, larvae, pupae and adults of *T. castaneum* at the HCN dosage of 20 g.m^{-3}. After exposure the insects were transferred into Petri dishes. Experimental conditions were 22ºC ± 1ºC, 60% r.h. We checked adult and larvae mortality after 48 h and pupal and egg mortality after 288 h (12 days). We compared mortality for each exposure period and insect stage by Kruskal-Wallis using Statistica Version 10.

Efficacy of HCN on Long horn beetle *Hylotrupes bajulus* (Cerambycidae)

The test was performed in the fumigation chamber at Lucebni zavody Draslovka a.s. Kolin with HCN at 2% (concentration 24 g.m^{-3}) for 24 h. All stadia were taken from the laboratory *Hylotrupes bajulus* stocks; they were cultivated and provided by the personnel of Timber Institute s. e. (Na Florenci 7-9, 111 71 Praha 1- Czech Republic). **Larvae:** Larvae (6/block) were fixed in 10 wooden blocks. Two untreated blocks were taken as control. The size of each block was 150±2 x 100±2 x 25±2 mm. The mortality check was made after 24 h of wooden block ventilation. **Adults:** We tested 10 adults (5 males, 5 females) and 2 adults were left untreated as a check. The mortality check of the exposed adults was made after 24 h of wooden block ventilation. **Eggs:** 10 batches of eggs (laid by 10 females) were divided as follows: 1/3 of each batch served as untreated controls and the remaining 2/3 were used for fumigation treatment. After the 24-h exposure the treated eggs were transferred to the Timber Institute s. e. laboratory and left to see if larvae emerged.

RESULTS

HCN wood penetration and desorption

It took about 15 h for HCN to penetrate into the spruce blocks to about half the chamber concentration level (Fig. 2). Desorption of HCN from 5 spruce blocks after fumigation and the short headspace ventilation is shown in Fig. 3.

Efficacy of HCN on *Tribolium castaneum*

The results are summarized in Table 1. We found significant differences among various tested stages for the 15-min exposure period (N = 48, H = 37.81; p = 0.0001) and the 60-min exposure period (N = 48, H = 20,04; p = 0,0002). The multiple comparison test revealed that pupae were statistically more tolerant than the other stages. No significant difference was evident for the 120-min exposure period (N = 48, H = 3.00; p = 0.3916) or for the 180-min exposure period which achieved total kill.
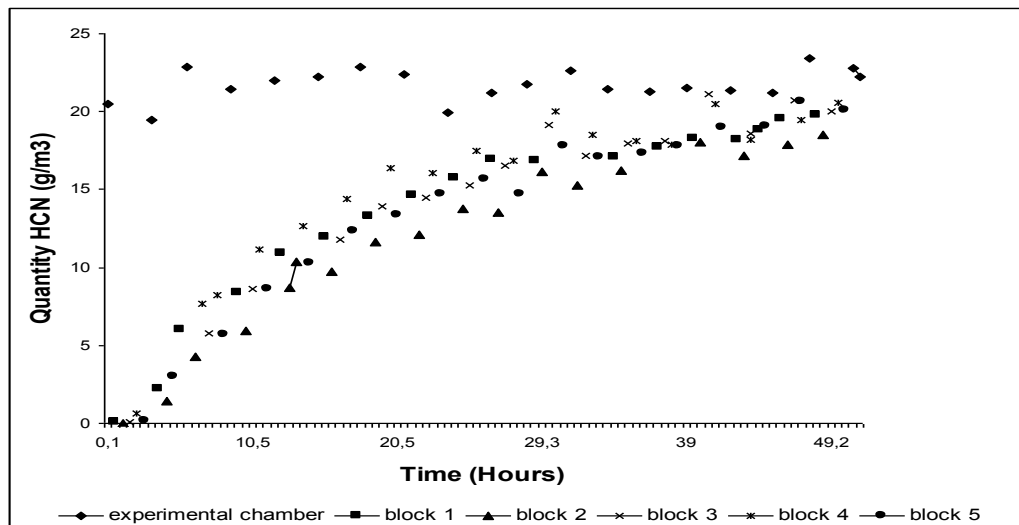
Fig. 2- Temporal dynamics of HCN concentration in the headspace and in the center of the experimental spruce blocks. Five blocks were used per fumigation chamber (650 L). (Explanations: "experimental chamber" = HCN concentration in the headspace of fumigation chamber; "block" = HCN concentration in the center of spruce block 100 x 100 x 150 mm).
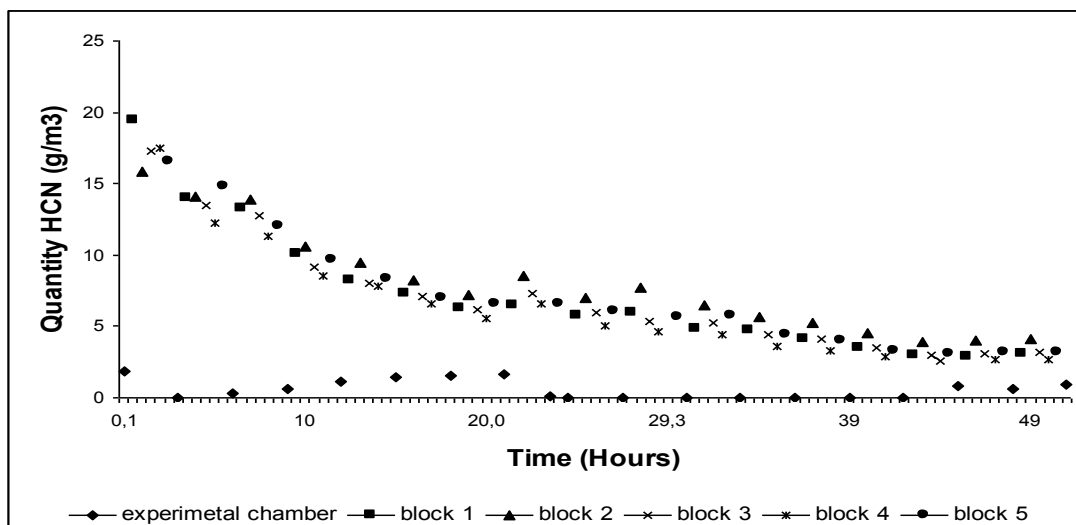
Fig. 3- Desorption of HCN from 5 spruce blocks after fumigation treatment and short headspace active ventilation (Explanations: "experimental chamber" = HCN concentration in the headspace of fumigation chamber, "block" = HCN concentration in the center of spruce block 100 x 100 x 150 mm).

Table 1. Mortality of various stages of *Tribolium castaneum* after various exposures of HCN (dose 20 g m^{-3})

Tabulka	Exposition time (min)				
	15	60	120	180	Control
Imago	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0	0.0±0.0
Egg	98.3±1.1	100.0±0.0	100.0±0.0	100.0±0.0	42.5±8.0
Larva	97.5±1.8	100.0±0.0	100.0±0.0	100.0±0.0	5.0±3.4
Pupa	48.3±5.9	90.8±3.1	98.3±1.7	100.0±0.0	10.8±2.6

Efficacy of HCN on long horn beetle *Hylotrupes bajulus*
The results are summarized in the Table 2. The 24-h HCN exposure achieved 100% mortality of all test samples (i.e. eggs, larvae, male and female adults) of *H. bajulus*.

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Table 2. Mortality of various stages of *Hylotrupes bajulus* after a 24-h exposure to HCN (dose 20 g m^{-3})

Tabulka	Experiment	Control
Imago	100.0±0.0	0.0±0.0
Egg	100.0±0.0	2.0±1.3
Larva	100.0±0.0	0.0±0.0

DISCUSSION

In this study we have confirmed 100% biological efficacy of a 24-h exposure period to 2% HCN (concentration 24 g.m^{-3}) on all tested stages of *Hylotrupes bajulus* treated inside the small wooden blocks. With the other test species *Tribolium castaneum*, 100% mortality was reached for all developmental stages, including eggs and pupae after 180 min of HCN exposure in the fumigation chamber. The most tolerant stage was the pupa; its survival was recorded after 120 minutes of HCN exposure.

In the past HCN has been used for special stored product fumigations. However, only few studies are available on HCN absorption and penetration in different commodities: Kunz et al. (1964) studied the penetration of HCN through a grain sorghum bulk. Although HCN has been employed for structural wood fumigation in historical buildings, only one study has been published concerning the rate of HCN absorption by timber (Capoun and Krykorova, 2008). They determined considerably higher HCN absorption in spruce wood than in pine wood. The highest HCN content was determined at a depth of approximately 1.5 cm while content of HCN was low in the surface layer because of desorption between the time of exposure to the fumigant and sampling. However, this study did not provide any HCN measurements from the fumigated headspace. We showed that HCN required (under normal room pressure and temperatures) almost 50 h to reach the central part of the exposed spruce blocks (100 x 100 x 150 mm) at the concentration that was equal to the head space concentration (Fig. 2). Ren et al. (2011) compared rate of penetration of four industrial fumigants (SF, PH$_3$, C$_2$N$_2$, CH$_3$Br) into pinewood (*Pseudotsuga menziesii*) blocks (10 cm × 10 cm × 30 cm). Each fumigant penetrated to all parts of the block, but the speed and extent of penetration were different. The fumigants that most rapidly achieved an even concentration throughout the block and chamber were PH$_3$ and C$_2$N$_2$. We cannot compare our results for HCN directly with this work since Ren et al. (2011) used a different type of wood. Nevertheless, it seems that the rate of HCN penetration in spruce blocks (obtained in our experiments) was similar to the rate of sulfuryl fluoride penetration into pinewood obtained by Ren et al. (2011).

SUMMARY

In our work we showed high efficacy of HCN on various stages of the serious timber pest *Hylotrupes bajulus* and *Tribolium castaneum*, and described the temporal rate of HCN penetration inside spruce blocks. The results can be used to establish alternative timber fumigation technologies and protocols to methyl bromide.

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JAPAN IMPLEMENTATION PRACTICES ON ACCURATE RECORDING OF METHYL BROMIDE USE AND ITS REDUCTION IN QUARANTINE PHYTOSANITARY MEASURES

Akio Tateya

Syngenta Japan K.K.
21F, Office Tower X, 1-8-10, Harumi, Chuo-ku, Tokyo 104-6021 Japan
*Corresponding author's e-mail: akio.tateya@nifty.com

ABSTRACT

Decision XXIII/5 Paragraph 1 of Montreal Protocol on the substances that deplete ozone layer encourages parties to follow the recommendation of the Commission on Phytosanitary Measures of the International Plant Protection Convention that data on current usage of methyl bromide as a Phytosanitary measure should be accurately recorded and collated, including information on the quantities of methyl bromide used in kilograms, a description of the articles fumigated and target pests. When plants are imported, they are subject to plant quarantine inspection at the entry. If quarantine pest insects are intercepted by plant quarantine inspector, they are treated with Quarantine Phytosanitary measures such as fumigation by methyl bromide, hydrogen cyanide or aluminum phosphide. Japan established those recording system for quarantine fumigation and undertakes properly as one of the operation practices. In this presentation, items entered in fumigation record sheet are shown such as name of the company of pest control operation, plant articles, chamber category of gas holding capability and air tightness, methyl bromide application amount (kg), dose rate (g/m^3) and name of target pest insects. Japan recording and collating system meets all requirements of the Decision XXIII/5 paragraph 1 of Montreal Protocol. Japan has made much effort to reduce methyl bromide use as much as possible. Fumigation chamber holders are expected to keep high gas holding capability for the minimum use and emission of methyl bromide. Dose rates are set in fumigation schedule in view of a kind of plant article, grain temperature, fumigation duration time and gas holding capability etc. Furthermore various means to reduce methyl bromide use are shown in every aspect such as choice of appropriate size of fumigation chamber to the commodity and improvement of non quarantine pest insects. Japan Government enforces those regulations to require people concerned to implement to make effort for reduction of methyl bromide use.
Key words: Plant quarantine treatment, IPPC, Commission on Phytosanitary Measures, methyl bromide, accurate recording of the use of methyl bromide, decision of Montreal Protocol, gas holding capability, air tightness of fumigation chamber, fumigation schedule, dose rate.

INTRODUCTION

When plant and plant products are imported in Japan, they are subject to plant quarantine inspection at the entry. If quarantine pest insects are intercepted by plant quarantine inspector,

they are treated with Quarantine Phytosanitary measures such as fumigation by methyl bromide, hydrogen cyanide or aluminum phosphide. Japan has undertaken recording system as one of the quarantine practices. In this presentation, all items entered in fumigation record sheet are shown very in detail.

Japan has made much effort to reduce methyl bromide use as much as possible. Amounts of methyl bromide use for the quarantine treatment (tones) are shown in table 1[*1]. Dose rates are prescribed very in detail in terms of various factors for ensuring fumigation effectiveness and minimum use and emission of methyl bromide. Fumigation chambers are expected to keep air tightness very high. Dose rates are prescribed very in detail as fumigation schedule on kinds of plant article, grain temperature, fumigation time and category of gas holding capability and air tightness of fumigation chamber etc. because fumigation effectiveness could definitely depend upon those factors. Extracts of prescribed dose rates are shown in table 2 and table 3[*2]. In addition, very many way of fumigation practices are actually implemented to reduce methyl bromide use.

Table 1. Amount of methyl bromide use for the quarantine treatment (tones)[*1]

Year	1995	2000	2003	2004	2005	2006	2007	2008	2009	2010	2011
Amount	2,448	1,591	1,403	1,306	1,165	1,039	867	706	542	511	547

Table 2. Dose rates (g/m3) for bagged commodity in the chamber of gas holding capability of category super A at the loading rate of more than 0.5 tones/m3 with fumigation time of 48 hours[*3]

Commodity	Grain temperature (℃)		
	T<10	20>T≧10	T>20
Bagged rice, wheat, cocoa and coffee bean etc.	19	15	12
Bagged maize, milo and millet etc.	25	20	15
Bagged soybean, kidney and peanut etc.	31	26	19
Bagged buchwheat, safflower seed and rice, maize and soybean flour etc.	38	30	22

Table 3. Dose rates (g/m3) for bulk commodity in the silo of gas holding capability of category super A at the loading rate of more than 0.5 tones/m3 with fumigation time of 48 hours[*3]

Commodity	Grain temperature (℃)		
	T<10	20>T≧10	T>20
Bulked rice and wheat etc.	27	22	16
Bulked maize, milo and millet etc.	36	29	22
Bulked soybean, kidney and peanut etc.	39	31	23

Import plant inspection

When plant is at the entry, consignee should submit import plant inspection application form No. 4*[3] to plant quarantine inspector. Then consignment is inspected. If quarantine pests are found intercepted, names of the pests are entered in the form. Consignee is required to undertake Phytosanitary measures to the consignment.

Phytosanitary treatment

Being entrusted by consignee, pest control operator submits Phytosanitary treatment plan for the approval of plant inspector. When it is approved by the inspector, consignment is brought into fumigation chamber. Pest control operator to undertake quarantine treatment is certified by the plant quarantine authorities. Fumigation chamber for the consignment is also designated by plant quarantine authorities. Consignment is undertaken quarantine treatment by the pest control operator. When be completed, fumigation success or failure is checked by plant quarantine inspector with the confirmation of the test insect condition of life or death and/or with the level of remaining gas concentration at the fumigation termination time. When fumigation treatment is judged success, consignment is subject to custom clearance.

Methyl bromide fumigation record sheet

When plant quarantine fumigation is undertaken, fumigation operation recording sheet of form 3*[4] is required to submit to the director of plant quarantine station by the people of designated fumigation chamber prior to fumigation. Items, which are supposed to be entered concerning methyl bromide use, are included below.

   Name of the company of pest control operation:
   Name of vessel:
   Name of plant article treated with methyl bromide:
   Its quantities (metric tones):
   Name of fumigation chamber holder:
   Name of fumigation chamber:
   Chamber capacity (m3):
   Loading rate (tones/m3) of consignment in the chamber:
   Chamber category in terms of gas holding capability and air tightness:
   Methyl bromide application amount (kg):
   Application dose rate (g/m3):
   Existence of circulation apparatus: Yes or No
   Ventilation use: Yes or No
   Date of dose application: Year/ Month/ Day/ Time
   Chamber space temperature: degree centigrade
   Grain temperature: degree centigrade
   Name of facility owner, people responsible to pest control operation and workers in the presence of dose application:
   Remaining gas concentration at the termination of fumigation treatment: mg/l
   Test insect condition: Life or death
   Fumigation judgments by plant quarantine inspector: Success or failure
   Name of plant quarantine inspector:
   Names of target pest insect are entered in the import plant inspection application sheet by plant quarantine inspector.

Full compliance of fumigation recording practice to Decision XXIII/5 paragraph 1

Information mentioned above to be required to enter in the fumigation recording sheet is fully complied with the ones suggested in the Decision XXIII/5 paragraph 1 which mentions only the quantities of methyl bromide used in kilograms, name of the articles fumigated and target pests.

Various efforts to reduce use and emission of methyl bromide in the use of quarantine treatment

Methyl bromide fumigation as Phytosanitary treatment of plant quarantine is strictly put into practice for the consignment in which quarantine pest insects are found intercepted. Quarantine pests of 780 species and non quarantine pests of 226 species have been clearly listed[*3] on the basis of pest risk analysis based in International Standards of Phytosanitary Measures (ISPM) 2. Pests, which are not listed, are regarded as quarantine pests by the clarification. Many species of grain insect pests have been come to be classified as non-quarantine pest in very earlier time, so that methyl bromide use has been reduced significantly. This is one of the big factors to decrease methyl bromide use in quarantine treatment. Also it is also one of the factors to reduce use of methyl bromide that import commodity, in which quarantine pests could not be found intercepted, has got increased and particularly unsown timber import, which is subject to plant quarantine, has been getting significantly decreased.

Registration of methyl bromide for quarantine use

Methyl bromide for exclusive use of quarantine treatment was specifically registered in December 24th, 1994 which was independently registered away from methyl bromide for general regulated use. Therefore, it is not allowed to the general regulated use such as soil treatment or post harvest treatment. Methyl bromide for quarantine use has been specified with the red label put on the cylinder or the cartridge, so that it is easily recognized as quarantine use. Pest control operator is strictly required to use methyl bromide for quarantine use exclusively and not to misappropriate it to the regulated use.

Dose rates in grain fumigation schedule

Dose rates are respectively set in the fumigation schedule to ensure complete control under various fumigation conditions[*2]. They are respectively set under consideration of many different factors such as grain loading type of bagged in chamber or bulk in silo, fumigation duration time, categories of plant articles in view of methyl bromide gas absorption, grain temperature, loading rate in chamber, category of air tightness and methyl bromide gas holding capability of the chamber and existence of installation of air circulation in the chamber. Factors related to fumigation conditions are mentioned below.

(1) Fumigation duration: Dose rates are set differently in view of fumigation duration time of 24, 48 and 72 hours. The longer fumigation time is taken, the less dose rate is set.

(2) Grain loading of bagged in chamber or bulk in silo: Usually, grain bags are piled up in the chamber and grain in bulk is put in the silo. Fumigant gas penetrates and spreads easier in the grain bags than in bulk, so dose rate for grain bag is set less than grain bulk in silo.

(3) Plant category with gas absorption: Dose rate is set in view of plant categories of methyl bromide gas absorption. To the plant articles of the less absorption of methyl bromide gas, the less dose rate is set. Dose rate to soy bean is more than wheat because soy bean absorbs more methyl bromide because soy bean has more protein contents than wheat. Flour absorbs more gas than grain, so dose rate of wheat flour is set more than wheat grain.

(4) Grain temperature: When grain temperature is higher, pest insect is more sensitive to the fumigant gas. So the higher grain temperature is, the less dose rate is set.

(5) Loading rate: It means loading volume of consignment in the chamber. It is expressed by tones/m3. More volume of grain is loaded in the chamber, more methyl bromide gas could be absorbed. Therefore more dose rate is necessary.

(6) Capability of gas holding and air tightness of fumigation chamber and air tightness: In Japan high capability of gas holding and high air tightness is extremely expected to fumigation chamber by the quarantine authorities because it should be kept higher level of gas with less leakage outside. Fumigation chambers for quarantine use are designated by the director of plant quarantine station with the way of the check either by gas holding capability or air tightness.

(7) Circulation system in the fumigation facility: Dose rate is set less with the existence of air circulation installation in the facility. Gas is easily distributed in the chamber with circulation system. So dose rate is set less for the chamber with the installation of circulation system.

Improvement of gas holding capability of fumigation chamber

Fumigation chambers for quarantine use are categorized by the check of gas holding capability[*4]. Methyl bromide is put to the non loading chamber or silo at the dose rate of 10 g/m3 and remaining gas concentration is determined at 48 hours later of dose application. Designation standards of respective category in view of gas holding capability are set by the level of remaining gas concentration. When it is determined more than 85% of application dose rate, chamber is categorized as the class super A. With the remaining gas found more than 70 %, chamber is categorized as the class A, and chamber with more than 55% of remaining gas is as the class B, and more than 40% of remaining gas is as the class C. Chamber with the remaining gas of less than 40% is not designated to the use for quarantine treatment.

Improvement of air tightness of fumigation chamber

Upon the request from chamber owner, check of air tightness of the chamber could be applied instead of the check of gas holding capability[*4]. For fumigation chamber to which air is sent inside, air pressure gets raised up to 55 mm Aq. and then it is left to lower to the height of 50 mm Aq. At five minutes later, to the chamber where gauge shows higher than 45 mm Aq., the chamber is categorized as class super A. When gauge heights shows between 5 and 45 mm Aq., the chamber is categorized as class A.

For silo or grain elevator, air pressure is raised up to 550 mm Aq. by sending air to stop and left as it is. Gauge comes down to shows down to 500 mm Aq. and left to keep on gauge down. Twenty minutes later from time gauge shows 500 mm Aq., the silo, where gauge shows not less than 400 mm Aq., is categorized as super A. The silo, where gauge shows between not less than 200 mm Aq. and not more than 400 mm Aq., is categorized as class A.

Various measures for the least use and emission of methyl bromide

Various measures have been taken to reduce use and emission of methyl bromide for quarantine use as follows.

(1) Improvement of gas holding capacity and air tightness: Fumigation chamber facility holders have been encouraged to improve gas holding capability and/or air tightness. Currently majority of fumigation chambers, which are designated for quarantine treatment by the authorities, belongs to category of super A or class A.

(2) Operation by fumigation licensed expert: Fumigation operation is required by the instruction of licensed experts to secure safe operation and reliable fumigation effectiveness.

(3) Effort to avoid fumigation failure: If fumigation treatment by methyl bromide be judged failure, it is not allowed to repeat methyl bromide fumigation, but to use alternative method. Success of fumigation treatment should be confirmed by the check that all test insects be found dead and level of remaining gas concentration be found remained more than the prescribed level. If fumigation results are not met with those conditions, fumigation treatment is judged failure and it should be taken further disinfestations procedure. In this case it is usually to use aluminum phosphide or carbon dioxide which take longer time and more cost.

(4) Encouragement of the use of fumigation chamber appropriate to the size of commodity:

Methyl bromide use amount is applied adaptable to the size of fumigation chamber, not to the size of consignment. To small size of consignment, size of fumigation chamber should be appropriately small, not too big compared to the size of consignment.

(5) No mixed loading with the commodity of more absorption: If plants of different category of the absorption are mixed loading, dose rate is applied adaptable to the plant category of which absorption is bigger no matter how small size of the plant article is loaded. For example, maize absorbs more methyl bromide than wheat and rice. Wheat absorption is classified same as rice. So dose rate of rice and wheat is less than maize. Therefore dose rate for mix loading with bagged rice with wheat is rather less than dose rate for mixed loading of rice and maize no matter how small size of maize is put in the chamber to which dose rate is applied to maize.

(6) Encouragement to apply heat treatment for wood packing materials instead of methyl bromide fumigation: Heat treatment is much more encouraged than methyl bromide fumigation unless treatment is done under unavoidable circumstance that such as size of wood packing materials is too big to put in the facility of heat treatment or consignment is already put in the wood packing materials. In 2011, only 3,343 kg of methyl bromide was reported to use for the treatment of exporting wood packing materials which is only 0.6% of methyl bromide used in plant quarantine treatment .

(7) Improvement of list of non-quarantine pest insects and diseases: List of non quarantine pest insects has been elaborated by pest risk analysis based on International Standard of Phytosanitary Measure-2. Japan Government had made a list of non quarantine pests in the grain in early time to which methyl bromide had been used much resulting in vivid reduction of methyl bromide use.   At present non quarantine pest insects and diseases were listed 226 species[*3]. Improvement of the list of non-quarantine pest could be devoted a lot to make less use of methyl bromide.

(8) Development of phosphine gas generator from aluminum phosphide: Tablets or small balls formulation are used in the quarantine treatment as alternative to methyl bromide, however, people prefer methyl bromide fumigation to aluminum phosphide because it takes much longer time. Recently, some installation unit of phosphine gas generator from aluminum phosphide, which is set outside attached to the chamber, had been developed. Use of this unit will save dose rate and shorten fumigation duration which could be implemented soon.

(9) Development of alternatives to methyl bromide: To treat unsown timber and lumber, several alternatives have been developed which are of methyl iodide, mixture of methyl isothiocyanate and carbon dioxide (Ecofume) and mixture of methyl isothiocyanate, carbon dioxide and sulfuryl fluoride (Ecotwin). They are all registered for quarantine uses, however, it is under preparation to amend import plant quarantine regulation.

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PORT INTERCEPTION, QUARANTINE AND TREATMENT DEVELOPMENT FOR EXOTIC INSECTS IN THE UNITED STATES

Scott W. Myers*[1] and Hannah Nadel[1]

[1]USDA APHIS PPQ CPHST, Otis Laboratory, Buzzards Bay, MA, U.S.A.
*Corresponding author's e-mail: *Scott.W.Myers@aphis.usda.gov*

ABSTRACT

The USDA APHIS is tasked with developing regulatory policy and science-based solutions to prevent introductions and establish quarantines and eradication programs when introductions do occur. Despite the implementation of phytosanitary measures to prevent the introduction of invasive species into the US, interceptions at ports of entry remain common for a number of insect taxa. Frequency of interception can trigger restrictions on trade and revisions to phytosanitary treatment schedules. Recent introductions of several tortricid pests of fruit and ornamental plants, a spike in introductions of Khapra beetle, and the establishment of a number of devastating wood boring insects have generated special interest in reducing the threat of invasive insects to our agricultural commodities and natural resources.

Here we report on the status of projects developed to evaluate insect interceptions at US ports by improving our existing capacity to identify immature insects and evaluating efficacy of phytosanitary treatments on imported commodities. In collaboration with a number of US ports we are collecting live exotic insects as they are intercepted, rearing immature stages to adult to facilitate identification, and using DNA sequencing to identify species already represented in genetic databases or to catalog unknowns and expand our current database. As a part of this process we are we looking for patterns in interceptions to document pathways of introduction, identify commonly intercepted insects, and determine whether they result from phytosanitary treatment failures or a lack of treatment altogether. Results are improving our identification capacity and will help shape future efforts in treatment development.

SESSION 9

Integrated commodity management

Chairpersons:
Bhadriraju Subramanyam, USA
Christos Athanassiou, Greece
A. Guray Ferizli, Turkey

INTEGRATED COMMODITY MANAGEMENT

Digvir S Jayas*, Fuji Jian

Department of Biosystems Engineering, University of Manitoba, Winnipeg, MB, R3T 5V6 Canada
*Corresponding author's e-mail: *Digvir_jayas@umanitoba.ca*

ABSTRACT

Agricultural produce of all kinds fall within the broad definition of the commodity. Integrated commodity management requires an understanding of the physical and bio-chemical properties of the commodity, storage environment of the commodity, and all tools which can be used to manipulate the storage environment to extend the storage life of the commodity and to maintain the quality of the commodity. Using bulk grain as an example, the relationship among physical properties of grain kernels, grain bulk, environment of stored grain, and methods of managing stored grain was analyzed and an approach to integrated management of grains was presented. The paper highlights the directions for further research for integrated commodity management.

Keywords: Physical and bio-chemical property, storage grain management, commodity.

INTRODUCTION

Agricultural commodities refer to bulk products grown or produced on farms. There are many different types of agricultural commodities, including staple grains and most feed products from plants and livestock and products derived from livestock. Agricultural commodities are usually used to sustain life on the earth.

Under most situations, agricultural commodities are stored, transported, and processed before finally consumed. For example, grain will be stored for several months to one year in Canada. About 900 million tonnes of grains are in storage throughout the world at any given time (Jayas et al., 1995). Grain storage is a repeated interim phase in the complex logistics of moving grain from producer to processor and grain products from processor to consumer. Microorganisms, insects, toxins, chemicals, and heavy metals can contaminate and/or infest the grain and products during this period (White, 1992). Infestation and/or contamination can cause extensive morbidity and mortality, and economic destruction of food manufacturers and agricultural industries. In any country, grain owners (farmer and elevator manager) have a moral obligation to supply grain that are safe, while food processors have a duty to convert those grain into products which can be eaten by consumers without risk of compromising individual health. Therefore, the main purpose of grain storage management is to safely store and transport grain and products to provide a stable and safe food supply on a daily basis to consumers who might live in other villages, towns, cities or countries.

A sound program of commodity management requires an understanding of the characteristics of the commodity, storage environment of the commodity and all tools which

can be used to manipulate the storage environment to extend the storage life of the commodity and to maintain the quality of the commodity. From the view of engineering design and economic efficiency, all applied management methods and tools should be based on the physical and bio-chemical property of commodities (Cenkowski and Zhang, 1995) and the property of its storage environment. In this article, we used bulk grain as an example to analyze the relationship among physical and chemical properties of grain kernels, grain bulk, environment of stored grain, and methods of managing stored grain. This analysis should help to understand the integrated approach to grain commodity management.

PHYSICAL CHARACTERISTICS OF GRAIN

Stored grain is bulk granular solids and has flowability under certain elevation potential. Their physical characteristics include the mechanical, thermal, aerodynamic, storage, and quality properties. Data related to these physical properties are required in design and evaluation of machines and equipment used during harvesting, handling, storing, separating, cleaning, and processing of agricultural commodities (Cenkowski and Zhang, 1995; Mohsenin, 1986). During engineering design and evaluation, the limitation to achieve a high accuracy is the availability of adequate data on the engineering properties of grain and oilseeds (Jaros et al., 1992). For example, the geometric properties such as size and shape are one of most important physical properties considered during the separation and cleaning of agricultural grains (Mohsenin, 1986). To a large extent, the geometric properties determine the interactions among and between particles, and with the surrounding air and structures of the machine. These interactions, in turn, influence almost all engineering properties of grain (Cenkowski and Zhang, 1995). In theoretical calculations, agricultural seeds are assumed to be sphere or ellipse. However, the shape of kernels might not be the exact sphere or ellipse and there might be differences between any two kernels (Fortes and Okos, 1980). The shape and size of the seeds affect drying rate, grain and air flow rate, and loads on bin walls (Cenkowski and Zhang, 1995).

To properly use these physical characteristics, each term of the physical characteristics of bulk and kernel of grain should be properly defined and measurement methods should be standardized or described. For example, the term of storage life and germination of grain is well defined and their measurement methods are developed (Karunakaran et al., 2001). However, the definition of storability might be vague even though it has been used in many publications. It is usually used to qualify the storable ability of grain. In most situations, storability might refer to (but not exactly) storage life or vitality (germination). There is also no recognized or described method to determine the storability of grain. There is no method to compare storability of different grain types or the same type of grain at different storage conditions if storage life and/or germination are not used. This unclear definition limits the application of the storability term.

Different procedures and applications impact the physical properties of the agricultural commodity. Moreover, physical properties of the kernels might keep changing during applications (Fortes and Okos, 1980). For example, size and shape of kernels might change during drying, loading, and processing. Size and shape influence the grain bulk density. Different methods for determining bulk density may give different results (Nelson, 1980). In a storage facility, the bulk density is difficult to interpret because the degree of packing depends on the method used to fill the facility (Jayas et al., 1989). Therefore, to properly use the data of the physical properties, measurement condition should be well described and should be close to its application condition.

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Any tools, machines, and equipment used should fit well with the physical property of the material. For example, bulk grain can be loaded or unloaded by applying its flowability. When grain is bagged, the bagged grain becomes un-flowable and can be transported inside bags. Even though both methods of grain handling (transfer grain in bags or in bulk) have advantages and disadvantages, it should be used at different stages with different applications. For example, bagged grain is best transported in a small amount and usually used in the retail handling, while bulk grain is handled in large amounts (e.g. 100 t) during harvesting, storing, and processing. Some countries currently handle bagged grain instead of bulk grain. This degrades the handling capacity and efficiency when a large amount grain is transported. Both bags and bag storage space are also expensive, particularly where manpower costs are high. Therefore, some countries have been gradually converting their handling system from bag system to bulk system over the last 20 years. For example, China converted from the bag storage system to the bulk system in the 1990s. India and Brazil currently used both systems.

BIO-CHEMICAL CHARACTERISTICS OF GRAIN

Grain consists of carbohydrates (starch, sugar, and cellulose and non-cellulosic polysaccharides), lipids, fats, protein, vitamins, fibre, minerals, and water. Just as the various cereal species differ in appearance at the macro and micro levels (such as their size and shape), so these differences in chemical composition. These differences influence their storage life, milling and baking quality, and govern the procedure of processing and handling (Wrigley and Batey, 2010). The chemical components are also a source of biological available energy making it susceptible to consumption by animal and microorganisms and damage by insect pests and microorganisms (White, 1995). These bio-chemical composition differences govern their suitable storage environment. For example, different insect species have different preferences on different types of grain. Different chemical components provide different water activity and this explains why different crops have different storage life under the same temperature and moisture content.

Grain at harvest and during parts (if not all) of its storage periods are living, albeit quiescent, organisms that respire and age. After they lose germination ability or are broken, they lose the protection of their structure or of their immunity. Changes in grain components during storage could be seed dormancy, lipids, carbohydrates, proteins, nutrition and feed quality (thiamine, niacin, pyridoxine, inositol, biotin, and vitamin E), and water (Serna-Saldivar, 2010). Therefore, management methods should also be based on the bio-chemical characteristics of the stored commodity.

Agricultural commodities usually consist of certain amounts of water and also usually absorb water when under high RH environment and lose water when under dry conditions. Their chemical activities during storage and transportation are influenced by water activity and temperature (Tipples, 1995). Therefore, controlling water activity and temperature of stored commodities is the basic requirement of integrated commodity management. Universal guidelines for controlling temperature and humidity conditions to suit the various food commodities are impossible because these conditions and the operating environments vary from place to place and commodity to commodity. However, some basic instructions can be followed such as keeping all food commodities in dry conditions, storing wet and dry foods separately, cross-ventilation in the warehouse, sun roofs and covering food commodities during transportation.

GRAIN STORAGE ENVIRONMENTS

Storage environment includes its physical, chemical, and biological environment. These three parts interact with each other (Fig. 1). There is a high risk of grain deterioration due to chemical reaction and infestation of insects and microorganisms when the storage environment is under optimum physical, chemical and biological conditions (White, 1995). For example, insects will infest the grain only when the physical and chemical environments are in their development and multiplication zones (Fields, 1992). Biological and chemical environments are usually subordinate to the physical environment, but in their turn exert a great influence on the physical environment (Fig. 1). Therefore, the efficient way of controlling the bio-chemical environment is to control the physical environment.
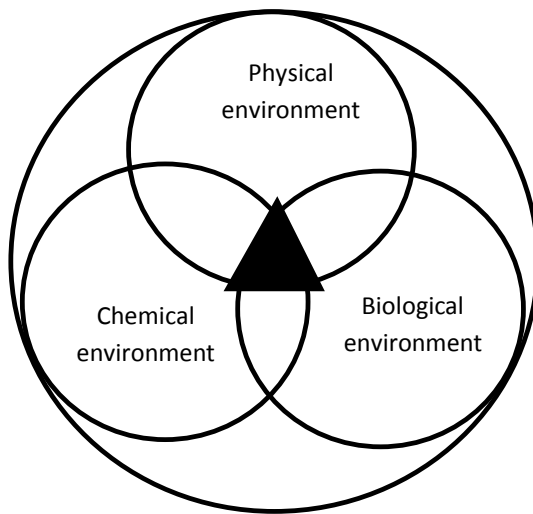
Fig. 1- Environment of stored grain. The triangle shows the high risk of grain deterioration due to chemical reaction and infestation of insects and microorganisms. The edge of the largest circle shows the zones of low chemical reaction and biological multiplication.

Bio-chemical activity of commodities is directly influenced by the water activity and temperature of the commodity. Therefore, managing temperature and moisture content of stored commodities will control both the bio-chemical activity and the environment of the commodity. For example, key issues with grain storage are infestation with microorganisms and insects. Infestation by microorganisms and insects can be controlled by manipulating temperature and moisture content (or relative humidity) (White, 1995). Maintaining these at optimum levels inside and outside of the grain is the secret to successful and cost effective storage. Both temperature and moisture control have advantages and disadvantages (Table1). Theoretically, moisture control will be easier (Serna-Saldivar, 2010) and last longer than the temperature control (Table1). Therefore, the first option for proper grain storage should be the control of grain moisture to safe levels.

Table 1. Comparison of temperature and moisture control of stored bulk grain in bins

	Temperature control	Moisture (RH) control
Control methods	Aeration, chilling (refrigeration)	Drying, mixing, airtight
Limitation of the control methods	Ambient temperature is the limitation under most situations	No limitation if grain will be dried to desired moisture content
Influenced by ambient condition inside bins	Influenced by ambient temperature and follows seasonal temperature	Exchange with ambient air or get wet from precipitation
Prevention from ambient influencing	Difficult to prevent	Can be prevented by good storage facilities
Persisted time	Can keep grain at modified temperature for a short time period (say few days)	Can keep grain at modified moisture for a longer time (say few months)
Total grain mass	Not influenced	Influenced by removing or adding water

The stored grain environment is not a closed system. Its environment is always influenced by ambient environment (Jian et al., 2009). When the environment of the grain is different than the ambient, exchange always occurs until equilibrium is reached. To prevent any undesired influence by the ambient environment, proper storage facilities and/or structures are required. Storage facilities and structures also provide a suitable tool and environment for the control of the physical environment of the storage grain. Even though grain can be stored at different stages inside different structures with defined units such as silos, warehouses, containers, bags, and even in piles on the ground or underground, storage structures should be suitable for the volume, type, physical and bio-chemical properties, processing stage of stored grain, and the nature of the distribution process from the storage. Good designing of storage facilities is the basic requirement for proper grain moisture control. There is currently a trend to decrease the investment on storage structures by using silo bags or other temporary storage facilities as long-term storage facilities. This trend will sacrifice the tools of physical environmental controlling. For example, there will be a challenge in monitoring the temperature and conducting aeration inside silo bags.

INTEGRATED MANAGEMENT (IM)

Integrated management (IM) of stored agricultural commodities (such as grain) can be defined as the use of available technology and tools with ecosystem approach to cost-effectively minimize the losses caused by pests, fungi, and handling damage. It is the practice of monitoring and treating commodity with the goal of reducing cost and inputs. The concept of the IM should be based on the understanding of physical and bio-chemical properties of the stored agricultural commodity. During handling and storage, IM should take advantage of the physical and bio-chemical properties of the commodities. Cost-effectiveness requires that all

costs and benefits, including sociological and environmental effects, should have been taken into account.

Integrated management should consist of many components such as integrated pest management (IPM) and integrated mold management (IMM) without contradiction with each other. Ecosystem approach should be applied when the environment of the stored commodity can be viewed as an ecosystem (Jian and Jayas, 2012). The concept of ecosystem approach is not the same as the IM. The main difference between ecosystem approach and the IM is that the ecosystem approach uses the ecosystem concept to manage the stored commodity and IM uses the ecosystem concept as an option. A program developed based on IM might be adapted and improved using ecosystem approach because many recommendations developed in IM program are also based on the concept of the ecosystem. Therefore, compared with IM, ecosystem approach adds complexity to management but brings additional tools for the task. For example, ecosystem approach should be used to manage the stored grain ecosystem. IM should be used to manage the handling and transportation of the grain.

The key of integrated commodity management is to successfully control the physical, chemical, and biological environment of the stored commodities. For example, even though there are many available methods of handling, safe storage, monitoring, and treatment (Fig. 2), the selection of the applied methods should be based on the physical and bio-chemical property of the grain, the storage environment, and final goal of the storage. Physical environmental control with ecosystem approach should be the primary choice. Controlling both temperature and moisture content or either one would be the basic requirement of a successful IM program.

INTEGRATED MANAGEMENT DURING HANDLING AND TRANSPORTATION

Transport of commodities occurs in many ways, from being hand-carried by individuals to being transported by large bulk carriers. The mode of transport used should be appropriate for the origin and size of a shipment and the distance and terrain over which it needs to be moved. Commodity is usually handled and transported in a short time period. The total mass of the transported commodity is usually smaller than that in storage facilities. Therefore, ambient environment might dominate the environment of the transported commodity. During transportation and handling, commodity might also be in contact with different facilities and tools, and being cut, crushed, impacted, and/or sheared (Fig. 3). For example, grain might gain moisture if it is handled during a rainy day. There is a high risk of contamination, damage, and infestation of pests and microorganisms during handling and transportation (Fig. 3). The integrated management during this time period should focus on the control of the physical environment of the handled grain (such as preventing grain from being in rain) and should eliminate any source of contamination and infestation of pests.

Compared with the storage stage, the advantage of the handling and transportation stage is that grain is usually in a small batch, can be easily moved from one location to another location, will be handled in a few days or hours, can be sampled easily, can be treated in batch or during handling, and insect populations might not have enough time to grow. Therefore, an IM program of grain during handling and transportation should take these advantages and avoid quality and quantity damages.

Drying methods

Hot air, natural air with or without heater, solar, and oven drying.

Temperature and moisture modification

Aeration, chilling (refrigeration), turning, mixing, and drying.

Handling and transportation

Bulk grain: auger, elevator, conveyor, pneumatic system (such as grain vacs), grain truck, vessel, and grain rail car.

Bagged grain: conveyor, truck, vessel or boat, and crane.

Monitoring

Inside silo: Temperature, moisture content (temperature plus RH cable), CO_2 and Phosphine, grain sampling, insect species and density.

Monitoring by sampling: moisture content, germination, FAV, protein, foreign materials, dockage, broken rate, insect species and density, microorganism species and infestation.

Pest (insects, rodent and bird) and mould control

Physical method: sanitation, cleaning, temperature and∕or moisture content control, control atmosphere, heat treatment, chill the grain, physical handling, irradiation, insect traps, impact, packaging, inert dusts.

Chemical method: fumigation, contact pesticide and fungicide, biorationals (such as pheromone).

Biological control: pathogens, parasites, predators.

Control atmosphere: airtight or hermetic storage, low oxygen storage, high CO_2, combustion gases.

Methods of integrated management of grain

Fig. 2- Available methods of integrated management of grain.
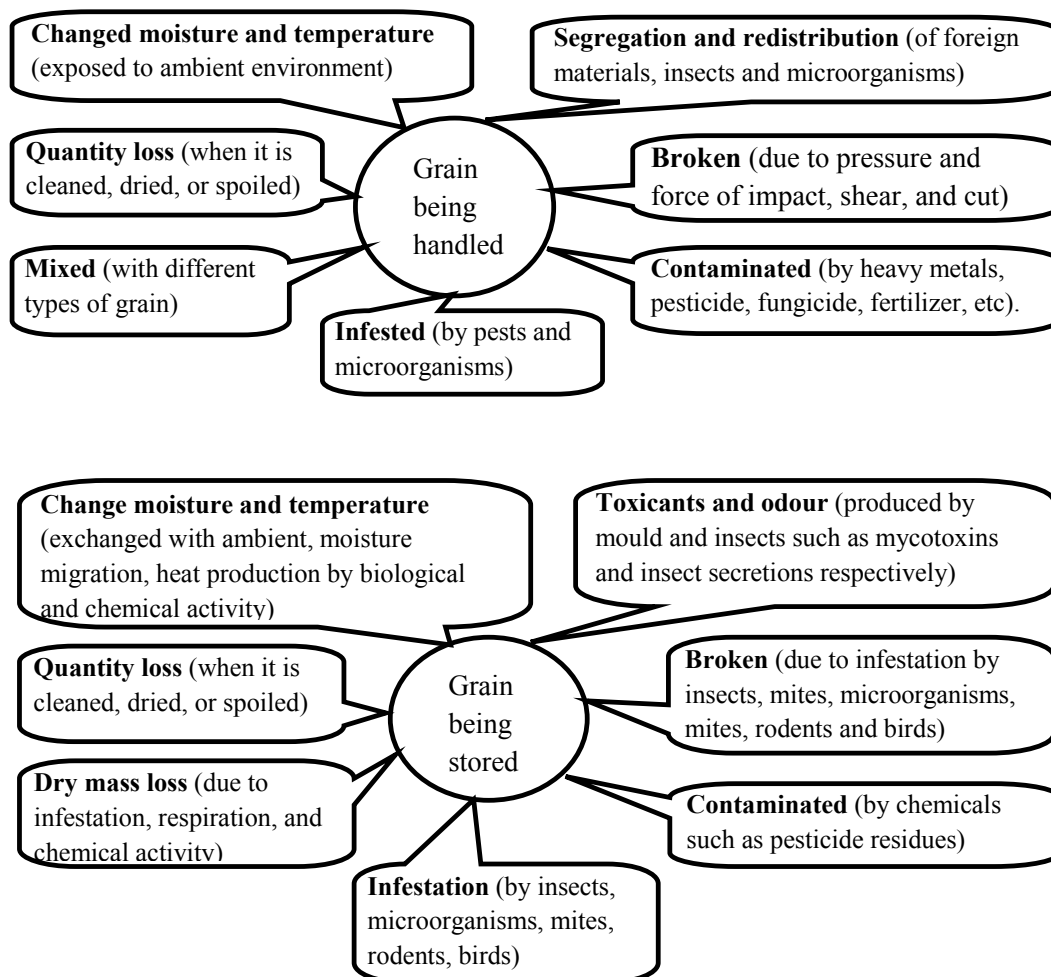
Fig. 3- Sources of quality and quantity change during grain handling (top) and storage (bottom).

INTEGRATED MANAGEMENT DURING STORAGE

During grain storage, there are many sources which will result in the quality and quantity loss of the stored commodity (Fig. 3). Even under "optimized" storage conditions it is impossible to eliminate qualitative changes. They can only be minimized in storage at low temperatures and moisture content. Even though there are management issues causing physical and economical loss of stored grain, key issues are usually mechanical damage due to handling and infestations with microbes, insects, birds and rodents (Jayas et al., 1995). Damage from these factors is inter-related.

Experience has shown that losses during storage are not easily reduced in the absence of well-integrated policies and an IM program. Even though there are many choices to conduct a sound grain storage management, integrated management with ecosystem approach should be

the first choice of the grain storage management (Jian and Jayas, 2012). All methods applied should take advantage of the relationship between the physical and bio-chemical environment and fit well with the physical and chemical property of the grain. For example, grain moisture should be controlled during the entire storage period. Sanitation should be practiced throughoutthe entire procedure of handling, transportation, and storage. Any action of treatment should be based on the result of monitoring and inspection.

Coordination between the three principle approaches - physical, biological and chemical - to the protection of agricultural commodities such as grain must be known when a program of integrated pest management is developed. The interaction between living and non-living factors and integration of various control techniques, within the framework of integrated pest management, has become a focus for research in the stored-products field. The importance of a multidisciplinary approach to stored grain research has also been stressed (White, 1992). Entomology, mycology, chemistry, engineering and food science are commonly involved, but effective integration of technical solutions is often lacking. Intergradation of technical solutions requires the thorough understanding of the physical and bio-chemical properties of commodities and their storage environment. Outlining of inner links and inherent laws behind the IM principles might help with the integration. Identification of the basic issues and their control strategies might promote the application of IM. For example, IM of commodity should focus on the physical control combined with ecosystem approach.

FUTURE RESEARCH

Integrated management theory uses the concept of economic control thresholds (ECTs). For example, an ECT of IPM is most simply defined as the level of pest damage which justifies, in cost/benefit terms, the expenditure of resources upon control actions. It is always a variable threshold because the costs and benefits of any action will depend upon the situation and its circumstances. The ECT at different situations should be studied. Losses of quantity and certain quality parameters should be objectively determined. For example, for insect control in grain storage the ECT is likely to be at or very close to zero in most situations. This will limit the application of biological control.

Monitoring is the basic requirement of the sound IM program. However, reliable monitoring applied in grain storage is only the temperature (Neethirajan et al., 2009). Quality of grain inside stored bins could not be determined without sampling. Therefore, affordable and reliable sensors which can be used under different situations should be developed. Relationship among the physical and chemical properties, and the physical and bio-chemical environments of the stored commodity should be used in IM programs. A successful monitoring program can help to reach this goal.

ACKNOWLEDGEMENTS

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ONE-YEAR PROTECTION OF STORED WHEAT WITH SEVERAL GRAIN PROTECTANTS

Zlatko Korunic[1]*, Irma Kalinovic[2], Vlatka Rozman[2], Anita Liska[2]

[a] Diatom Research and Consulting Inc., 14 Tidefall Dr. Toronto, ON, Canada, M1W 1J2
[b] University of Josip Juraj Strossmayer in Osijek, Faculty of Agriculture in Osijek, Department for Plant Protection, Kralja Petra Svacica 1d 31000 Osijek, Croatia
*Corresponding author's e-mail: *zkorunic@rogers.com*

ABSTRACT

This study was initiated in order to determine how long several grain protectants will provide acceptable protection against *Sitophilus oryzae* (L.), *Rhyzopertha dominica* (F.), and *Tribolium castaneum* (Herbst.), when applied to clean white winter wheat of 13.6% moisture content. The treatments in the experiment were: mixture of diatomaceous earth and deltamethrin (DE/DM insecticide) applied at 100 ppm containing 90 ppm of DE and 0.1 ppm of deltamethrin active ingredient (a.i.); spinosad technical 92% powder applied at 1 ppm a.i.; Storicide II a mixture of chlorpyrifos methyl (CM) and deltamethrin (DM), applied at 3 ppm CM and 0.5 ppm DM a.i.; Actellic 5 E (pirimiphos methyl) applied at 10 ppm a.i. Bioassays were initiated immediately after treatment (zero day), 30, 120, 180 and 360 d after the initial treatment and were maintained at 30±1°C and 70±5% air relative humidity during the twelve months. The treatment of wheat with DE/DM mixture and Storicide II provided effective protection against the adults and the progeny of all three species. Actellic at zero day controlled adults and the progeny of *S. oryzae* and *T. castaneum* (96% to 100%). However it did not control the adults and the progeny of *R. dominica* (38% adult's mortality and 96% progeny reduction). The effectiveness of Actellic on 360 d old deposit on grains was reduced against adults of *S. oryzae* to 12%, *R. dominica* to 45%, and *T. castaneum* to 38% and did not control completely their progeny. Spinosad did not control the adults and the progeny of *S. oryzae* and *T. castaneum* at zero day and 360 d. However, the effectiveness against adults and the progeny of *R. dominica* was 100% at zero and 360 d. The treatment of wheat with DE/DM mixture and Storicide II provided 100% protection against the adults and the progeny of all three species.

Key words: Grain protectants, Storicide II, diatomaceous earth and deltamethrin mixture, Actellic 5E, spinosad, *Sitophilus oryzae*, *Rhyzopertha dominica*, *Tribolium castaneum*, wheat

INTRODUCTION

Integrated pest control (IPM) strategy is used to protect stored agricultural commodities. Different measures are included in IPM strategy such as prevention, monitoring and control (Mueller, 1998). The use of grain protectant insecticides is an important part of IPM strategy. Due to new regulations fewer options are available for providing long term protection of grain (Ignatowicz and Olejarski, 2010). Grain protectants had proven effective against grain insects

when used alone and in a combination (Daglish, 1998; Korunic, 1998; Chintzoglou et al., 2008; Subramanyam et al., 2003). Pesticides residues in food are recognized as a major safety concern (Fishwick, 1988; Fields, 1999).

The objective of this study was to determine if grain protectants Storicide II (mixture of chlorpyrifos methyl and deltamethrin), Actellic 5E (pirimifos methyl), spinosad technical powder and the mixture DE/DM (diatomaceous earth and deltamethrin) applied at registered and recommended concentrations can protect wheat grain during 12 months of storage controlling the adults and the progeny of *Sitophilus oryzae* (L.), the rice weevil, *Rhyzopertha dominica* (F.), the lesser grain borer and *Tribolium castaneum* (Herbst), the red flour beetle.

MATERIALS AND METHODS

Mixed-sex adults of *S. oryzae*, *R. dominica* and *T. castaneum*, 7 to 21 d old, were used in the experiment. *Sitophilus oryzae* and *R. dominica* were cultured on wheat with approximately 14% moisture content (m.c.). *Tribolium castaneum* was cultured on white flour with 5% brewer's un-activated yeast. Insect rearing was conducted at 30±1ºC and 70±5% air relative humidity (r.h.). Un-infested clean eastern white wheat from Ontario, Canada, with 13.6% moisture content (m.c.) was used in the experiment. Moisture content of the grain was measured using a dielectric moisture metre (AACC method 44-11). Dockage was removed by sieving the grains for 45 seconds in a sieve with 2.36 mm openings (8 mesh).

The insecticides tested in the experiment were: chlorpyrifos-methyl + deltamethrin (Storicide II, 216 mg active ingredient (a.i.) of chlorpyrifos-methyl in 1 ml and 37 mg a.i. of deltamethrin in 1 mL (Bayer Crop Science, Research Triangle Park, NC), which is registered in the USA on wheat and rice at the rates of 3 ppm of chlorpyrifos-methyl and 0.5 ppm deltamethrin applied at 3 ppm a.i. of chlorpyrifos methyl and 0.5 ppm a.i. of deltamethrin; spinosad technical materials 92% a.i. (the producer BioSeen, China), which is registered in the USA and has a label rate of 1 ppm for wheat, maize and rice applied at 1 ppm a.i.; pirimiphos-methyl (Actellic 5E, 480 mg a.i. in 1 mL, which is registered in the USA on maize at 8 ppm.) (Agriliance, St Paul, MN), and in some European countries, applied at 10 ppm of a.i.: mixture of diatomaceous earth (DE) and deltamethrin technical (DM) (formulation developed by Z. Korunic) applied at 100 ppm containing 90 ppm of DE and 0.1 ppm of DM a.i.

At the beginning of the test, five of 14 kg groups of wheat were weighed, and the grain m.c. was determined. Four groups were treated with insecticides and one group served as untreated (control) group. Immediately after the initial treatment, 600 g of grain was removed from each container containing treated and untreated grain. This 600 g grain was evenly divided between three 500 mL jars (3 replicates containing 200 g grain per jar). After introducing 50 adult insects of each species into jars, jars were maintained at 30±1°C and 70±5% r.h. The containers with treated and untreated grain (groups) during twelve months were maintained under the same conditions (30±1°C and 70±5% r.h.). Bioassays had been initiated 0, 30, 120, 180, 270 and 360 d after the initial treatment.

To determine mortality in each treatment, grain was sieved 7, 14 and 21 d after insects were introduced, and the number of dead and live insects was recorded. All dead insects were removed 7 and 14 d post-introduction and all dead and live insects were removed 21 d after introduction. The jars were maintained under the same conditions for an additional 21 d (totally for 56 d after introduction) before being sieved again to determine the number of adults' offspring generated.

Mortality and progeny data were subjected to analysis of variance (ANOVA) according to the GLM (General Linear Model). Significant differences in the means were separated by using LSD test (least significant difference). Data processing was conducted by SAS/STAT software 9.1.3 (2003).

RESULTS AND DISCUSSION

The results of the effectiveness of tested grain protectants against the progeny and the adults of *S. oryzae*, *R. dominica* and *T. castaneum* immediately after treatment (zero day), after 180 d and 360 d post-treatment and the exposure of adults during 7 and 21 d to treated and untreated grain are presented and discussed in present study. These results showed clearly the values of tested insecticides for long term grain protection. Immediately after the treatment (zero day), 180 and 360 d, the mixture DE/DM and Storicide II successfully controlled the adults of *S. oryzae*, *R. dominica* and *T. castaneum* and their progeny (Table 1 and 2). The mixture DE/DM was developed to mitigate the disadvantages of DE on grain and to reduce deltamethrin residues in grains (Korunic and Rozman, 2010).

Actellic applied at 10 ppm of a.i. at zero day successfully controlled adults and the progeny of *S. oryzae* and *T. castaneum*. However, the same concentration didn't control the adults and the progeny of *R. dominica* (38% adult's mortality after 21 d and 97% progeny reduction). At 360 d and the exposure period of 21 d the effectiveness of Actellic against *S. oryzae* and *T. castaneum* was significantly reduced from 100% at zero days to 12.6% at 360 d (*S. oryzae*) and to 38.6% at 360 d (*T. castaneum)*. The effectiveness against adults of *R. dominica* was pretty low during all 12 months but the progeny was reduced more than 95% (Table 1 and 2).

Spinosad applied at 1 ppm did not control the adults (88% at zero day and 99% at 360 d and the progeny of *S. oryzae* (approximately 38% progeny reduction) and the adults (22% at zero day and 38% at 360 d) and the progeny (approximately 20% to 40% reduction) of *T. castaneum*. However, spinosad successfully controlled the adults and the progeny of *R. dominica* during 12 months (Table 1 and 2).

Table 1. The mortality of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* at zero day, 180 and 360 d post-treatment after 7 and 21 d of adults' exposured to grain protectants treated and untreated grain

Formulation	Concentr. (ppm)	*Sitophilus oryzae* Average adult mortality (%) ± SE					
		Zero day post-treatment***		180 d post-treatment****		360 d post-treatment*****	
		7 d	21 d	7 d	21 d	7 d	21 d
Untreated	0	0.6 ± 0.6^d	10.0 ± 3.0^c	10.6 ± 3.5^c	28.6 ± 2.4^b	4.6 ± 1.7^c	6.6 ± 2.4^c
*DE/DM	100	99.3 ± 0.6^a	100.0 ± 0.0^a	98.6 ± 1.3^a	100.0 ± 0.0^a	98.6 ± 0.6^a	100.0 ± 0.0^a
Actellic E5	10	100.0 ± 0.0^a	100.0 ± 0.0^a	29.3 ± 2.9^b	96.0 ± 2.0^a	2.0 ± 1.1^c	12.6 ± 4.6^x
**StoricideII	3CP;0.5DM	100.0 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a	100.00 ± 0.0^a	100.0 ± 0.0^a	100.0 ± 0.0^a
Spinosad	1	71.3 ± 6.3^b	88.6 ± 2.9^b	92.0 ± 4.6^a	97.3 ± 1.7^a	36.6 ± 4.0^b	99.3 ± 0.6^a

Formulation	Concentr. (ppm)	*Rhyzopertha dominica* Average adult mortality (%) ± SE					
		Zero day post-treatment***		180 d post-treatment****		360 d post-treatment*****	
		7 d	21 d	7 d	21 d	7 d	21 d
Untreated	0	2.0 ± 1.1^c	2.6 ± 0.6^c	4.0 ± 1.1^e	24.6 ± 1.7^d	6.0 ± 1.1^f	18.0 ± 1.1^e
*DE/DM	100	97.3 ± 1.7^a	100.0 ± 0.0^a	78.0 ± 1.1^b	100.0 ± 0.0^a	97.3 ± 0.6^{ab}	100.0 ± 0.0^a
Actellic E5	10	7.3 ± 1.7^c	38.0 ± 7.2^b	23.3 ± 5.8^d	45.3 ± 6.9^c	14.0 ± 1.1^e	45.3 ± 1.3^d
**StoricideII	3CP;0.5DM	98.6 ± 1.3^a	100.0 ± 0.0^a	69.3 ± 5.4^{bc}	100.0 ± 0.0^a	70.0 ± 6.9^c	100.0 ± 0.0^a
Spinosad	1	100.0 ± 0.0^a	100.0 ± 0.0^a	85.3 ± 6.9^{ab}	100.0 ± 0.0^a	92.0 ± 3.4^{ab}	100.0 ± 0.0^a

Formulation	Concentr. (ppm)	*Tribolium castaneum* Average adult mortality (%) ± SE					
		Zero day post-treatment***		180 d post-treatment****		360 d post-treatment*****	
		7 d	21 d	7 d	21 d	7 d	21 d
Untreated	0	0.6 ± 0.6^e	5.3 ± 1.3^d	3.3 ± 1.7^e	15.3 ± 5.2^d	2.6 ± 1.7^d	11.3 ± 2.9^d
*DE/DM	100	68.6 ± 5.8^b	100.0 ± 0.0^a	50.6 ± 3.7^b	100.0 ± 0.0^a	52.6 ± 4.0^b	100.0 ± 0.0^a
Actellic E5	10	100.0 ± 0.0^a	100.0 ± 0.0^a	11.3 ± 1.3^d	40.6 ± 2.4^{bc}	8.6 ± 2.9^d	38.6 ± 4.6^{bc}
**Storicide II	3CP;0.5DM	100.0 ± 0.0^a	100.0 ± 0.0^a	56.0 ± 5.0^b	100.0 ± 0.0^a	58.6 ± 3.3^b	99.3 ± 0.6^a
Spinosad	1	0.0 ± 0.0^e	22.0 ± 1.1^c	11.3 ± 1.3^d	41.3 ± 2.4^{bc}	10.0 ± 1.1^d	38.0 ± 2.0^c

* DE/DM – diatomaceous earth/chlorpyrifos methyl; **3ppm chlorpyrifos; 0.5 ppm deltamethrin

*** Means in the columns for zero day post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test

****means in the columns for 180 d post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test

*****Means in the columns for 360 d post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test

Table 2. The progeny of *Sitophilus oryzae*, *Rhyzopertha dominica* and *Tribolium castaneum* at zero day, 180 and 360 d post-treatment

Formulation	Concentr. (ppm)	*Sitophilus oryzae* Average adult number (progeny) ± SE		
		Zero day post-treatment*** after 56 d	180 d post-treatment**** after 56 d	360 d post-treatment***** after 56 d
Untreated	0	429.3 ± 37.7[c]	500.3 ± 41.3[c]	508.0 ± 5.1[d]
*DE/DM	100	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Actellic E5	10	0.3 ± 0.3[a]	1.3 ± 0.3[a]	4.6 ± 1.6[b]
**Storicide II	3CP;0.5DM	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Spinosad	1	163.6 ± 11.8[b]	181.0 ± 7.1[b]	176 ± 34.3[c]

Formulation	Concentr. (ppm)	*Rhyzopertha dominica* Average adult number (progeny) ± SE		
		Zero day post-treatment after 56 d	180 d post-treatment after 56 d	360 d post-treatment***** after 56 d
Untreated	0	229.3 ± 18.0[c]	279.6 ± 10.0[c]	312.6 ± 10.1[c]
*DE/DM	100	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Actellic E5	10	7.3 ± 1.7[b]	7.3 ± 2.0[b]	12.0 ± 2.5[b]
**Storicide II	3CP;0.5DM	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Spinosad	1	1.3 ± 0.6[a]	1.0 ± 0.5[a]	0.6 ± 0.3[a]

Formulation	Concentr. (ppm)	*Tribolium castaneum* Average adult number (progeny) ± SE		
		Zero day post-treatment after 56 d	180 d post-treatment after 56 d	360 d post-treatment***** after 56 d
Untreated	0	101.0 ± 4.0[c]	99.6 ± 8.7[d]	107.3 ± 6.9[d]
*DE/DM	100	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Actellic E5	10	0.6 ± 0.6[a]	2.0 ± 0.5[b]	3.3 ± 0.8[b]
**Storicide II	3CP;0.5DM	0.0 ± 0.0[a]	0.0 ± 0.0[a]	0.0 ± 0.0[a]
Spinosad T	1	40.0 ± 17.0[b]	24.0 ± 4.0[c]	28.0 ± 3.7[c]

* DE/DM – diatomaceous earth/chlorpyrifos methyl; **3ppm chlorpyrifos; 0.5 ppm deltamethrin
*** Means in the columns for zero day post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test
****Means in the columns for 180 d post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test
*****Means in the columns for 380 d post-treatment followed by the same letters are not significantly (P>0.05) different as determined by the LSD-test

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OPTIMIZATION OF HS-SPME-GC METHOD FOR DETECTION OF STORED GRAIN INSECTS

Yonghao Niu[1,2,3*], Giles Hardy[3], Lei Hua[2], Robert Trengove[3], Manjree Agarwal[1,3], Hui Cheng[1,3], Yonglin Ren[1,3,4]

[1]Australia Cooperative Research Centre for National Plant Biosecurity, LPO Box 5012, Bruce, ACT 2617
[2]College of Plant Protection, Northwest A&F University, Yangling, Shaanxi, 712100, China
[3]School of Biological Science and Biotechnology, Murdoch University, South Street, Murdoch, WA, 6150, Australia
[4]Australia Department of Agriculture and Food, Western Australia, 3 Baron-Hay Court South Perth, WA, 6151
*Corresponding author's email: Y.Niu@Murdoch.edu.au

ABSTRACT

Headspace solid phase micro-extraction (HS-SPME) coupled with gas chromatography (GC) is a useful sample preparation, volatile extraction and separation method for analysis of volatile compounds from stored grain insects and their hosts. However, for using this high-quality analytical method, there is a need to optimize a range of factors to ensure good extraction efficiency. These factors include fibre selection, column selection and sample preparation. In this paper, six types of polar and non-polar fibres (100 μm PDMS, 85 μm PA, 85 μm CAR/PDMS, 65 μm PDMS/DVB, 50/30 μm PDMS/CAR/DVB and 7 μm PDMS) were used to conduct the HS-SPME of volatile chemicals from wheat, wheat flour and two species of stored grain insects *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (Fabricius). The results showed that the 50/30 μm PDMS/DVB/CAR fibre not only extracted the maximum number of volatile organic chemicals (VOCs), but also captured the largest mass of VOCs for subsequent detection by GC. Optimum sample sealing time, fibre extraction time, desorption time and temperature were 24 h, 4 h, 5 min and 250°C, respectively. The GC results of volatiles from different samples gave different patterns of GC spectrum, which indicated that different volatile compounds were released from the different samples. Therefore, this study provides a detailed sequence of HS-SPME-GC optimization steps that can be applied towards the development of HS-SPME-GC methods to detect stored grain insects.

Key words: Wheat, wheat flour, *Rhizopertha dominica*, *Tribolium castaneum*, volatiles, solid phase micro-extraction, gas chromatography

INTRODUCTION

Stored product insects are endemic to grain industries throughout the world. The detection and quantification of stored product insects in stationary or moving grain masses have proven to be a difficult task (Brett, 2009). The typical approaches for detecting insects in stored grain are based on collecting representative samples of grain from stacks, trucks and rail bogies,

and manually inspecting these samples for adult insects by sieving, flotation and Berlese-funnels (Neethirajan et al., 2007). These techniques can easily trap or detect adult insects but not suitable to find immature insects. X-ray imaging and near infrared reflectance (NIR) spectroscopy have been studied for the detection of stored grain insects as they can detect hidden insects (Milner et al., 1950). However, the operation of these technologies is relatively complicated and there has been no success with in-situ detection.

A good potential detection method is to analyse the air within a grain mass for specific VOCs released by insects. Insect odours or aromas, which are identified through volatile chemical signals, could be used to demonstrate the presence of insects in grain storage facilities. Headspace solid phase micro-extraction (HS-SPME) coupled with gas chromatograph (GC) is probably the method that could compromise between cost and sensitivity (Reuss, 2003). This sample preparation and volatile detection method has been used to examine volatile secretion from stored grains and grain insects because sample preparation is rapid, sampling is integrated, and the extraction, concentration and introduction of the samples to an analytical instrument occurs in one solvent-free step (Risticevic et al., 2010). However, use of GC in combination with SPME requires optimization of various sample preparation and GC parameters which affect the extraction efficiency and GC sensitivity. Therefore, the objective of this research was to provide a detailed sequence of HS-SPME-GC optimization steps that can be applied towards the development of HS-SPME-GC methods to detect stored grain insects.

This paper reports a systematic laboratory study on the a) selection of a suitable fibre which can absorb the maximum number of volatiles from wheat, wheat flour, *T. castaneum* (H.) and *R. dominica* (F.); b) development of optimum sample preparation procedures; and (c) the evaluation of optimum GC conditions to achieve the maximum number and best resolution of peaks.

MATERIALS AND METHODS

Grain pre-treatment

The newly harvested (2011-2012) wheat (Australia Standard Wheat I) used for this experiment was procured from CBH (Co-operative Bulk Handling), Western Australia. The moisture content was 11.5% (w/w, Electronic Moisture Meter, PFEUFFER, HOH-Express 50, Kitzingen, Germany). The wheat sample was placed in sealed glass jars (4 L) and stored in a fridge at -4°C for one week to kill any live insects, and then stored at 4°C until use.

The wheat flour was made from the same frozen wheat described above. A coffee grinder was used to make wheat flour and stored in large jars at 4°C. Before use, the sample was conditioned at room temperature (25±2°C) for 24 h.

Insects culture

The insect species *R. dominica* (strain No. MUWRD 7) and *T. castaneum* (MUWTC-8) were obtained from the Stored Grain Research Laboratory, School of Biological Sciences and Biotechnology, Murdoch University, Perth, Australia. About 200 adults of *R. dominica* and *T. castaneum* were added into 400 g whole wheat and 350 g wheat flour in 500 mL bottles with a meshed lid, respectively to obtain mixed age insect population. The experimental insects were reared in the dark at 30°C and 60% r.h., kept for 4-5 weeks until adults of the next generation emerged.

Glassware and SPME fibres

One hundred mL Erlenmeyer flasks (Fisher Scientific, Quickfit, U.K.; Cat. No FE 100/3) equipped with cone/screw-thread adapter (Crown Scientific, Code ST 5313, Wantirna South VIC 3152, Australia) with 7/16" blue septa (Grace Davison Discovery Sciences, Cat. No. 6518, Vic 3178, Australia) were used for samples preparation. The measured volume of each Erlenmeyer flask and inlet system was calculated from the weight of water required to fill the container. The SPME fibres tested were 100 μm Polydimethylsiloxane (PDMS; Cat. No. 57300-U); 85 μm Carboxen/Polydimethylsiloxane (CAR/PDMS; Cat. No. 57334-U); 85 μm Polyacrylate (PA; Cat. No. 57304); 65 μm Polydimethylsiloxane/Divinylbenzene (PDMS/DVB; Cat. No. 57326-U); 50/30 μm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS; Cat. No. 57348-U); 7 μm Polydimethysiloxane (PDMS; Cat. No. 57302), all from Analytical Sigma-Aldrich, Sydney, Australia, and all were conditioned prior to use, in accordance with the manufacturer's recommendations.

Apparatus and instruments

A 6890 model Agilent GC manufactured by Agilent Technology (Palo Alto, CA, USA) equipped with a Zebron ZB-WAX plus column (Dimensions: 30 m×0.25 mm I.D. × 0.25 μm film thickness, polar column) and Flame Ionization Detector (FID) was used to analyse the volatile profiles extracted by HS-SPME.

General procedures

The HS-SPME-GC method included the following procedures: (1) incubate samples for different period, (2) extract VOCs from the head space above the sample within the flask using fibres, (3) setup and precondition GC and insert fibres into GC inlet for a certain desorption period, and then remove the fibre and (4) save the GC chromatogram and export into Microsoft Excel spread sheet for further analysis.

Evaluation of fibres and sample processing conditions

Four types of sample including 80 g wheat, 70 g wheat flour, 100 *R. dominica* adults and 100 *T. castaneum* adults were placed in 100 mL Erlenmeyer flasks separately sealed with cone/screw-thread adapter with injection port, and the samples were kept at 25°C in a temperature controlled room.

For fibre selection, the above four samples were extracted; however, only the wheat sample was systematically tested further under different sample processing conditions, such as sample sealing time, extraction time and desorption time. Three replicates for each treatment were conducted.

GC condition

The following GC conditions were used: hydrogen was used as the carrier gas at a constant speed of 40 mL/min, in the split-less mode. The GC inlet was operated at 250°C and Flame Ion Detector (FID) temperature was 250°C. The oven temperature program used was: 45°C for 5 min, increasing from 45°C to 250°C at 5°C/min and being held for 5 min at each increment with a total run of 51 min.

Optimization Scheme

Sample preparation and extraction procedures were tested as follows: (1) six types of fibres used for extraction of volatile compounds (2) samples were sealed for 12, 24 and 48 h, (3)

extractions were for 0.5, 1, 2, 4 and 8 h, and (4) fibre remained in the GC inlet for the desorption of volatiles for 1, 3 and 5 min.

Data Analysis

The GC data including retention time, peak height and peak area were collected and integrated by the chromatography software Agilent Chemstation, and then exported to Microsoft Excel for further analysis. The repeatability of replicates from the same sample was verified by checking the chromatogram pattern features such as detected peak retention times, peak heights, and peak areas.

<div align="center">RESULTS AND DISCUSSION</div>

Effect of fibres

A typical chromatogram of volatiles by six fibres from the wheat sample is given in Fig. 1. Similarly chromatograms were obtained for other samples. Fig. 2 showed the percentage of GC total peak areas from different fibres in four tested samples. All results for wheat, wheat flour, *R. dominica* and *T. castaneum* showed that the 50/30 μm CAR/DVB/PDMS fibre was an optimum fibre compared to the other 5 fibres: 100 μm PDMS, 85 μm CAR/PDMS, 85 μm PA, 65 μm PDMS/DVB and 7 μm PDMS fibres. The 50/30 μm (PDMS/DVB/CAR) fibre is a three phase fibre, it can extract wide range of components from C2 to C20 (Risticevic et al., 2010) and showed high sensitivity and selectivity for the determination of volatile compounds from all four samples. In the last few years, HS-SPME coupled with GC has been widely used to analyse VOCs from stored grain pests and their hosts, but to date complete VOCs profiles from non polar or polar compounds in stored grain pests and hosts have not been established. Previous research has used either 1 or 2 phase fibre for the detection of metabolites from *R. dominica* and *T. castaneum* (Villaverde et al., 2007; Seitz, 2004) and there is a possibility of missing out some compounds hence the need for optimum fibre.
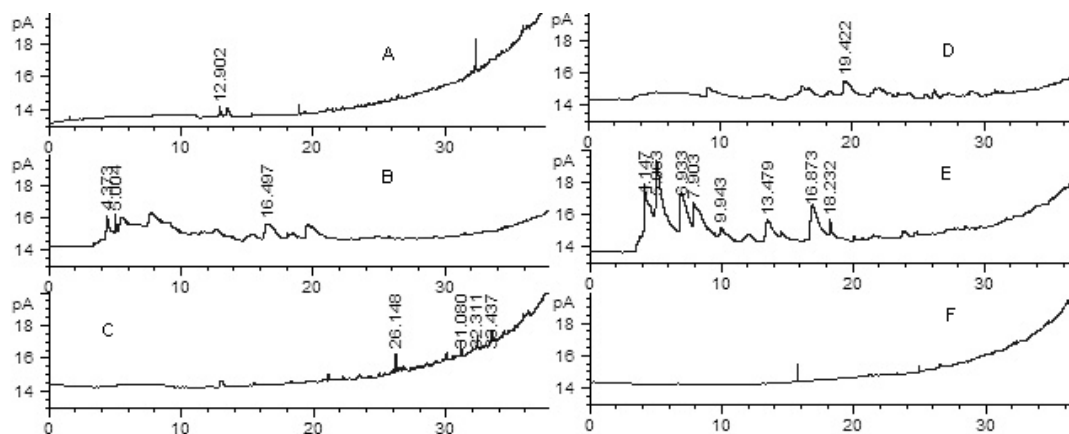
Fig. 1- Chromatograms of wheat volatiles by HS-SPME-GC using six fibres. A. 100 μm PDMS, B. 85 μm CAR/PDMS, C. 85 μm PA, D. 65 μm PDMS/DVB, E. 50/30 μm DVB/CAR/PDMS and F. 7 μm PDMS.
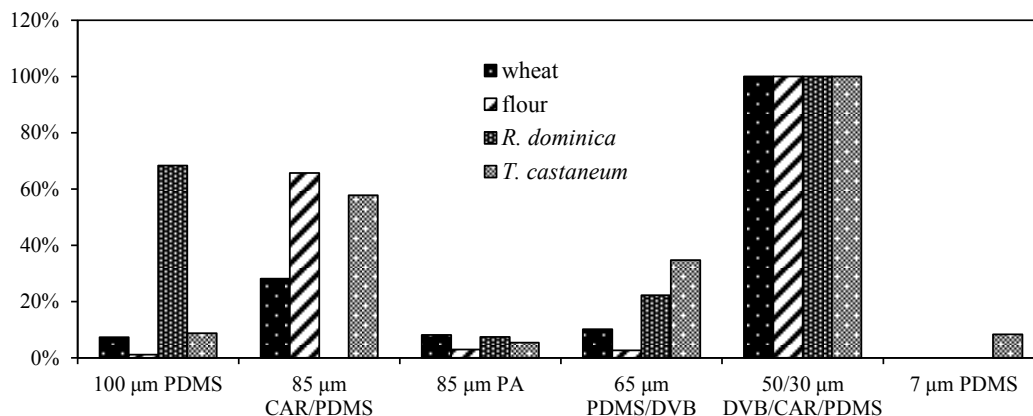
Fig. 2- The percentage comparison of GC total peak areas for six fibres in four samples (The total peaks area of 50/30μm DVB/CAR/PDMS used as 100%).

Effect of period of sample sealing

Total peak areas from the different samples sealed for 12, 24 and 48 h are compared in Fig.3. The chromatograms of the three different sealing times are not shown. If the total peaks area of 24 h sealing time was used as 100%, the peak area of the 12 h sealing time had 27% of 24 h, and 48 h sealing sample had 28% of 24 h. This result showed that 24 h sealing period achieved higher efficiency for VOCs extraction from the wheat sample. That is, the equilibrium between the wheat and its volatiles within the flask had an impact on the final volatile extraction by the SPME fibre.
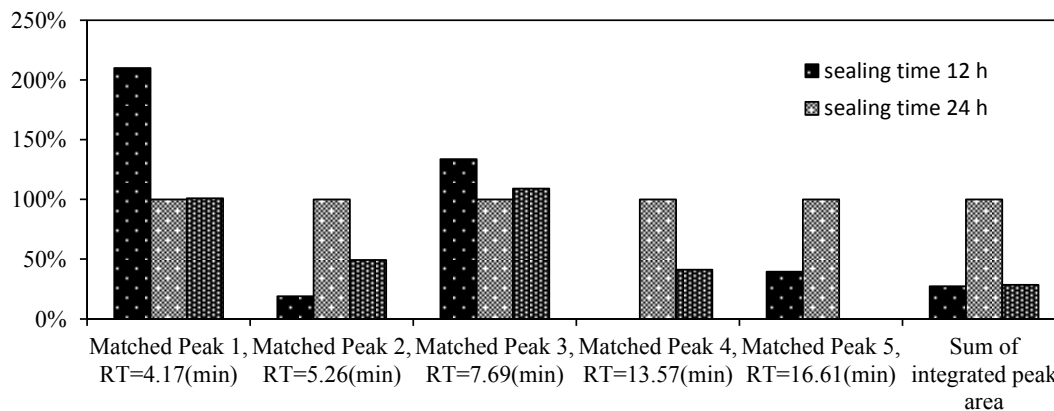
Fig. 3- The percentage comparison of GC total peak areas with 12, 24, and 48 h sealing time in sample preparation (The total peaks area of 24 h sealing time was used as100%).

Effect of extraction time

Fig. 4 compared the percentage data of the major peaks and the total GC peak areas with different extraction time for the wheat sample using the 50/30 µm CAR/DVB/PDMS fibre. An extraction time of 4 h was the optimum. The extraction time is the time-limiting step of the SPME procedure and is one of the most crucial steps of the development of the SPME method (Kudlejova et al., 2007). During extraction time, sample components in the head-space of the sample transferred to the fibre coating. Thus, different extraction times can affect the fibre absorption results.
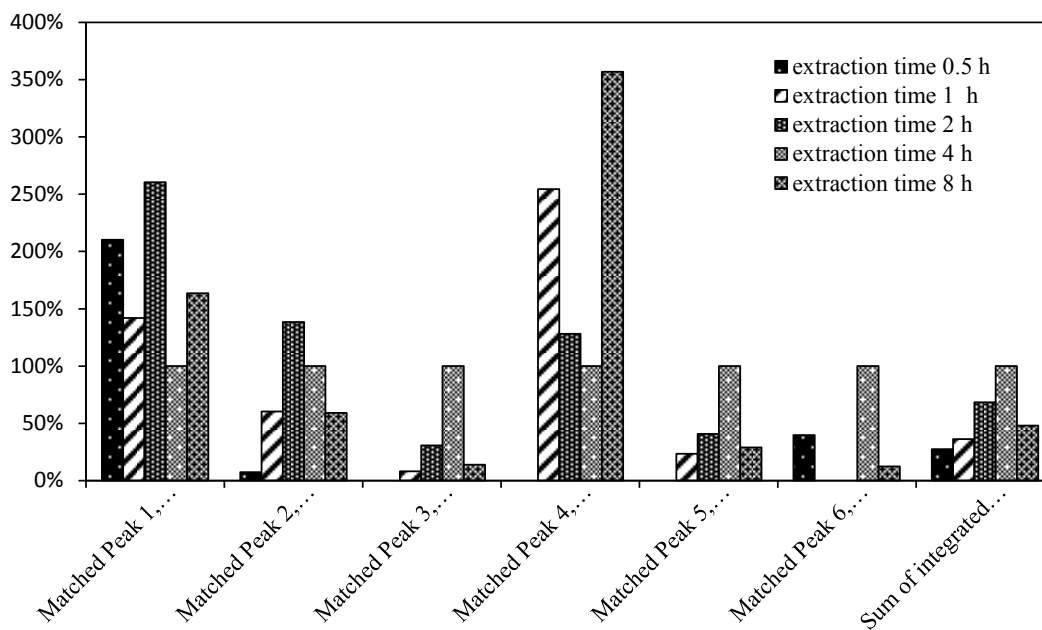
Fig. 4- The percentage comparison of GC total peak areas with 0.5, 1, 2, 4 and 8 h extraction time from the wheat sample (The total peak area of 4 h extraction time was used as 100%).

Effect of desorption time

The volatiles of wheat sample by 50/30 µm CAR/DVB/PDMS fibre using different desorption time in the GC inlet are different. Fig. 5 demonstrated the percentage of total GC peak areas for different desorption times of 1, 3 and 5 min. The results showed that more components were detected at 5 min desorption time. Desorption time can influence analytes desorption efficiency, this finally influenced how many compounds were transferred into the GC column. In fact, there are some factors that can affect compounds transfer efficiency such as desorption temperature, carrier gas linear flow rate and desorption time (Kudlejova et al., 2007). In this experiment, same desorption temperature and injector gas flow rate were used for analysing compounds, only different desorption times were tested. The result showed that if the other conditions were same, different desorption time can affect the fibre desorption efficiency.

726
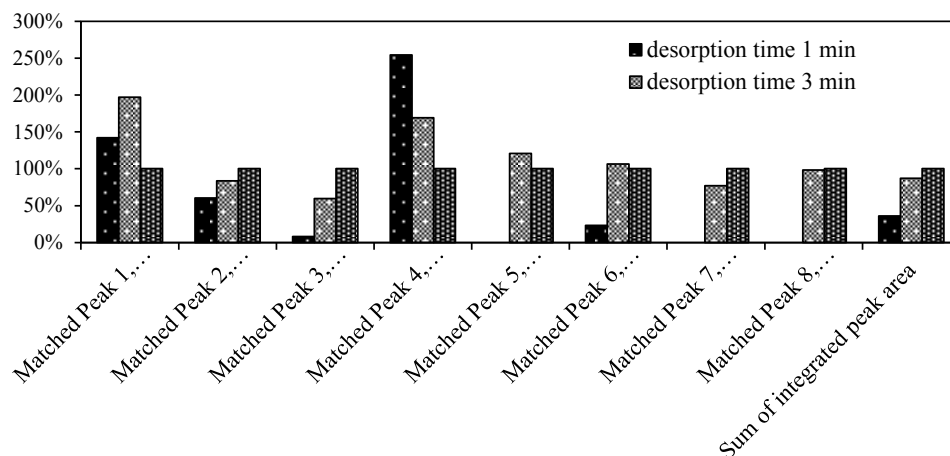
Fig. 5- The percentage comparison of GC total peak area with 1, 3 and 5 min desorption time from the wheat sample (The total peak area of 5 min desorption time was used as 100%).

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OLFACTORY RESPONSES OF *PLODIA INTERPUNCTELLA* (HÜBNER, 1813) (LEPIDOPTERA: PYRALIDAE) TO DRIED APRICOT VOLATILES

Agnès Flore Ndomo[1,2*], Bernhard Weissbecker[3], Stefan Schütz[3], Maximilian von Fragstein[3], Christoph Reichmuth[1], Christian Ulrichs[2], Cornel Adler[1]

[1]Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Ecochemistry, Plant Analysis and Stored Product Protection, Königin-Luise-Str. 19, D-14195 Berlin, Germany
[2]Division of Urban Plant Ecophysiology, Faculty of Agriculture and Horticulture, Humboldt University, Lentzallee 55/57, 14195 Berlin, Germany
[3]Institute of Forest Zoology and Forest Conservation, Georg-August-University of Göttingen, 37077 Germany
*Corresponding author's e-mail: *agnes.ndomo@jki.bund.de*

ABSTRACT

Dried apricot is one of the stored fruit most endangered to be infested by the Indianmeal moth, *Plodia interpunctella* (Hübner). The aim of the present study was to identify volatile compounds from dried apricot that elicit an electrophysiological response in *P. interpunctella* adults. The volatiles were collected from the headspace of a glass vial with dried apricots using closed-loop-stripping analysis. Measurement by coupled gas chromatography-mass spectrometry together with an electroantennographic detector (GC-MS/EAD) revealed that ten volatile compounds were consistently EAG-active. They were representatives of six different groups of organic compounds. These were four alcohols (1-butanol, 1-pentanol, 1-hexanol, 3-methyl-1-butanol), two esters (ethyl benzoate and 3-methyl-1-butanol acetate), one acid (acetic acid), one ketone (3-hydroxy-2-butanone), one pyrazine derivative (trimethylpyrazine) and one benzenoid compound (benzyl alcohol). In general, antennae of females responded more strongly than those of males. These EAG-active compounds can be considered as olfactory cues for *P. interpunctella*. They will be used in behavioral bioassays in order to determine whether they are attractive, or repellent towards the moth.

Key words: Volatiles, electroantennography, dried apricot, *Plodia interpunctella*

INTRODUCTION

Due to the damages caused by the larvae which feed on a wide variety of foods (stored cereals, beans, nuts, dried fruits, dried flowers, dried vegetables and some spices), the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), is considered the most important insect pest in the food and feed processing industry worldwide (Reichmuth et al., 2007). Since larvae develop within the stored foods, the early control of adults is required for an effective control of the population to avoid egg laying. Insect attractants can play an important part in the management of insect pests. Their use, especially attractant food volatiles, is now receiving great attention (Olsson et al., 2006; Uechi et al., 2007; Germinara

et al., 2008) since certain products are able to produce hundreds of volatile compounds. Moths use volatiles from food materials to locate suitable partners and oviposition sites (Olsson et al., 2006; Uechi et al., 2007). For instance, it has been shown that extracts from chocolate as well as specific components attract *P. interpunctella* and *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) (Olsson et al., 2005; Olsson et al., 2006; Uechi et al., 2007). *Plodia interpunctella* adults also orientate to volatile blends emitted by grains of cereals (Uechi et al., 2007).

Despite this behavioral evidence and the identification of many volatiles emitted by grains of several cereals little attention has been given to the response of *P. interpunctella* to dried fruit volatiles. The identification of electrophysiologically active volatile compounds emanating from food can be made by coupled gas-chromatography with two detectors: mass spectrometry and an electroantennographic detector (GC-MS/EAD) as described by Weissbecker et al. (2004). This technique presents two advantages, determining simultaneously which volatile in a complex mix an insect is able to perceive and the identification of the compound responsible for the induced EAG response (Weissbecker et al., 2004). Using the same technique, we identified in the present study active volatile compounds in dried apricot, which elicit an electrophysiological response in *Plodia interpunctella* adults.

MATERIALS AND METHODS

Insects
Adults of *P. interpunctella* were obtained from the insect culture of the institute. They were kept at 25±1°C temperature and 65±5% relative humidity (r.h.) in the dark. Insects were sexed during the last instar larval stage, when the male gonad is dorsally visible as a dark spot in fifth abdominal segment, while there is no spot visible in females (Reichmuth et al., 2007). Males and females were kept in different glass jars and chambers until the emergence of new adults. Twenty adult moths (10 males and 10 females) of 2 d-old were used for bioassays.

Fruit sample
Dried apricot fruits (*Prunus armeniaca* L.), used in the present study was originated from Malatya, Turkey. The pitted samples were purchased from a retail organic food market in Berlin, Germany. They were mixed varieties of "Hacihaliloglu", Cöloglu", "Soganci", "Hasanbey", "Kabaasi" and "Cataloglu". Dried apricots were kept in original packages at +5°C until analysis.

Sampling of volatiles
Dried apricots were cut by hand into small pieces (approx. 10 x 10 mm) and batches of 100 g were immediately introduced into a 250 ml glass flask with neck outlet (30 mm ID) and closed with a custom made polytetrafluoroethylene (PTFE) stopper. Volatiles were sampled from the headspace using the closed-loop stripping analysis (CLSA) method (Boland et al., 1984). Miniature pumps were used to pump air at a flow rate of 1 l/min from the flasks to CLSA tubes loaded with 1.5 mg activated charcoal in which the volatiles were trapped. The sampling time was 45 min. Odor was then eluted from the charcoal with a mixture of dichloromethane and methanol (2:1).

Gas chromatography mass spectrometry / electroantennographic detection (GC MS/EAD) of odor sample

Odor samples collected from dried apricot were analyzed by gas chromatography (GC) coupled to mass spectrometry (MS) and electroantennographic detection (EAD) as described by Weissbecker et al. (2004). The unit used consisted of a 6890N gas chromatograph and a 5973N quadrupole mass spectrometer (both Agilent, Santa Clara, USA). One µl of the odor extract was automatically injected into the injector port. The GC employed the following temperature program: start: 50°C, hold for 1.5 min, ramp 7.5 °C/min to 200°C, hold for 5 min. It was equipped with a split/splitless injector operated at 250°C in a pulsed-splitless mode and an INNOWAX column (30 m length, 0.25 mm ID and 0.25 µm film thickness). Helium was used as carrier gas at a constant flow of 1 ml/min. The effluent of the column was splitted between the quadrupole mass spectrometer and a modified "olfactory detection port" (ODP-2, Gerstel, Mülheim, Germany). The ODP was used to mix the effluent from the GC with the humidified air. The air flow carrying the odors was directed to the insect antenna which was fixed in a special antenna holder (Färbert et al., 1997) within the sensor containment. Signals from the antenna were amplified by a factor of 100 and recorded using the A/D-convertor (Agilent) and the HP ChemStation software. Peaks of the chromatogram were identified by using the NIST mass spectral library (National Institute of Standards and Technology, Gaithersburg, MD, USA) and the Mass Finder 3.0 software (Hochmuth, 2004) together with the library "Terpenoids and related constituents of Essential Oils" (König et al., 2004).

In addition a non-polar column, HP-5MS (Agilent Technologies, Santa Clara, USA), 30 m x 0.25 mm ID., 0.25 µm film thickness using the GC-MS method described above and the same analytical conditions parameters as the HP-INNOWAX column was used to ensure the identification of all (polar and non-polar) volatiles present in the substrates.

The identity of volatile compounds that elicited an EAG response was confirmed by matching their mass spectra and retention time to those of authentic standards on the two different columns used. The linear retention indices were calculated according to the formula of Van Den Pool and Kratz (1963).

RESULTS

The present results show that dried apricot (mixed variety of Hacihaliloglu", Cöloglu", "Soganci", "Hasanbey", "Kabaasi" and "Cataloglu", emitted 36 different volatiles detectible by GC-MS. Out of the 36 volatiles, ten were able to elicit EAG responses in 17 of the 20 insects tested (Fig. 1). They belonged to the chemical classes of esters, alcohols, ketones, pyrazine derivatives and acids. Two constituents distinctly elicited higher EAG responses in both male and female *P. interpunctella*, 3-methyl-1-butanol [1] and 1-hexanol [6] (Fig. 1). The identity of the compounds was confirmed by injection of purchased authentic compounds. A positive verification of the identity of EAG-active compounds made by comparing the spectra with those of corresponding synthetic compounds in the NIST library was assumed when approximately the same retention time and mass spectrum could be found.

Table 1. List of electrophysiologically active substances found in the headspace of dried apricot

N°	Component	Linear retention indices (LRI)[a]	Chemical class
1	3-methyl-1-butanol acetate	1119	Esters
2	1-butanol	1136	Alcohols
3	3-methyl-1-butanol	1202	Alcohols
4	1-pentanol	1241	Alcohols
5	3-hydroxy-2-butanone	1288	Ketones
6	1-hexanol	1341	Alcohols
7	trimethyl pyrazine	1396	Pyrazine derivatives
8	acetic acid	1439	Acids
9	ethyl benzoate	1664	Esters
10	benzyl alcohol	1869	Alcohols

[a] Linear retention indices (LRI) were calculated from the chromatograms obtained with a polar column; the identity of the respective compounds was confirmed by comparing its mass spectra and retention indices with that of its authentic standard. Peak number correspond to the number in Fig.1
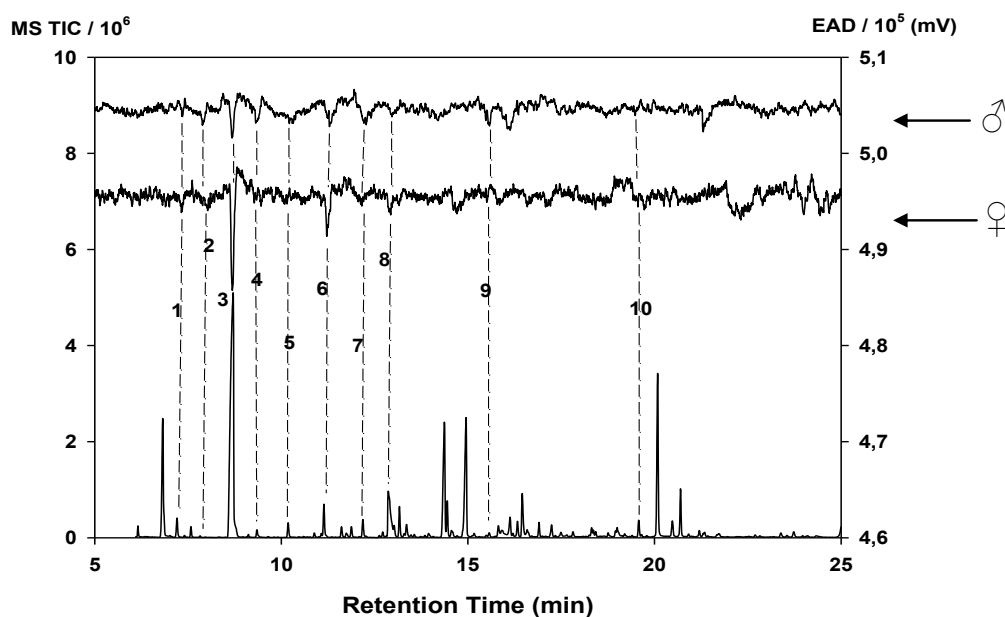
Fig.1- GC-MS/EAD recording of *P. interpunctella* in response to volatiles sampled from dried apricot headspace. Upper inverted trace: EAD signal ($/10^5 + 0.07$ mV) of the *P. interpunctella* male; lower inverted trace: EAD signal ($/10^5$ mV) of female; bottom line: MS-TIC (total ion current). Compounds labelled are as in table 2: [1] 3-methyl-1-butanol acetate; [2] 1-butanol; [3] 3-methyl-1-butanol; [4] 1-pentanol; [5] 3-hydroxy-2-butanone; [6] 1-hexanol; [7] trimethylpyrazine; [8] acetic acid; [9] ethyl benzoate; [10] benzyl alcohol.

DISCUSSION

The antenna of *P. interpunctella* adults responded electrophysiologically to 10 volatile compounds found in dried apricot headspace. This obviously means that primary receptor cells located on the insect's antenna were stimulated by these volatiles. However, these compounds might be attractive, repellent or deterrent to *P. interpunctella* adults. Some of the EAG-active volatiles have earlier been identified. The alcolhols 1-pentanol and 1-hexanol are found in wheat flour (Uechi et al., 2007), in roasted almonds and dried apple (Ndomo, unpubl. data) and in cereals (Germinara et al., 2008). Sexual dimorphism in the perception of volatiles has been observed (Reinecke et al., 2005). The EAD peaks obtained with females were mostly stronger than those of males (Fig. 1). Biologically, this could make sense as females need to find suitable substrate for oviposition (Uechi et al., 2007; Olsson et al., 2006) while males may react to food volatiles just with the intention of finding females as mating partners because adult moths do not feed. In pitfall olfactometers, only mated females of *P. interpunctella* were attracted to volatiles of wheat flour (Uechi et al., 2007). In addition, alkanals (C_6-C_{10}) and 2E-alkenals (C_7-C_{11}) were as attractive as individual aldehydes or mixtures, as well as the mixture of many alcohols with aldehydes (Uechi et al., 2007). Isoamyl alcohol and acetic acid found in the present study in dried apricot were used to trap *P. interpunctella* female in mixture with acetic acid (1:1) (Toth et al., 2002). In their study on chocolate volatile attractants for *P. interpunctella* and *E. kuehniella,* Olsson et al. (2006) found that benzyl alcohol, nonanal and phenylacetaldehyde stimulated oviposition in the females of *E. kuehniella*. Therefore, benzyl alcohol [10] (Table 1) found in the present study as EAG-active compound could be attractive to *P. interpunctella.* However, depending on the concentration, some volatile compounds may act as attractant or repellent. 1-pentanol found in our sample, acted as attractant at low concentrations and repellent at higher ones towards *Sitophilus granarius* (L.) (Germinara et al., 2008). The EAG-active compounds found in dried apricot could be considered as olfactory cues which orientate the moth in the direction of stored dried apricots. However, this statement can be confirmed only after performing behavioral bioassays with different concentrations of pure compounds. Since olfaction in insects is mediated by receptors present on the antennal sensilla, the present study represents an important step towards the development of lures baited with attractant food volatiles for the monitoring or control of *P. interpunctella*.

ACKNOWLEDGEMENTS

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VISUAL IDENTIFICATION GUIDE FOR STORED-PRODUCT BEETLES

Elliott BG[1]*, Bousquet Y[2], Timlick B[1], White NDG[3], Fields PG[3]

[1] Canadian Grain Commission, 303 Main St, Winnipeg MB, R3C 3G8, Canada,
[2] ECORC, Agriculture and Agri-Food Canada, 960 Carling Ave., Ottawa, ON, K1A 0C6, Canada,
[3] Cereal Research Centre, Agriculture and Agri-Food Canada, 195 Dafoe Rd., Winnipeg, MB, R3T 2M9, Canada,
*Corresponding author's e-mail: brent.elliott@grainscanada.gc.ca

ABSTRACT

Stored-product insects can be difficult for non-experts to identify because they may not be familiar with the technical language of keys. We have developed two visual keys using photographs to illustrate the distinguishing characters. One key identifies 14 of the most common stored-product insects, and another, more detailed key, identifies 32 stored-product beetles. The keys will be available online and as a smart phone application.

Key words: Key, identification, guide, mobile phone

INTRODUCTION

Currently, to identify a stored-product insect, you can use keys (Bousquet 1990, Gorham 1991) that require a good knowledge of the technical terms used to describe the distinguishing characters. The alternative is to use photographs or line drawings of insects (White et al. 2001) that may not correctly identify the insect. Therefore, there exists a need for a simple, visual key to stored-products insects, that is both easy to use and is more directed than current publications. Complex, text-oriented keys often prove difficult for new users and for some more experienced users terminology may also present a challenge. By using photographs of the features of an insect needed for identification, we hope to make insect identification easier, while retaining the critical information needed for correct identification.

RESULTS AND DISCUSSION

The two keys are directed at different audiences. The first, simple key is targeted at people working in the grain-storage industry who do not have the technical knowledge to use insect keys (Bousquet 1990, Gorham 1991). This key is brief and covers most of the orders of arthropods (14 groups or species) that are associated with stored grain. It also goes into greater detail with the most common stored-product beetles. The second key describes 32 beetles associated with stored grain and is modified from Bousquet (1990). The second version is aimed more at those with a strong background in entomology. In both versions of the key, the terminal couplet ends with the name of the species (scientific and common names) and links to descriptions of the species' biology.

Fig 1.1. Large insect.

Fig 1.2. Smaller insect.

| 1 (A) | Larger beetles (11 mm or more). | 2 |
| 1' | Smaller (up to 10 mm). | 3 |
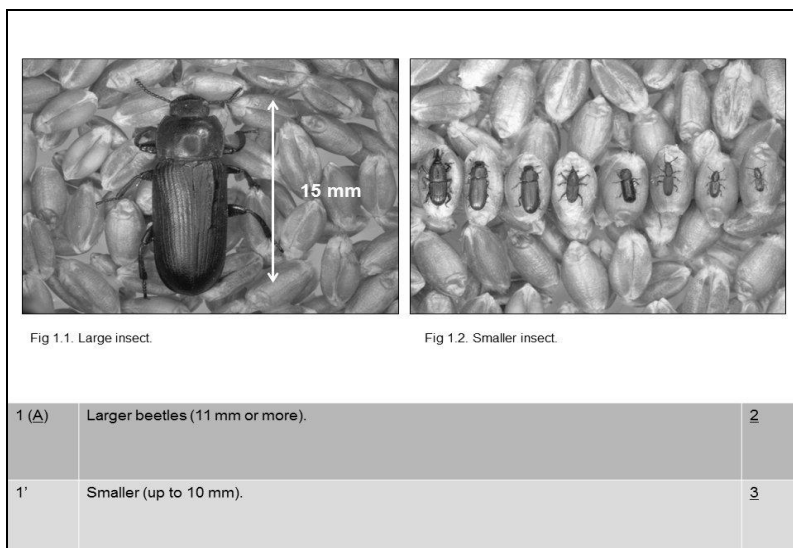
Fig. 1- Sample screenshot from key showing choice between large (1(A)) or small insects (1'). Decision will lead to a selection of option 2 or option 3 leading to subsequent couplets. Selection of (A) will take user to preceding couplet. Wheat is used as a background to give scale.

Both keys were developed first using text versions of the keys. This was followed by developing an extensive image library of the insects. Images were taken to illustrate the whole insect from various aspects and their distinguishing features. Over 2000 images were taken over a period of several months.

Fig 15.1. *Tribolium confusum*, eye one or two facets wide.

Fig 15.2. Eye three or four facets wide.

| 15 (14) | Eye one or two facets wide laterally at level of incision. | *Tribolium confusum* Confused Flour Beetle |
| 15' | Eye three or four facets wide laterally at level of incision. | 16 |
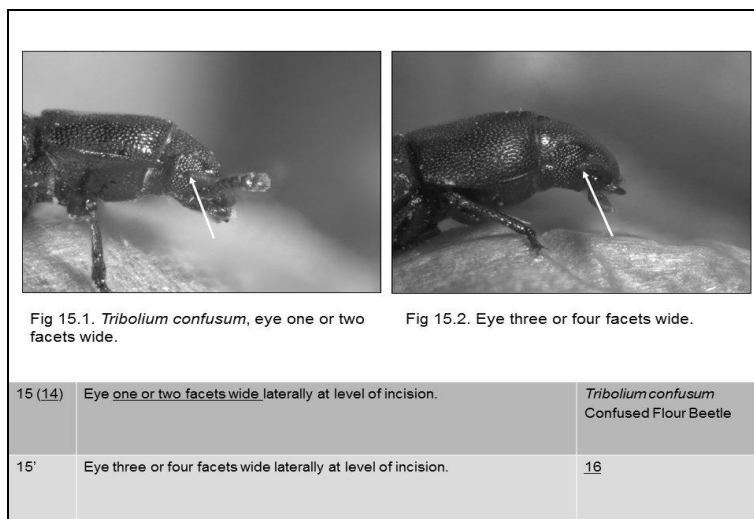
Fig. 2- Sample screenshot of couplet 15 with figures 15.1 and 15.2 from key with choices 15(14) and 15' showing distinguishing feature that identifies *Tribolium confusum* Jacquelin du Val or option 16 leading to another couplet.

The key will be available to the general public via two main avenues: publication in the online journal, Canadian Journal of Arthropod Identification and on the Canadian Grain Commission website. Smart phone applications will be developed for the simplified key.

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STORAGE OF FOOD GRAINS IN INDIA UNDER CENTRAL POOL: PRESENT STATUS AND FUTURE STRATEGIES

B.B. Pattanaik*

Central Warehousing Corporation, New Delhi-110 016 (India)
*Corresponding author's e-mail: *pattanaik181@yahoo.com*

ABSTRACT

Sustained crop production efforts have resulted in self sufficiency in food grains in India with production increasing to 257.45 million tonnes during 2011–2012 (advance estimate) comprising of 93.90 million tonnes of wheat, 104.32 million tonnes of rice and the remaining being coarse cereals, pulses, etc. As on July 1, 2012 the total stock of food grains in the central pool was 80.50 million tonnes. Storage capacity available with major storage agencies in the public sector is 54.91 million tonnes thus requiring utilization of private and cooperative sector storage and storing some quantity of food grains in open temporary storage structures. Handling, storage and transportation of food grains in India is done mostly in bags of 50 kg capacity. Food grains are liable to deterioration due to various biotic and abiotic factors. Pre-requisites of scientific storage of food grains for loss minimization is by execution of regular hygiene monitoring, prophylactic, and curative treatments. Popularity of phosphine in India is because of its versatility, ready to use tablet/sachet formulations that allow safe and easy handling, good penetrability and easy to apply correct dose. Limitations of phosphine are: (i) difficulty in selection of correct dose because of wide variation in susceptibility of life stages of different insects (adults being more susceptible than pre-adult stages) and (ii) insect resistance to phosphine has been most marked in the developing countries due to repeated exposure to sub lethal concentration in poorly sealed fumigation chambers, or use of unsuitable and damaged fumigation covers and improper sealing. Strategies for resistance management have been recommended with required phosphine gas dose and monitoring the gas concentration during the extended exposure period. It is, therefore, imperative that the storage agencies and pest control agencies follow best fumigation management practices to avoid control failures.

Key words: Sustained crop production, Central pool, Food grain deterioration, Biotic and abiotic factors, Phosphine popularity, Limitations of phosphine, Insect resistance to phosphine, damaged fumigation covers, improper sealing, sublethal doses, short exposure periods, phosphine gas monitoring.

POST FUMIGATION PRODUCT MANAGEMENT: HYGIENE & INFESTATION MANAGEMENT IN LEAF OPERATIONS (HIMILO): AN IPM SYSTEM AND ON-LINE QUALITY AUDIT TO PROTECT FUMIGATED TOBACCO STOCKS FOR LONG PERIODS FROM CIGARETTE BEETLE (*LASIODERMA SERRICORNE* (F.)

Vithal P.S.R.V.S*, Sugata Roy, Amitava Modak, Venkat P.S.R.A, Venkat Rao Vegi, Ramana Murthy R.S.V, Rajashekar R

*Hygiene and Quality Assurance, ITC Limited – Agri Business Division — ILTD, PO.Box:317, GT.Road, Guntur-522004. Andhra Pradesh, INDIA.
Corresponding author's e-mail: psrvs.vithal@itc.in*

ABSTRACT

There is a global need to protect fumigated stored products from reinfestation by developing suitable local systems with due consideration to operations flow, warehouse structure, hygiene, infestation monitoring, need based prophylactic sprays and effective and safe fumigation practices to reduce pesticide usage and residues in stored products.

The ITC Limited – Agri Business Division - ILTD has developed Hygiene & Infestation Management In Leaf Operations (HIMILO) system to protect fumigated tobacco stocks from reinfestation by Cigarette beetle (*Lasioderma serricorne* (F.)) and got it internalised up to warehouse level with training, audits and constant improvements with a policy and execution structure from senior management to warehouse level covering 19 warehouse complexes – 3.8 million sq. ft. (>350,000 m^2) warehouse area - 214 unit compartments – 150 million kg of Tobacco.

Post fumigation reinfestation of fumigated tobacco stocks by Cigarette beetle (*Lasioderma serricorne* (F.)) was assessed using three scales: (i) quality audit rating system with an infestation risk level rating on a scale from 0 to 5, (ii) compliance (%) rating on critical control points (CCP) from 100 to 0, (iii) with corresponding compliance level from 5 to 0 which was developed to take into account the education, knowledge and skills of warehouse staff.

Quality (infestation) audit considered critical control points in several areas as follows: (a) Prevention: warehouse structure, mesh screening and maintenance (11 CCPs-1000 points); (b) Prevention: hygiene – Cleaning – Tobacco stacks and warehouse (12 CCPs-1000 points); (c) Monitoring: Serrico traps for Cigarette beetle monitoring (IPM) (8 CCPs-1000 points); (d) Prevention: Prophylactic insecticide spray – hard surface (Deltamethrin 2.5% WP- 90 days cycle) and space sprays (Permethrin 25% EC and Pyrethrum 1% EC - 30 days cycle) (8 CCPs-1000 points); (e) Control: Fumigation (one gram PH3/M3 -10 day exposure) (17 CCPs-2000 points); (f) Shipments: own cigarette factories (8 CCPs-850 points); and (g) Shipments: Exports (6 CCPs-650 points)(h) for a grand total of 7500 points.

Fumigated tobacco stocks can be protected effectively for long periods from Cigarette beetle (*Lasioderma serricorne* (F.)) by organization's policy and commitment of management staff to quality audit system.

Key words: Post fumigation protection, Reinfestation, Quality (infestation) audit system, Critical control points, warehouse staff, Cigarette beetle (*Lasioderma serricorne* (F.)), Protective covers, Organization policy and commitment.

AUTHOR INDEX