Proceedings

CAF

International Conference on Controlled Atmosphere and Fumigation in Stored Products

Nicosia, Cyprus, 21–26 April 1996

E.J. Donahaye, S. Navarro and A. Varnava (editors)

PROCEEDINGS OF AN INTERNATIONAL CONFERENCE ON CONTROLLED ATMOSPHERE AND FUMIGATION IN STORED PRODUCTS

Nicosia, Cyprus, 21–26 April 1996



UNDER THE AUSPICES AND SPONSORSHIP OF
The Cyprus Grain Commission,
Nicosia, Cyprus

IN COLLABORATION WITH
The Permanent Committee of Conferences on
Controlled Atmosphere and Fumigation in Stored Products

INTERNATIONAL CONFERENCES ON CONTROLLED ATMOSPHERE AND FUMIGATION IN STORED PRODUCTS

1980 Rome, Italy
1983 Perth, Australia
1989 Singapore
1992 Winnipeg, Canada
1996 Nicosia, Cyprus

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) *Proc. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products,* Nicosia, Cyprus, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus

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ISBN 9963-8202-0-4
PRINTCO Ltd., Nicosia, 1997
Printed in Cyprus

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Gulielmo XYLANDRO, "Cyprus", woodcut (75x120 mm) page 779, *Στράβωνος Γεωγραφικών βίβλοι Ἑπτά καί Δέκα,* **Basle 1571.**

This small woodcut map originated in 1561 in Ioannis Honterus' *Cosmographia* published in Basle by Henricus Petri. A decade later Petri published G. Hylandro's edition of Strabo's geographical work and the same small map of Cyprus was republished along with five other woodcuts of Greek islands. The map is wholly based on Ptolemy's map of the island. It is printed at the lower part of page 779 where the text on Cyprus begins set in two columns: Greek on one and a Latin translation on the other. The contents of the map are ancient but the three main medieval towns of Nicosia, Famagusta and Limassol are also indicated.

Map from the" Map Collection of the Bank of Cyprus Cultural Foundation".
Text from the book of A. Hadjipaschalis and M. Iacovou "Maps and Atlases", Nicosia,1989.

AN ANCIENT MAP OF CYPRUS

Λίμνης Ἀσκανίης ὧν χείλεσιν· ἔνθα Δολίων
Υἱὸς Σιληνοῦ νάσατο Μηλίης.

Καλοῦσι δὲ φησι Δολιονίδα καὶ Μυσίην, τὴν πρὸς Κύζικον ἰόντι ἐς Μιλητούπολιν. Εἰ δὴ οὕτως ἔχει ταῦτα, καὶ ἐκμαρτυρᾶται τοῦτο τῶν δ' εἰκυιμένων νεῶν, οὐ τοῦτο τῶν ποιητῶν, τί ἐκώλυε τὸν Ὅμηρον τὰ τῆς Ἀσκανίας μεμνῆσθαι καὶ Ξάνθου λεγομένης εἶρηται δὲ οὐ πρότερον περὶ τούτων οὐδὲ περὶ Μυσῶν οὐ φρυγῶν λόγῳ ὡς τετύχηκε τῶν ὡρῶν. Λοιπὸν δὲ τὴν πρὸς νότον παρακειμένην τῇ χερρονήσῳ τὴν πρὸς δύσιν νῆσον τὴν Κύπρον. Εἴρηται δ' ὅτι ἡ πρὸς ἑσπέραν θάλαττα, τοῦ καὶ Αἰγύπτου καὶ Φοινίκης, καὶ Συρίας, καὶ τῆς λοιπῆς παραλίας μέχρι Ῥοδίων, συμβαίνει πως δύσιν ἔκτε τὸν Αἰγυπτίου καὶ Παμφυλίου, οὐ τὸ Παμφυλίας, καὶ τὸ κατὰ τὸν ἰσικὸν κόλπον. Ἐν δὲ ταύτῃ δύσιν ἡ Κύπρος νῆσος τὰ μὲν πρὸς ἑσπέρα μέρη, συμπίπτοντα ἐγγύς τῇ Τραχείᾳ Κιλικίᾳ, κατὰ δὴ τὸ πρὸς ἐχάτῃ τῇ ἠπείρῳ δύσι, τὰ δὲ ἑῶα καὶ ἰσικῷ κόλπῳ, τὰ δὲ ἑσπερία καὶ Παμφυλίῳ κατὰ μὲν σύμφωνα καὶ ἐλάγιστα, τὰ δὲ νότια καὶ Αἰγυπτίῳ. οὕτω μὲν σύμπασαν δύσιν ἀπὸ μὲν ἑσπερῶν καὶ Λιβυκῷ καὶ Καρπαθίῳ κατὰ δὲ τὰ νότια καὶ τῶν ἑώων μορῶν, ἥ τε Αἰγυπτία δύσις, οὐ ἡ ἐφεξῆς παραλία μέχρι Σελινικείας τε καὶ Ἰσσοῦ. πρὸς ἄρκτον δ' ἥ τε Κύπρος καὶ τὸ Παμφύλιον πέλαγος. οὗτο δὲ ἀπὸ τῶν ἀκρωτηρίων πρὸς τὰ ἄκρα τῆς Τραχείας Κιλικίας, οὐ τῆς Παμφυλίας, οὐ Λυκίας μέχρι Ῥοδίων· ἀπὸ δὲ τῆς δύσεως, τῆς Ῥοδίων νήσου, ἀπὸ δὲ αὐλῶνος τῆς κατὰ Ἀσκανίαν ὑπὸ τὴν Δολίονα Sileno natus & Melia incoluit.

Vocant autem, inquit, Dolionidē & Mysiam, quâ apud Cyzicum Miletopolin itur. Hæc si uera sunt, & testimonio eorum quæ hodie sunt comprobātur, & poëtarum: quid obstabat quin Homerus huius Ascaniæ mētionem faceret, non eius quam Xantus refert. Sed hîc finem facio, cùm supra de hac re dixerim in Mysorum & Phrygum enarratione. Restat ut huic peninsulæ uersus meridiem adiacentem insulam Cyprum describamus. Dictum fuit, mare quod continetur Aegypto, Phœnices, Syria & ora quę usque ad Rhodo oppositam continentem porrigitur, quodammodo componi ex Aegyptio, Pamphylioq; mari, & eo quod sinum facit Isicum. In eo est insula Cyprus, cuius septentrionales partes ad asperam accedunt Ciliciam & continentis extremitatem: orientales Isico sinu, occidua Pamphylio, meridionalia Aegyptio mari alluuntur. Id ab occasu cum Africo & Carpathio mari confluit: ab austro & oriente Aegyptum habet, oramq; deinceps Seleuciamusq; & Issum: uersus septentrionem Cyprus est & mare Pamphylium. Hoc à septentrione continetur extremitatibus asperæ Ciliciæ & Pamphyliæ ac Lyciæ usque ad Rhodiam, ab occasu Rhodo insula, ab ortu Cypro, quâ Paphus est & Amathus, ab austro & oriente Aegyptio mari, quod deinceps Seleuciam attingit, & Isicum sinum usque ad Issum.

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ABOUT THE CONFERENCE

The International Conference on Controlled Atmosphere and Fumigation (CAF) continues to serve as the leading international forum for reporting advances in the research and development of gaseous treatments applied to the preservation of stored commodities. These proceedings contain the presentations made at the 5th gathering, which took place in Cyprus (1996), the previous conferences having been held in Italy (1980), in Australia (1983), in Singapore (1989) and in Canada (1992).

In previous conferences the topics were limited to controlled atmosphere (CA) and fumigation techniques applied to grain storages. However, during a meeting of the CAF Permanent Committee in Winnipeg, Canada, in 1992, a decision was taken to widen the scope of the conference to include other agricultural products that utilize CA or fumigation, provided the treatments are directed to the control of pests. With this approach in mind, the CAF Conference Scientific Committee decided to add two sessions to the conference program. One session covered quarantine and regulatory issues and the second covered sampling and trapping for monitoring insect populations. In the total of 71 presentations at the conference, a good balance was achieved between those devoted to fumigants (28 papers) and those pertaining to CA technologies (35 papers).

Recent decisions by the international community to phase out methyl bromide (MB) because of its involvement in the depletion of the atmospheric ozone layer were reflected at the conference by the presentation of fresh information, particularly in relation to the development of new fumigants such as methyl phosphine, methyl isocyanate and carbonyl sulphide. Furthermore, participants at the conference had the opportunity to discuss alternative new techniques and treatments for the possible replacement of MB.

The conference program included a pre-conference visit to Germany, organized by the Institute of Stored-Product Protection in Berlin, in coordination with the CAF Organizing Committee in Cyprus, under the sponsorship of Dr C. Reichmuth. This interesting program, which included professional visits to silos, granaries, a museum and laboratories in Germany, was matched in Cyprus with visits to storage centers accompanied by sightseeing visits to historical and tourist sites. All these activities contributed an additional social flavor to the conference.

The conference was held in the Cyprus International Conference Center in Nicosia, which provided excellent facilities and audio-visual equipment. The official handling agency, Aeolos Cyprus Travel Ltd., is to be congratulated on its smooth running of the organizational and logistic aspects of the conference.

The preparation of the conference from its inception until its realization was largely dependent on the enthusiasm, devotion and support of representatives from two institutions: the Cyprus Grain Commission in Nicosia and the Israel Agricultural Research Organization (ARO) in Bet Dagan. Dr Andreas Varnava, Secretary of the Organizing Committee, by his tireless devotion to the solution of the numerous issues related to the preparation of the Conference, by creating a friendly atmosphere that characterizes the rich hospitality of Cyprus, by his professional background as grain storage specialist within the Cyprus Grain Commission, and by his uncompromising determination that

contributed to the success of the Conference, deserves special mention. As Chairman of the Scientific Committee and Secretary of the Permanent Committee, I see it as my privilege to thank both institutions and especially Dr Andreas Varnava.

The cooperation of the Cyprus Grain Commission and its willingness to sponsor the conference deserve full recognition, and in this respect I should like to take this opportunity to thank the Chairman of the Cyprus Grain Commission and Chairman of the Organizing Committee, Mr Andreas Tryfonides.

The conference participants were honored with an opening address by the Minister of Finance, Mr Christodoulos Christodoulou. This was followed by opening speeches by the Chairman of the Organizing Committee, Mr Andreas Tryfonides, the Chairman of the CAF Conference Permanent Committee, Dr Jonathan Banks, and by myself as Chairman of the Scientific Committee.

Concerning our preparation of the scientific program, I should like to express our gratitude to Dr Sam Angel of the ARO, Israel, for his help in organizing the scientific sessions, and to Mrs Miriam Rindner, Mr Avi Azrieli and Mr Raphael Diaz of the Department of Stored Products, ARO, and Ms Joanna Olympiou of the Cyprus Grain Commission for their valuable assistance. On behalf of the Scientific Committee and the Organizing Committee, I should also like to thank Mrs Phylis Naiman, Ms Fritzi Grosser and Dr Carol Efrati for their dedication to highly professional language editing and technical production of the book of Proceedings.

The Scientific Committee aspired to preparing a most diverse and interesting series of sessions for the conference, and it is my special privilege to thank Dr Jonathan Donahaye of ARO, Dr Andreas Varnava of the Cyprus Grain Commission, and Drs John Zyngas and Nicos Iordanous of the Cyprus Ministry of Agriculture for their full cooperation throughout the preparation of these sessions.

The volume of Proceedings includes all papers accepted by the Scientific Committee whose authors participated in the conference. The contributors were most helpful in providing both manuscripts and diskettes and in promptly answering queries and comments by the Scientific Committee on matters relating to their papers. In order not to offend, we have retained both American and English forms of spelling according to author preference. However, we have striven for uniformity in scientific terminology and in adoption of the metric system throughout.

The stated terms of reference of this conference — to report on advances in CA storage and fumigation in agricultural commodities — was fully achieved within the framework of the session meetings, while informal discussions held before, between and after sessions provided an excellent opportunity for dialogue among foremost professionals in their disciplines. Between the formal and the informal aspects, we believe the CAF Conference achieved its objectives in full, and we are confident that these Proceedings contain a very important series of original and review papers and will be an invaluable source of reference for years to come.

Shlomo Navarro,
Chairman of the Conference Scientific Committee

INTERNATIONAL CONFERENCE ON CONTROLLED ATMOSPHERE AND FUMIGATION IN STORED PRODUCTS

Cyprus International Conference Center
21–26 April 1996, Nicosia, Cyprus

PRESS RELEASE ON CAF CONFERENCE

INTERNATIONAL CONFERENCE ON AGRICULTURAL PRODUCTS HOLDS ITS MEETING IN NICOSIA

The International Conference on Controlled Atmosphere and Fumigation in Stored Products is the leading forum to report on advances in research and development of gaseous treatments to preserve durable agricultural products in storage.

Following a pre-conference visit to the Institute for Stored Products Protection in Berlin, Germany, the 5th CAF Conference was held in Cyprus at the International Conference Center, Nicosia, from 21 to 26 April 1996, under the auspices of the Ministry of Finance, sponsored by the Cyprus Grain Commission.

Currently there is worldwide pressure to reduce or phase out the use of fumigants, and particularly methyl bromide. Scientists from 26 countries have gathered in Nicosia with the objective of reporting on advances in research and development.

The conference pointed the way forward in agricultural products protection. Use of gases is increasingly the method of choice for treatment of pests infesting stored agricultural products. Research presented here lays the basis for techniques for use in the next decade.

The conference covered important global issues of pest control in stored products with the technique of fumigation or the use of controlled atmospheres:

— *presenting alternatives to methyl bromide.*

— *documenting the recent state of the art in protecting grain and other commodities from insect attack.*

— *giving an overview of the political and technical constraints on the use of fumigants.*

— *indicating the need to observe and avoid the appearance of resistance.*

Among the new techniques and substances which were covered, the use of carbon dioxide under pressure for rapid disinfestation and the production methods available for nitrogen were seen as promising approaches to including controlled atmospheres as a component of integrated pest management after harvest as an alternative to fumigants.

Substances like methyl phosphine, methyl isothiocyanate and carbonyl sulphide were presented as possible alternatives to methyl bromide in some areas of application.

Methyl phosphine was presented as a new fumigant with the potential of combatting the problem of resistance to phosphine.

Fumigant application techniques, such as recirculation and flow-through techniques with phosphine and electronic devices for gas detection, are on their way to establishing effective controlled fumigation techniques for the future. Thorough sealing techniques with pressure testing for gas tightness serve as crucial requirements for sound fumigation practice.

The papers presented at this international meeting will be published as a book of the conference proceedings. This document will provide information important to scientists and policy makers alike in all countries.

ADDRESS BY THE CHAIRMAN OF THE PERMANENT COMMITTEE FOR CONFERENCES ON CONTROLLED ATMOSPHERES AND FUMIGATION IN STORED PRODUCTS

DR JONATHAN BANKS

Excellencies, colleagues, ladies and gentlemen,

We live in interesting and difficult times. World grain prices are high, and world grain stocks are disturbingly low. There is speculation about whether we have now entered a time when stocks will remain low while increasing affluence, population and environmental degradation will lead to demand's exceeding grain supply. Paul Erlich, in the early 1970's, predicted that such a point would be reached about now. History will soon show if he was correct.

Whether or not demand will tend to exceed supply, storage of basic foodstuffs, particularly cereal grains, will continue to play a critical role in human well-being on a world scale. This will be so whether such storage evens out the inevitable high and low productions of the seasons and years or whether it maintains food security for years of scarcity. In this conference, we are focusing on the technology for using gases for the protection of grain and other products in storage. This includes use of toxic gases, fumigation, and altered atmospheres in Controlled Atmosphere (CA) or Modified Atmosphere (MA) systems. The area of our study may seem only a small part of the grain storage system. Nevertheless, it is a crucial part of many nations' strategies to protect their grain from pest attack and permit trade in grain at the standard demanded by today's discerning markets.

Within our field of endeavor, there is a state of evolution, change and improvement — much of it the subject of our conference over the next few days. We meet against a background of increasing certainty that methyl bromide use will be highly restricted, if not completely banned, in the near future. This is because of its damaging effects on the atmospheric ozone layer. For developed countries, it is to be eliminated by 2010 and severely curtailed by 2005 — only a few harvests away. While it is possible there will be exemptions from control for "critical agricultural uses", it is by no means certain that grain treatment will be one of the permitted uses.

Other influences on our field of work include environmental concerns over emissions from fumigations. Regulatory agencies are increasingly calling for very low levels of fumigant as maximum standards of concentration in work spaces and outside storages under gas. Shifts in the way legal liability is viewed mean that we have to develop

increasingly sophisticated monitoring systems to cope with the need for systems not only to be safe, but to be shown to be so. Moves towards Quality Assurance are placing further demands on the technology for the monitoring and effective conduct of fumigation, with increasing accountability for grain storage managers and fumigators.

Overall, the requirements are for safer, better, faster, more controlled, more effective and, of course, cheaper treatments. The restrictions on fumigation with toxic gases are even forcing reconsideration of CA systems as alternatives — systems that until recently were regarded as impractical "poor cousins" of fumigation.

All is not bad. Some new and unexpected areas are opening up. One obvious example is the apparently successful pursuit of new fumigants to challenge — and even potentially replace — our principal tools, methyl bromide and phosphine. At our last meeting, four years ago, it was still widely held that there would be no new fumigants. Now there is at least hope that this is not so. Discarded materials, too, may be rejuvenated.

Clearly, there is no shortage of work for those involved in R & D in the area of fumigation and CA.

Despite this need, several of the institutions that have played leading roles in the recent past in the field covered by this conference have been reorganised, changed or shut. There has been a considerable reduction of effort, and the personnel of these institutions, with their expertise, have often been scattered with the obvious loss of that important "critical mass" that is said to make a centre productive. No doubt new centres will arise, though the process may be both slow and difficult. It will fall to new technologies of communication and to meetings such as this to keep the synergies going so as to continue to make progress in our field.

Part of the problem that we face is loss of expertise. Some of this is inevitable due to retirements. At this conference, three members of the Permanent Committee, Dr E.J. Bond, Mr E. Ripp and Dr E. Jay, are not here to contribute their experience, guidance and enthusiasm today. All are retired and none could participate. We must proceed without them. They will be much missed.

In conclusion, may I thank the members of the local Organizing Committee for their obviously successful efforts in arranging these meetings. Organizing such meetings is an onerous but important task, one critical to success. Our thanks, too, are due to the Cyprus Government, the Ministry of Finance and the Cyprus Grain Commission for their hospitality, support and sponsorship of this meeting. Cyprus is a particularly fitting place for us to meet. It holds a special place for me, personally, in the recent history of CA's, as the home of the world famous Ctesiphon bins, though I recognise that hermetic systems have been used here for millennia. We are to see the Ctesiphon bins on one of our field visits.

Finally, on behalf of the Permanent Committee, I welcome you all to this fifth CAF conference — the successor to meetings in Rome, Perth, Singapore and Winnipeg. Let us work together to ensure that this conference, like its predecessors, is productive, creative and influential.

CLOSING REMARKS BY THE CHAIRMAN OF THE CYPRUS ORGANIZING COMMITTEE

MR ANDREAS TRYFONIDES

Mr Chairman, distinguished participants, ladies and gentlemen,

I am pleased to be here with you again at the closing of our CAF Conference. I hope that our promise to you at the beginning of a fruitful and enjoyable stay in our island has been kept.

If we have failed to keep our promise to you of good weather full of sunshine and warmth, please forgive us for our inability to go against God's will and for the success of the Cyprus Growers' and Water-Supply Authorities' desperate prayers for the rain which was much needed because of this year's drought.

If you have noted any deficiencies in the organizational arrangements, please bear with us. We have tried to do our best despite our limited resources.

I sincerely hope you have experienced the evidence of our genuine effort to extend to you all, our friends, the warmth of our heartfelt, traditional Cypriot hospitality.

I understand that the 71 scientific presentations during the CAF Conference were very significant, not only for all of us, who were fortunate enough to be here, but also for all our friends in other countries which, as you know, face the same crucial problems in preserving durable agricultural products in storage. Here I need not re-emphasize the real worldwide benefit to be derived from these scientific findings.

On our part, we, the organizing committee, would like to pledge to you that we will spare no effort in preparing, printing and distributing the Proceedings as soon as possible, always in consultation with — and with the unreserved support of — the CAF Permanent and Scientific Committees.

At this point, I would like to take the opportunity to express my sincere thanks and appreciation to Drs H.J. Banks and S. Navarro for their valuable assistance, guidance and general contribution to the success of our Conference. I would also like to thank all the chairpersons, co-chairpersons and rapporteurs for their own invaluable contributions. To my Cypriot colleagues in the Grain Commission and in the Ministry of Finance, who have participated in the Organizing Committee, I extend my deep appreciation.

Special thanks and appreciation should certainly be extended to Dr Andreas Varnava, the Secretary of our Committee, for his unquestionable contribution and the strenuous efforts which must have cost him much anxiety, sleeplessness and nightmares.

There is no doubt that the choice of our young, charming and able Joanna Tsingi-Olympiou for her secretarial task could not have been better.

Finally, I would like to thank you all once again for the honour of your presence here and for giving us the opportunity — and pleasure — of welcoming you to our beautiful long-suffering island.

I hope that when you go back to your countries and to your families, you will carry with you the best of impressions and happy memories of Cyprus. Let me assure you that you will always be welcome to visit us again as friends.

We wish you a safe return home and all the best to you and your families.

SESSION 1

BIOLOGICAL RESPONSES OF ARTHROPODS TO TREATMENT WITH CONTROLLED ATMOSPHERES AND/OR FUMIGATION

Chairpersons:
C.H. Bell, *United Kingdom*
C.S. Adler, *Germany*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 3–15

THE USE OF MIXED-AGE CULTURES IN THE MEASUREMENT OF RESPONSE TO PHOSPHINE

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ABSTRACT

The conventional way to measure the response of insects to pesticides has been to use selected stages with stable tolerance distributions so that the data can be subjected to rigorous statistical analysis using such methods as probit analysis. This approach may have merit when comparing the efficacy of different poisons or when determining changes in tolerance associated with selection for resistance. However, it is usually not suitable for determining dosages likely to be effective in practice.

A more effective method is to use mixed-age cultures and determine the time to population extinction. A major advantage of this method is that it is not necessary to know which stage is the most tolerant; in addition, it allows for changes in tolerance during the exposure periods. However, it is essential both that material used for testing contain all developmental stages and that adequate numbers of each stage are present to ensure a satisfactory degree of repeatability. This paper describes a suitable method for determining the dosages that can be expected to be effective in practice. Data are presented both to show the relative abundance of the different stages with a number of species and strains and to demonstrate the repeatability of the method.

INTRODUCTION

Simple bioassay techniques have been a fundamental tool of toxicologists for a long time. In principle they require only that samples of the test organism be exposed to a graded series of doses of the toxicant or drug and the response observed. These data are primarily used either to establish the relative potency of different drugs or poisons or to determine variations in the response of different populations of the organism. It is implicit in these tests that they are conducted so as to make the results repeatable. For example, there are standard procedures for applying the toxicants or drugs to organisms which are based on sound quantitative principles and techniques. Likewise, it is implicit that the organisms are sampled in such a way that their response can be expected to be repeatable. Clearly, choosing a stage with a stable tolerance distribution is a distinct advantage. For this reason, adults, because of their relatively stable physiology, are usually chosen for bioassay. This

is true for insects, provided that very young and very old individuals are excluded. The tolerance of adults of a strain of *Tribolium castaneum* exposed to phosphine (PH_3) (Table 1) provides a clear example. To achieve a relatively stable tolerance distribution with immature stages, a narrow age range is selected for testing: for example, 2-d-old eggs or 15-d-old larvae. It has been shown in many cases that the tolerance of eggs, larvae and pupae changes dramatically during the development of these stages (e.g. Lindgren and Vincent, 1966; Nakakita and Winks, 1981) and measurements of response that would represent the entire stage, i.e. the full age-spectrum of these stages, would be difficult to achieve as well as relatively meaningless.

TABLE 1
The influence of age on the mortality response of adults of *Tribolium castaneum* exposed to a range of PH_3 concentrations for 6 h at 25°C, 70 % r.h.

	Mortality	
Age (weeks)	LD_{50} (mg h L^{-1})	LD_{99} (mg h L^{-1})
1–3	0.205	0.455
20–22	0.196	0.398
48–50	0.198	0.475

Data from experiments of this kind are usually subjected to a method of analysis which normalises the response, following which a linearising transformation is used to facilitate comparisons. These linearising transformations are commonly probit and logit. These techniques have been effectively used, for example, to establish the dosage variables of fumigants, i.e. the relationship between concentration and time. This approach can be taken a step further and, using the techniques developed by Bliss (1940), examined as a probit plane which embraces all of the information in a family of probit lines. Probit plane analysis yields comprehensive models describing the response of a fumigant, for example, to a wide range of dosage variables (e.g. Winks, 1984).

These techniques also provide useful methods for determining the magnitude of differences in response, such as those induced by selection for resistance, and enable comparisons to be made between species in situations, such as stored products, where pest control must deal with a mixture of different species. However, it is in the area of pest control that the conventional bioassay techniques are of little value. It can be expected that the response of the different stages of insects will vary and that the tolerance of each stage, particularly the pre-adult stages, will change during development. This variability of tolerance has been observed most dramatically in the case of PH_3, where large changes occur between the tolerant egg and pupal stages and the much less tolerant larval and adult stages. In addition, the changes in both the egg and pupal stages as they age are both

rapid and considerable (Lindgren and Vincent, 1966; Nakakita and Winks, 1981; Winks, unpublished data). Clearly, to describe the response of a single stage comprehensively it would be necessary to develop a multifactorial model that would include the variables of concentration, time and age. If the most tolerant stage were known, such a model could yield a measure of the highest dosage needed for a high kill level of this age which might then serve as a guide to the dosages necessary to achieve control of the species in a practical fumigation. While such an approach might be mentally stimulating, the time and effort needed to obtain the data would be prohibitive.

Over the years, the study of insecticides used in agriculture and related fields has been characterised by the frequent assertion that laboratory data are of little value in determining dosages required in the field. This statement is frequently quoted because it is essentially true. It is exceedingly difficult, indeed almost impossible, to simulate field conditions in the laboratory, and even if one could, it is doubtful if the results could be analysed in a meaningful way. It is usually argued that laboratory data simply provide a guide to the relative potency of different insecticides and answer the question of whether tolerance changes have occurred in field populations. The answers concerning practical dosages are usually obtained from the field via screening trials and the like. However, because of their very nature, such data lack the control and precision that can be achieved under laboratory conditions. This is because it is exceedingly difficult, if not impossible, to control such variables as weather conditions, distribution of chemicals and the rate of absorption of the chemicals by the insects (a function of their mobility and the distribution of the toxicant) in such a way that rigorous analysis, like that implicit in probit plane analysis, is possible.

In contrast, the laboratory study of the toxicity of fumigants in grain and similar commodities is much more closely aligned to field usage of these toxicants. A fundamental requirement of fumigation techniques, both in the laboratory and in the field, is that the fumigant be confined within a definable space. Moreover, the range of climatic variation within a field enclosure (such as a silo) is not as extreme as that in a field crop. Within the time frame of a normal fumigation, although there may be some variation within the grain mass, temperatures will remain relatively stable. In addition, the range over which the temperature will vary in a grain mass is small, possibly no more than 10 degrees. In a fumigation enclosure the relative humidity (r.h.) is also relatively stable, and food is present in abundance. Furthermore, the gaseous toxicant moves to the insects and not the reverse as in the case of insecticides, so in most situations, the uptake rate of toxicant is related to the concentration of fumigant in the atmosphere surrounding the insect. The mixing and distribution of fumigant within the atmosphere of the grain is also a factor. Both processes may be accelerated with active distribution techniques (Winks and Russell, 1997). It is therefore possible to develop laboratory experiments that resemble field fumigations. The major limiting factor to this approach is the insects themselves. For a laboratory experiment to be useful in the context of developing field dosages, it clearly must embrace the full range of tolerance of all the species likely to be present. This necessity led to the development of a technique using mixed-age cultures.

Mixed-age cultures have been used in the past as a means of determining response. Many of the experiments have failed because they have not satisfied the requirement of repeatability. The experiments simply took laboratory cultures and exposed them to different dosages of the fumigant. It was assumed that all insect stages were present in sufficient abundance to obtain useful and repeatable data. It would appear that often these assumptions were not well-founded.

The benefits of laboratory techniques using mixed-age cultures are considerable; they can be used to establish dosages effective in the field in a relatively short time for any number of species at selected temperatures, moisture contents (m.c.), etc. To obtain similar data from field experiments would not only require a very long time but would be logistically very difficult. Merely to evaluate the dosage variables of concentration and time would require a large number of silos all of which would need to be infested with adequate numbers of insects of essentially the same species and all the silos would need to be at about the same temperature and m.c. Part of this difficulty has been met by using test insects in cages inserted into the grain, but again, using such techniques to evaluate the efficacy of different combinations of the dosage variables, together with the effects of different temperatures and m.c., is almost impossible. It is therefore clearly questionable whether any of the reported field trials carried out to evaluate the efficacy of PH_3 using application rates of 2 or 4 or some other number of tablets per t are of any real value. Quite apart from its practical significance, the difficulty poses particular problems for fumigant registration authorities who require efficacy data to support product recommendations. The dilemma is due, on the one hand, to the requirement for commercial usage data, and, on the other, to being presented with data that are of very limited value in the context of efficacy.

A far better approach to the question of determining field dosages of a fumigant is to use mixed-aged cultures in controlled laboratory experiments. Clearly, the object of a dosage of fumigant applied in the field is to kill all stages of all species present; similarly, a mixed-age test in the laboratory should be aimed at determining the minimum dosage necessary to kill all stages of the insect species or strain under test. However, to obtain meaningful data from laboratory experiments it is essential that a number of criteria be met. Firstly, all stages must be present. Secondly, there must be an adequate number of each of the stages and particularly of the more tolerant stages. Thirdly, the method of assessment must include the full range of possible development times. Given these principles, one distinct advantage of a mixed-age culture method is that no prior information is needed concerning either the tolerance of any particular stage or the rate at which the tolerance of the various stages changes.

DETAILS OF THE METHOD

Preparation of mixed age cultures

Mixed-age cultures were established by placing 300 adults on about 1,000 g of medium in 2-L culture jars. The medium was chosen to suit the particular species. However, for species that would normally be reared in a flour-based medium, this was replaced with

one containing wheat plus broken wheat so that air flows could be achieved without difficulty during the dosing phase of the experiment. Adults were left in the culture so that there would be a continuation of egg laying, at least until the cultures were used. The cultures were selected for use at a time when it was expected that F1 adults would be starting to emerge and could be observed. At this point, all stages should have been present including teneral adults. On a few occasions cultures of the same strain, established at a later date, were added to ensure adequate numbers.

During the development of the technique the relative abundance of each stage was determined prior to the start of each test. In addition, the size of the total population was determined. To ensure the composition of cultures of *Sitophilus* spp. and *Rhyzopertha dominica* prior to use, samples of the culture medium were taken and X-rayed, following, in essence, the method described by Katz *et al.*, 1950 and Milner *et al.*, 1950. The abundance of weevil eggs was occasionally determined during the development of the method by staining egg plugs with acid fuchsin. Egg numbers of other species were checked by visual examination under a stereo microscope of samples of medium taken from the cultures. These initial checks confirmed that adequate numbers of eggs could be expected so with subsequent use of the method, checks for eggs were not continued on a regular basis.

The number of pupae was checked more regularly using the X-ray technique for *Sitophilus* spp. and for *R. dominica* and by visual examination of samples of culture medium for other species. Greater emphasis was given to determining the number of pupae since, in the case of PH_3, the pupae are frequently the most tolerant stage, and even in those cases where eggs are more tolerant, the difference is small. The estimates of numbers derived from X-ray analysis were based on the number of grains observed to contain pupae, for example, compared with the total number of grains on the X-ray plate. To facilitate counts, the sample of culture medium (grains of wheat) to be X-rayed was spread out on a metal grid attached to the envelope containing the photographic film. The data obtained in this way for one strain of *S. granarius* is given in Table 2.

Dosing technique

The cultures were dosed in the apparatus shown in Fig. 1. This apparatus consists of a number of chambers through which a constant concentration of PH_3 is passed. The concentration was prepared by accurate dilution of a measured source of PH_3 in nitrogen with air through a Brooks' Mass Flow Controller. The air was 'purified' by passing it through an activated charcoal filter inserted in the inlet to the diaphragm pump. The air was drawn from outside the laboratory to avoid laboratory 'odours'.

The r.h. of the air/PH_3 mixture was achieved by saturating the gas mixture with water at a lower temperature, determined from psychrometric data, a temperature that yielded the required r.h. when the air/PH_3 mixture was returned to the test temperature. This method achieves a reliable and constant r.h. For example to obtain an r.h. of 57%, the equilibrium r.h. for 12% m.c. in Australian wheat at 25°C (Gay, 1946), the air/gas mixture should be saturated with water at 15°C.

TABLE 2

Estimate of number of eggs and pupae in mixed age cultures of *Sitophilus granarius*, strain SG_4, used in experiments to determine times to population extinction when exposed to constant concentrations of PH_3

Date of culture set-up	Experiment began	No. of grains per culture	Grains on X-ray	No. of eggs in sample	Estimated no. of eggs in culture	No. of pupae on X-ray	Estimated no. of pupae in culture
10/01/86	28/02/86	ca. 43000	826	107	5570	26	1354
28/04/92	08/06/92	ca. 43000	355	N/A	N/A	13	1575
23/08 and 11/10/85	05/11/85	ca. 43000	937	163	7480	10	459
23/11 and 07/12/84	08/01/85	ca. 43000	660	63	4105	22	1433
15/11/85	10/01/86	ca. 43000	780	72	3969	11	606
28/12/84 and 25/1/85	26/02/85	ca. 43000	459	116	10867	11	1031
22/06/90	03/08/90	ca. 43000	464	38	3522	61	5653
15/06/84	24/07/84	ca. 43000	1133	41	1556	50	1898
02/05/86	27/05/86	ca. 43000	699	150	9227	11	677
10/01/86	28/02/86	ca. 43000	826	107	5570	26	1354
27/07/84	11/09/84	ca. 43000	560	60	4607	82	6296
23/07/90	03/09/90	ca. 43000	282	45	6862	28	4270
26/06/85	02/09/85	ca. 43000	863	62	3089	24	1196

N/A = not assessed.

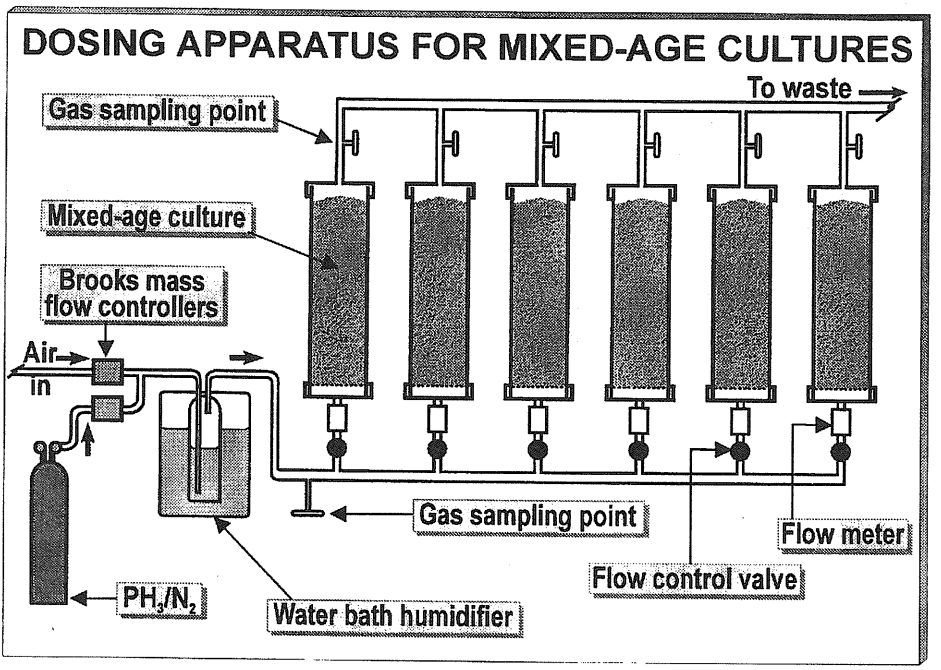


Fig. 1. Flow-through fumigation apparatus used for dosing mixed-age cultures of stored product pests with constant concentrations of PH_3.

The chambers were arranged in a bank and the flow to each controlled with a needle valve and monitored with an appropriate flow meter. Samples to check the concentration of PH_3 were drawn from the apparatus using a gas chromatographic technique based on the calibrated response of a flame photometric detector. At selected intervals (treatment times) the PH_3 flow to all chambers was stopped and samples of the culture medium taken for incubation. The samples were drawn by gently mixing the contents of the chamber, after which the required volume of culture medium was poured into a culture jar and sealed with a filter paper waxed to the rim of the jar.

Assessment of response

The samples were assessed for the presence of live adults as soon as they were drawn from the cultures, following which they were incubated and examined at approximately 8 weeks and 16 weeks. Observations at two intervals are not essential, but if only one interval is chosen, it must be long enough to ensure that all possible survivors have completed their development with allowance made for possible PH_3-induced delays. Observations at 8 weeks enabled decisions to be made concerning appropriate dosing times for subsequent experiments to be started before the full incubation period for the current experiment had elapsed.

The object of the method was to determine the minimum treatment time at a given concentration that would achieve complete kill of all stages present. This time provided an estimate of the *time to population extinction* (Winks and Hyne, 1994) and was determined from the first dosage interval that produced no survival (emergence), only provided that at least one subsequent sample or dosing interval also yielded no survival. Table 3 gives the response assessment in such a test. The data were examined graphically in the form of split-column plots (Fig. 2). The tops of the light or upper sections of the columns show the exposure time of the first sample from which no survivors were recorded (*time to population extinction*) and the tops of the dark or bottom sections of the columns show the exposure time of the previous sample from which survival was recorded.

In the early stages of developing the technique, the level of CO_2 in the effluent from the test chambers was considered. It was thought that when insect respiration ceased and the level of CO_2 in the effluent dropped to ambient levels, this would indicate population extinction, thus providing an indicator which would give a reasonable estimate of time to extinction. The CO_2 data obtained is provided in Fig. 3. It may be seen that there was poor correlation between the CO_2 levels and times to extinction from

TABLE 3

An example of the data recorded to determine times to population extinction of strains exposed to constant concentrations of fumigant in mixed-age culture experiments

	Exposure time (d)					
Strain	2	4	7	14	21	28
S. oryzae strain LS2						
No. survivors at end of treatment	194	0	0	0	**0**	0
No. emerged at 8 weeks	ca. 1000	ca. 1000	ca. 300	1	**0**	0
No. emerged at 16 weeks	N/A	N/A	N/A	0	**0**	0
S. granarius strain SG4						
No. survivors at end of treatment	190	0	0	**0**	0	0
No. emerged at 8 weeks	ca. 1000	ca. 500	32	**0**	0	0
No. emerged at 16 weeks	N/A	N/A	ca. 500	**0**	0	0
S. granarius strain SG46						
No. survivors at end of treatment	67	0	0	**0**	0	0
No. emerged at 8 weeks	ca. 1000	272	63	**0**	0	0
No. emerged at 16 weeks	N/A	N/A	N/A	**0**	0	0
S. zeamais strain SZM9						
No. survivors at end of treatment	119	0	0	0	**0**	0
No. emerged at 8 weeks	ca. 1000	ca. 1000	ca. 300	112	**0**	0
No. emerged at 16 weeks	N/A	N/A	N/A	N/A	**0**	0

N/A = not assessed. Columns with boldface numbers denote observed times to extinction for each strain. Test insects: *Sitophilus* spp. Experiment started on 4 August 1992. PH_3 concentration: 0.015 mg L^{-1}. Temperature: 25°C.

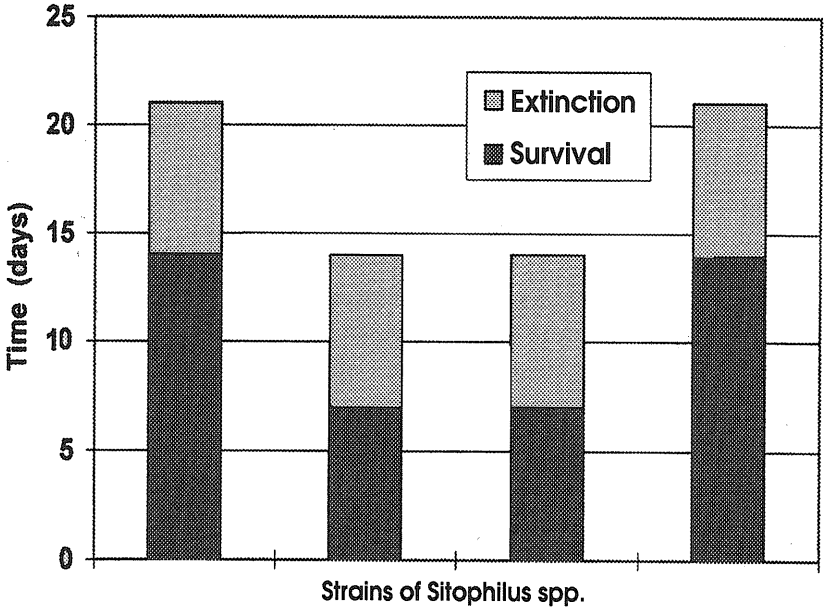


Fig. 2. Graphical presentation of data from mixed-age culture experiments showing the time at which extinction was recorded (top of the upper portion) and the previous sample time from which survival was observed (top of the bottom portion).

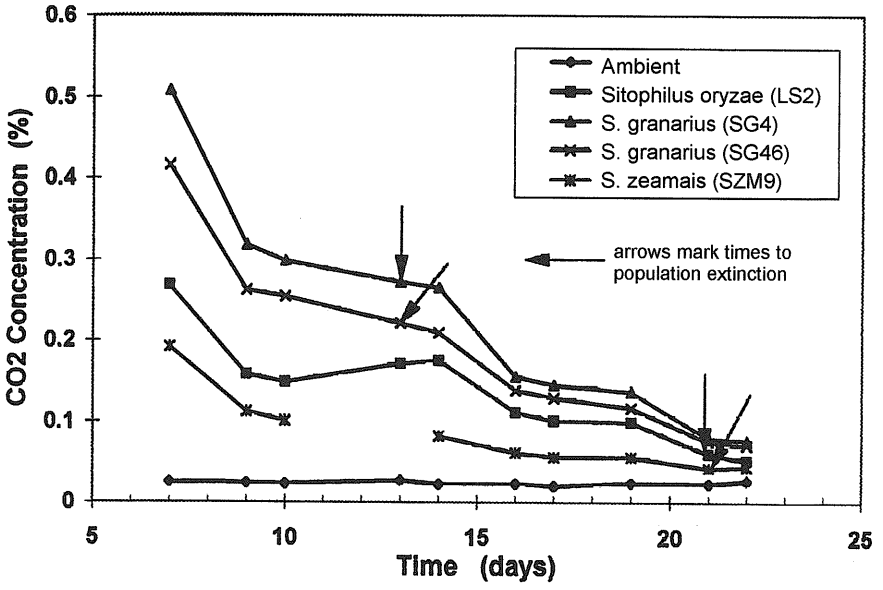


Fig. 3. Decay of CO_2 concentrations in effluent air from mixed-age cultures of *Sitophilus* spp. exposed to 15 g/L PH_3 at 25°C, 60% r.h.

incubated samples, possibly due to metabolic activity of fungi in the cultures. It was also difficult to discriminate between low levels of CO_2 in the effluent produced by one or two insects and the normal variation in atmospheric levels. The CO_2 level was thus rejected and incubated samples adopted as the basis for determining times to population extinction.

EVALUATION OF THE METHOD

The data in Table 2, as well as showing the relative abundance of the different stages of *Sitophilus granarius* in a series of different experiments, also provide a measure of the repeatability of the mixed-age culture technique. The data span a period of 8 years and are the result of the work of two to three laboratory technicians. The variation evident in the number of pupae was not considered excessive, and it may be seen that for each of the tests in question a sufficient number of pupae was present. Normally no more than six sub-samples were drawn from each culture (i.e. six dosage times). Therefore, from these data, approximately 80 to 1,000 pupae were expected in each sub-sample. This should have been sufficient to provide a reasonable indication of the time to extinction, assuming pupae to be the most tolerant stage. The same argument would hold if eggs were the most tolerant stage. A key aspect of the technique is the presence of a sufficient number of the most tolerant stage at the beginning or during the test to ensure a reasonable expectation that the result obtained is derived from the most tolerant age or stage. Clearly, the dosage times assumed and allowed for development during exposure to PH_3. This is exactly analogous to practical fumigations. Examples of the use of this technique are described by Winks and Hyne (1994, 1997). Winks and Hyne (1994) describe the use of this mixed-age culture technique to determine resistance factors in strains of *R. dominica* that were clearly more closely aligned with the difficulties of controlling such strains in the field than other factors derived from tests on adults.

A key question with this technique is the level of repeatability that can be achieved in estimating the time to population extinction. Figure 4 provides observed times to population extinction, together with the previous dosage time from which survival was recorded, for a resistant strain of *R. dominica* based on a number of experiments at different concentrations. The systematic decrease in times to population extinction with increasing concentration, together with the comparison of times at each of 0.015, 0.030 and 0.050 mg L^{-1} provide evidence that a reasonable level of repeatability can be achieved with this technique. It is significant that the experiments from which these data were extracted were conducted over a period of 4 years.

It is logistically difficult to obtain a large number of sub-samples at different treatment times. Thus the time between the previous sample from which survival was obtained and that from a later one from which no survival was obtained is frequently longer than is desirable. It follows that a precise estimate of the time to population extinction would be earlier than that recorded. This may be seen in the data for 0.03 mg L^{-1} in Fig. 4. Experiments have been repeated with different sample intervals to obtain closer estimates,

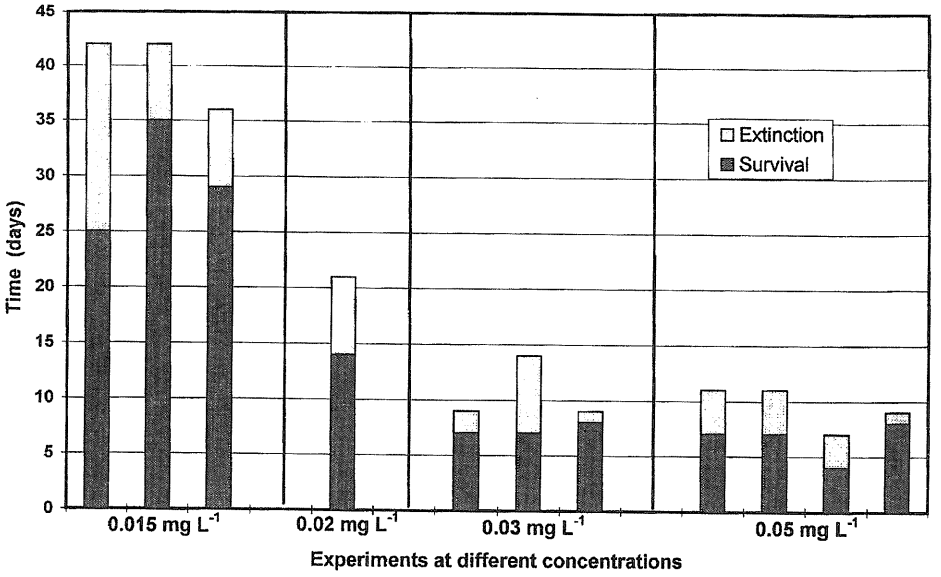


Fig. 4. Repeatability of times to population extinction of a Group 1 strain of *Rhyzopertha dominica* (RD316) exposed to constant concentrations of PH_3 at 25°C, 60% r.h.

but since these experiments can take up to 6 months to complete, obtaining such estimates is a protracted process.

This method has been used as the basis for determining minimum concentrations required for different exposure periods to control the range of pests likely to be found in Australian grain. These minimum concentrations (Table 4) have been submitted for incorporation in the revised guidelines for registration of PH_3-generating products issued by the Australian National Registration Authority.

CONCLUSION

Although experiments using the mixed-age culture technique require a longer period of time before results are available than do other methods based on selected stages with stable tolerance distributions, the data obtained from mixed-age cultures for PH_3 have enabled recommendations to be made for fumigations in the field and have thus been far more useful. Moreover, as described by Winks and Hyne (1994), this method also provides a far more meaningful measure of resistance to PH_3 than do those methods based on adults.

The most effective validation of the technique has been in the use of data derived in this way in practical control situations. These data have formed the basis of the recommendations for the use of PH_3 in general and SIROFLO® in particular in Australia for at

TABLE 4
Alternative minimum PH_3 concentrations in g m^{-3} with ppm in brackets at various dosage times to achieve complete control of infestations of a range of insect species

Group	Species	Temperature (°C)	Time (d) 5	7	10	14	21
1	*Sitophilus* spp., *Lasioderma serricorne*, *Trogoderma variabile*, *Dermestes* spp., Acarina (mites) and strains of Group 2 insects with low level of PH_3 resistance	>20	NA[1]	1.0 (700)	0.3 (200)	0.05 (35)	0.03 (20)
		15–20	NA	NA	NA	0.15 (100)	0.10 (70)
2	Susceptible *R. dominica* and *Tribolium* spp., *Oryzaephilus* spp., *Cryptolestes* spp., *Callosobruchus* spp., *Acanthoscelides obtectus*, *Palorus subdepressus*, *Carpophilus* spp., *Typhaea stercorea*, *Gnathocerus* spp., Ptinidae, Lepidoptera, Psocids	>20	0.15 (100)	0.04 (30)	0.035 (25)	0.03 (20)	0.015 (10)
		15–20	NA	0.15 (100)	0.07 (50)	0.05 (35)	0.02 (15)
3	*Bruchus pisorum* (pea weevil)	>20	NA	NA	NA	NA	0.04 (30)
		15–20	NA	NA	NA	NA	0.1 (70)

[1]NA means not applicable.

least the last 8 years. SIROFLO® has been widely adopted by most of Australia's Grain Handling Authorities and is currently used to treat approximately 8 million t of grain annually. It is the predominant fumigation method used in vertical silos in Australia and is now being extended into control in sheds. SIROFLO® has enabled Australia's grain industry to continue to meet the requirement for nil tolerance of insects in both domestic and export grain. Throughout this period PH_3 concentrations for use in SIROFLO® have been derived entirely from laboratory experiments using the mixed-age culture technique.

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ON THE EFFICACY OF SULFURYL FLUORIDE AGAINST STORED-PRODUCT PEST MOTHS AND BEETLES

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ABSTRACT

Sulfuryl fluoride (SO_2F_2) is a fumigant currently applied for the control of termites and wood-infesting beetles. In this study its efficacy against eight species of stored-product insect pests was studied. Each insect sample was exposed for 24, 48 or 72 h to concentrations ranging from 11.7 g/m^3 to 35 g/m^3. Concentrations of SO_2F_2 were measured using an infrared absorption spectrometer.

Complete control of adults of all tested species and of all larval stages and pupae of *Sitophilus granarius*, *Tribolium confusum* and *Tenebrio molitor* was achieved within a 24-h exposure period at 13 g/m^3. Some individuals of *Stegobium paniceum* and *Trogoderma versicolor* continued to develop to the adult stage, but no reproduction occurred. *Oryzaephilus surinamensis* survived and reproduced for all tested fumigation times at 18.6 g/m^3. *Plodia interpunctella* survived and reproduced within the 24-h and 48-h exposure times at 18.2 g/m^3. *S. granarius* and *Ephestia kuehniella* produced progeny within 6 weeks after fumigation for 24 h with 26.5 g/m^3 and 23.5 g/m^3, respectively. Complete control of eggs of *S. granarius* was achieved within the 24-h exposure time at 35 g/m^3. These results suggest that, as known from the treatment of wood-destroying insects, the eggs are the most tolerant stage of these species towards SO_2F_2.

INTRODUCTION

Sulfuryl fluoride (SO_2F_2) is a biologically active inorganic chemical that is odourless, colourless, non-corrosive and inflammable. It has low water solubility (750 ppm at 25°C), a high vapour pressure (15.98 bar at 21.1°C) and a boiling point of –55.4°C. Its density at 20°C is 1.36 kg/L, and its molecular weight is 102.06. The critical exposure route is via inhalation and the Threshold Limit Value is 5 ppm. On skin, the liquid can cause the tissue to freeze (Schneider, 1993).

SO_2F_2's potential for replacing methyl bromide (MB) in certain areas of stored-product protection is currently under investigation. In 1992, MB was listed under the Montreal Protocol as a significant ozone-depleting substance (UNEP, 1994), and in 1995 controls on the global use of MB were added to the treaty. Bromine from MB is estimated to be 50 times more effective at destroying ozone, on a per molecule basis, than chlorine from CFC's (WMO, 1995). Production and distribution of MB for fumigation will be phased out; therefore, alternatives are urgently needed. Non-chemical alternatives include controlled atmospheres (Prozell *et al.*, 1997), as well as physical and biological control methods. Chemical alternatives include phosphine (Benzing, 1997) and carbonyl sulfide (Plarre, 1997).

SO_2F_2 is fully oxidised and has no direct ozone-depletion potential. Furthermore, the degradation product of SO_2F_2, fluorine, has not been demonstrated to take part in ozone-destruction reactions (Fisher *et al.*, 1990; Bailey, 1992). The primary use of SO_2F_2 has been as a structural fumigant for the control of drywood termites. However, it is increasingly being adopted for the control of wood-infesting beetles and household insect pests. It is registered for use in Germany, Sweden and the USA. Research is currently being undertaken to evaluate the efficacy of SO_2F_2 on beetles infesting imported timber and on stored-product insect pests. This paper reports on a preliminary laboratory study undertaken to determine the fumigant's activity on eight stored-product insect pests.

MATERIALS AND METHODS

Six beetle species and the two moth species listed in Table 1 were included in the study. All insects originated from laboratory cultures maintained at the Institute for Stored-Product Protection.

TABLE 1
Insect species investigated in the study on the efficacy of sulfuryl fluoride

Species	Common name	Order	Investigated stages
Sitophilus granarius L.	Grain weevil	Coleoptera	Egg, larva, pupa, adult
Oryzaephilus surinamensis L.	Saw-toothed grain beetle	Coleoptera	Egg, larva, pupa, adult
Tribolium confusum J. Du Val	Confused flour beetle	Coleoptera	Egg, larva, pupa, adult
Stegobium paniceum L.	Drugstore beetle	Coleoptera	Egg, larva, pupa, adult
Trogoderma versicolor (Creutzer)	European larger cabinet beetle	Coleoptera	Egg, larva, pupa, adult
Tenebrio molitor L.	Meal-worm beetle	Coleoptera	Egg, larva, pupa, adult
Ephestia kuehniella Zeller	Mediterranean flour moth	Lepidoptera	Egg, larva, pupa
Plodia interpunctella (Hübner)	Indian meal moth	Lepidoptera	Egg, larva, pupa

Different stages of *S. granarius* and *T. molitor* were treated separately. A mixture of stages was exposed concurrently, together with the rearing substrate, for all other insects. Insects were exposed at 20°C to concentrations of SO_2F_2 ranging from 11.7 ± 0.4 g/m^3 to 35 ± 0.2 g/m^3 for 24, 48 and 72 h, respectively, in a 0.5-m^3 steel chamber, as described by Reichmuth (1981). The concentration of SO_2F_2 was determined using an infrared absorption spectrophotometer (Miran 1A). The lowest detectable concentration was 0.09 ppm. The extinction of infrared was measured and SO_2F_2 was absorbed at 11.5 µm. The fixed optical length was 20.25 m. Calibration was carried out by means of a calibration gas (SO_2F_2, 12560 ppm = 52.3 g/m^3) by injecting 5 ml of calibration gas three times to obtain three test points. Extinction was documented graphically using a recorder. The spectrum obtained with the spectrophotometer for 100 ppm SO_2F_2 is shown in Fig. 1. The calibration curve, a non-linear regression of the calibration data using the computer program TABLECURVE, enabled determination of the concentrations from the measurements.

In the trials, 5 ml of the atmosphere inside the fumigation chamber was measured three times. The average values of the three measurements were calculated, and the corresponding absolute values were determined using the calibration curve.

Following exposure, the insects were subjected to aeration for 3 h and then transferred, with additional standard substrate, and maintained at 20°C and 65% r.h. The efficacy of

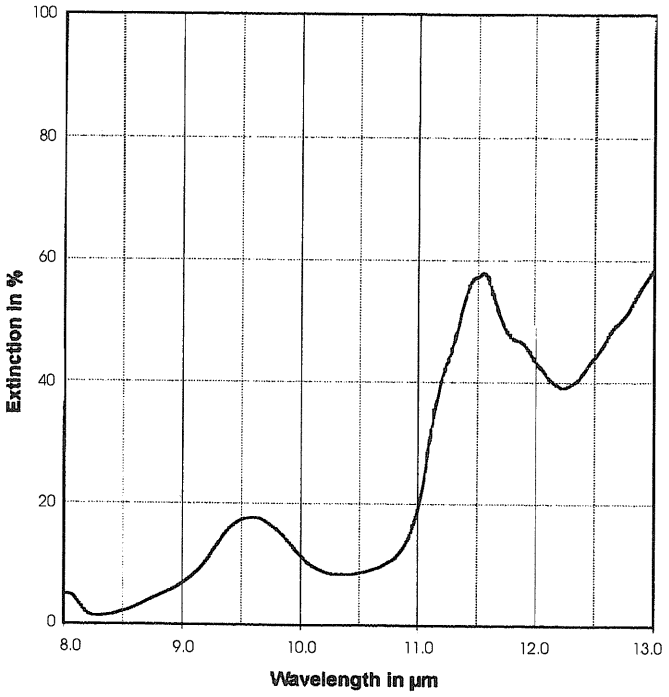


Fig. 1. Spectrum obtained with the spectrophotometer type Miran 1A for 100 ppm SO_2F_2, where SO_2F_2 is absorbing at 11.5 µm.

the SO_2F_2 on eggs, larvae, pupae and adults, as well as the ability of adults to produce progeny, was determined by counting dead, surviving and emerging adults 10–12 weeks after fumigation. Except for the fumigation, the untreated samples were handled in the same manner.

RESULTS

At the lower concentrations of SO_2F_2 (11.7–13.3 g/m^3) and the shortest exposure period (24 h), 100% mortality of larval, pupal and adult stages was achieved for *S. granarius*, *T. confusum* and *T. molitor* (Table 2). Some individuals of *S. paniceum* and *T. versicolor* continued to develop to the adult stage, but no reproduction occurred. Higher concentrations (23.5 g/m^3) at this exposure period were required to obtain 100% mortality of eggs,

TABLE 2
Efficacy of sulfuryl fluoride for 24, 48 and 72-h exposure at 20°C
(one trial per experimental design)

Exposure	Concentration (g/m^3)	Species/stage								
		1/As	2/As	3/As	4/As	5/L	5/P	5/A	6/E+L+P	7/E+L+P
24 h	11.7 ± 0.4	–	–	o	o	■	■	■	Δ	■
	13.3 ± 0.2	■	■	–	–	–	–	–	–	–
	18.2 ± 0.4	–	–	■	■	■	■	■	Δ	Δ
	18.6 ± 0.5	Δ	■	–	–	–	–	–	–	–
	23.5 ± 0.3	–	–	■	■	■	■	■	Δ	■
	26.5 ± 0.3	■	■	–	–	–	–	–	–	–
48 h	11.7 ± 0.4	–	–	o	■	■	■	■	Δ	■
	13.3 ± 0.2	■	■	–	–	–	–	–	–	–
	18.2 ± 0.4	–	–	■	■	■	■	■	Δ	■
	18.6 ± 0.5	Δ	■	–	–	–	–	–	–	–
	23.5 ± 0.3	–	–	■	■	■	■	■	Δ	■
	26.5 ± 0.3	■	■	–	–	–	–	–	–	–
	35.0 ± 0.2	–	–	–	–	–	–	–	■	■
72 h	11.7 ± 0.4	–	–	o	o	■	■	■	■	■
	13.3 ± 0.2	■	■	–	–	–	–	–	–	–
	18.2 ± 0.4	–	–	■	■	■	■	■	■	■
	18.6 ± 0.5	■	Δ	–	–	–	–	–	–	–
	23.5 ± 0.3	–	–	■	■	■	■	■	Δ	■
	26.5 ± 0.3	■	■	–	–	–	–	–	–	–
	35.0 ± 0.2	–	–	–	–	–	–	–	■	■

E = egg; L = larva; P = pupa; A = adult; As = all stages.
1 = *Oryzaephilus surinamensis*; 2 = *Tribolium confusum*; 3 = *Stegobium paniceum*; 4 = *Trogoderma versicolor*; 5 = *Tenebrio molitor*; 6 = *Ephestia kuehniella*; 7 = *Plodia interpunctella*.
o = surviving insects, no progeny produced; – = no experiment; ■ = 100% mortality; Δ = surviving insects, progeny produced.

larvae and pupae of *P. interpunctella*; 26.5 g/m^3 were required to obtain 100% mortality of all stages of *O. surinamensis*; and 35.0 g/m^3 were required to obtain 100% mortality of eggs of *S. granarius* (Fig. 2). Eggs of *E. kuehniella* survived the 24-h exposure to 23.5 g/m^3, which was the highest concentration tested.

Exposure for 48 h achieved 100% mortality for all stages of *T. versicolor* at 11.7 g/m^3, the lowest concentration evaluated. When exposed to 35.0 g/m^3, the highest concentration, for 72 h, eggs of both *S. granarius* and *E. kuehniella* were all killed (Table 2).

In both series of experiments with *E. kuehniella* and *S. granarius* (Figs. 3 and 4), the Ct-products for LD_{50} and LD_{95} increased slightly when the exposure period was prolonged.

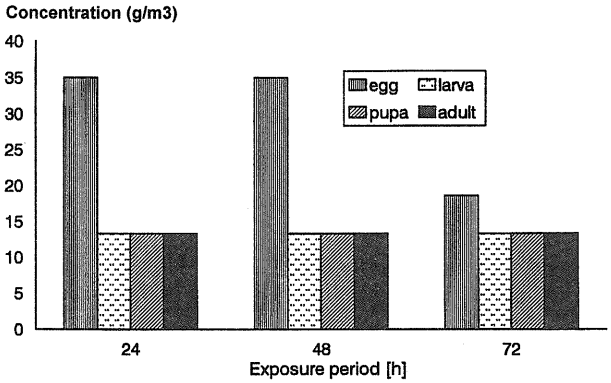


Fig. 2. Efficacy of SO_2F_2 against four developmental stages of *Sitophilus granarius* for exposures of 24, 48 and 72 h at 20°C (one trial per experimental design).

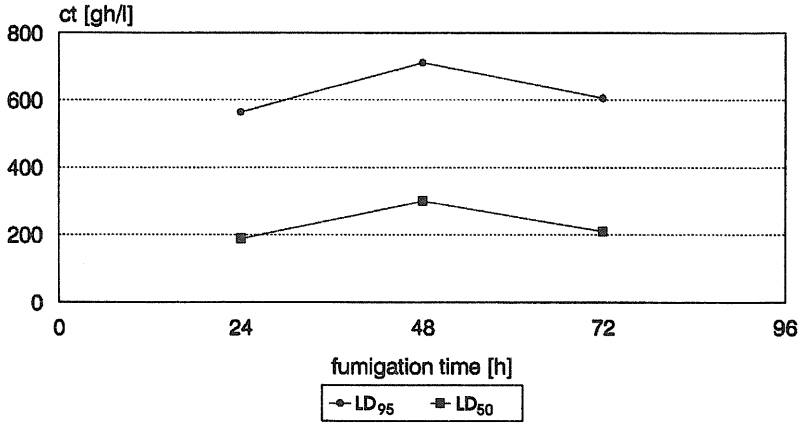


Fig. 3. LD_{50} and LD_{95} Ct-values for exposure of eggs of *Ephestia kuehniella* to SO_2F_2, in relation to fumigation time.

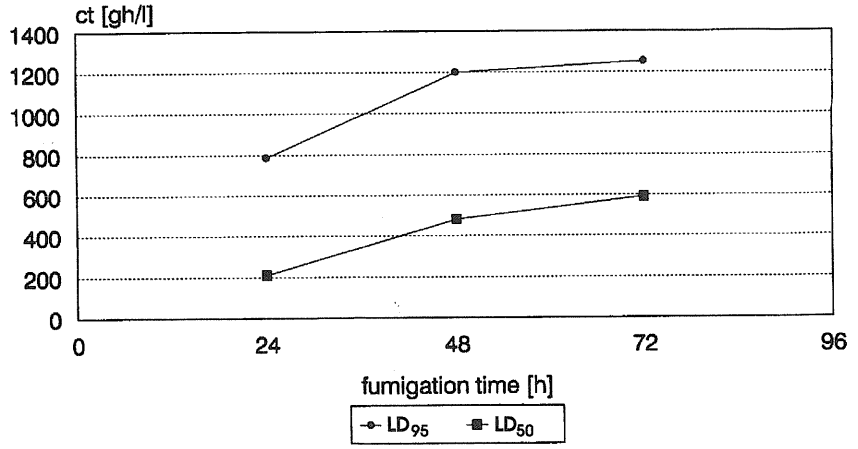


Fig. 4. LD_{50} and LD_{95} Ct-values for exposure of eggs of *Sitophilus granarius* to SO_2F_2, in relation to fumigation time.

DISCUSSION

This study demonstrated that larvae, pupae and adults were the most susceptible stages to SO_2F_2. The lowest concentrations (11.7–13.3 g/m^3) tested for 24 h produced 100% mortality of *S. granarius*, *T. confusum* and *T. molitor*. The eggs appeared to be the stage most tolerant to the fumigant. A dose of 35.0 g/m^3 for 24 h was required to achieve complete kill of *S. granarius* and *E. kuehniella* eggs. The results of this preliminary laboratory evaluation of the fumigant suggest that total control of all stages of the tested insects can be achieved by use of 350.2 g/m^3 for 24-h exposure at 20°C.

Insect pests are controlled by SO_2F_2 through disruption of the glycolysis cycle, leading to deprivation of metabolic energy (Meikle *et al.*, 1963). Its effect depends upon both the concentration which reached the target insect and the duration of exposure. The egg stage has been reported as being the most tolerant to SO_2F_2 for the stored-product beetles, *S. granarius* (Kenaga, 1957) and *T. molitor* (Outram, 1967), and also for various fabric and household pests (Kenaga, 1957; Lafage *et al.*, 1983; Thoms and Scheffrahn, 1994). Complete data for all developmental stages are not available for other stored-product insect pests, but data on certain developmental stages are available for *T. confusum* (Kenaga, 1957; Thoms and Scheffrahn, 1994), *Rhyzopertha dominica*, *O. surinamensis* (Kenaga, 1957), *Tenebroides mauritanicus* (Bond and Monro, 1961), *Dermestes maculatus* (Su and Scheffrahn, 1990), *Lasioderma serricorne* (Kenaga, 1957; Su and Scheffrahn, 1990), *Trogoderma granarium*, *E. elutella* (Thoms and Scheffrahn, 1994), *Sitotroga cerealella* and *E. kuehniella* (Kenaga, 1957). The results for *E. kuehniella* and *S. granarius* obtained in this study appear to indicate that the Ct-product increased when the exposure period was prolonged. Further laboratory studies on SO_2F_2 are required to validate the results of this preliminary observation.

SO_2F_2 has never been used for stored-grain fumigations due to both the cost competitiveness of other fumigants and the potential for residues (Schneider, 1993). However, the use of this fumigant for certain areas of stored-product protection, such as the fumigation of empty food processing and storage structures, is being investigated. Should the results of this study be confirmed, the performance of the fumigant under practical field conditions will be investigated.

ACKNOWLEDGEMENT

The authors are indebted to Gülyani Karacoglu and Gerhard Schmidt for their valuable assistance in setting up the experiments and carrying out the bioassay.

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SENSITIVITY OF NARCISSUS FLIES (GENERA: *EUMERUS* AND *MERODON*) TO METHYL BROMIDE

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ABSTRACT

Two species of narcissus fly attack the bulbs of *Narcissus* and *Amaryllis*, posing a serious quarantine threat when these crops are exported from Israel. The only available fumigant for rapid treatment is methyl bromide (MB). However, recent indications of phytotoxic effects on these bulbs required re-evaluating the fumigation schedule.

The sensitivities of the large narcissus fly (*Merodon eques* F.) and the small narcissus fly (*Eumerus* spp.) to MB were examined in the laboratory. Maggots of both species and pupae of *Eumerus* were obtained from infested narcissus bulbs and exposed in glass chambers at 28°C to different dosages of MB for 4 h periods. Because the *Merodon* develops inside the bulb, maggots of this species were transferred to cells hollowed out inside sound bulbs, and the bulbs were then placed inside the fumigation chambers. For *Eumerus*, where infestation is superficial, maggots and pupae were suspended in cages inside the fumigation chambers. Probit analyses of mortalities revealed that the dose required to obtain 99% kill (LD_{99}) was 20.4 g/m^3 for *Merodon* sp., whereas for *Eumerus* larvae and pupae it was 8.6 and 6.5 g/m^3, respectively. The high dosage required to kill *Merodon* may be attributed to the necessity that the gas penetrate to the target site.

INTRODUCTION

Of the insects attacking the narcissus bulb the most important economic pests are the large and small narcissus bulb flies. In Israel two species of the genus *Merodon* (the large narcissus bulb fly) have been recorded, *M. eques* F. and *M. geniculata*. The fly *M. equestris* F. does not occur in Israel but is present in Southern Europe, and by the thirties its range had extended to North America (Hodson, 1932). The species *M. geniculata*, recorded as restricted to North Africa and the Middle East, was believed to be the species present in Israel. However, recently large narcissus flies from a consignment of bulbs destined for the USA were identified as *M. eques*. This species is very similar to *M. geniculata* so the previous recordings of the latter species are in doubt. Since neither of these species has been recorded in the USA they would both represent a

serious quarantine threat. It is characteristic of the large narcissus fly that the damage caused is not clearly visible from outside. Generally a single larva in each infested bulb develops close to the base. Pupation takes place either in the bulb or in the soil. According to Hill (1987) the fully grown larva of *M. equestris* leaves the bulb and pupates in the soil, whereas Brosh and Hadar (1977) state that although the larva usually pupates in the soil, it sometimes does so within the bulb. During the bulb export season (June–July) only larvae are present in the bulbs; however, the possibility of pupation within the bulb in the autumn increases the danger that this insect could spread as a result of transportation unless quarantine measures are undertaken.

The genus *Eumerus* includes two species known collectively as the small narcissus bulb fly: *E. strigatus* Fall. and *E. tuberculatus* Rond. Both species are known in Europe, Asia and North America (Hodson, 1927). *E. amoenus* has been recorded from the Mediterranean basin including Israel (Hill, 1987). However, it has been suggested that the species found in Israel is a new one that has still to be described (Nestel *et al.*, 1994). Therefore this species should be considered of limited distribution until proven otherwise. It should be added that numerous species of both the genera *Merodon* and *Eumerus* that do not attack the narcissus bulb exist in Israel. The problematics of identifying the narcissus flies have been discussed recently by Nestel *et al.* (1994). It is characteristic of the small narcissus fly that damage is visible externally as a mass of rotting tissue within which can be found a group of developing maggots. Infested bulbs can be identified and removed fairly easily during sorting in the packing station, thereby reducing the danger that the small narcissus fly will be introduced into importing countries.

Clearly, uncertainty about the systematic status of the narcissus flies, incomplete knowledge of their distribution, and the failure of control measures in the field to produce a complete kill (Luria and Hokes, 1992), all contribute to the necessity that a fail-proof quarantine treatment be undertaken. The most widely used treatment for rapid insect kill before export is fumigation with methyl bromide (MB), and this has become the standard means of control in recent years.

The recommendation for commercial scale fumigation is exposure to 40 gm/m^3 for 4 h (Bond, 1984). In the past, dosage levels also took into account a decrease in concentration due to both leaky chambers and the sorption by the bulbs, but recently chambers made of flexible plastic have been introduced that are capable of containing the gas with minimum leakage. Therefore, in order to prevent bulb damage due to accumulation of high concentrations, it is necessary to amass laboratory information on the MB concentrations needed to kill the narcissus flies.

This study forms part of a series of experiments designed to establish recommended dosage levels that will ensure a complete kill of the flies on one hand and avoid phytotoxic effects on the bulbs on the other. The objective of this experiment was to determine the concentrations of MB lethal to the two insect species (*Merodon eques* and *Eumerus* sp.).

MATERIALS AND METHODS

Source

Larvae of the large narcissus fly (*Merodon eques*) and of the small narcissus fly (*Eumerus* sp.) used in the experiments were removed from infested narcissus bulbs obtained during sorting at the packing stations.

The large narcissus fly

Preparation. Live larvae were found and removed from the infested bulbs by cutting into the rotting material. Clean bulbs were then infested with these larvae as follows: a tunnel was bored through the bulb along its longitudinal axis using an instrument for cutting holes in rubber bungs. This tunnel was about 50 mm in length and 7 mm in diameter. A live larva was then inserted into the opening and the tunnel was sealed at both ends, with bulb material taken from the core, in order to prevent the larva from either abandoning the bulb or desiccating. From then until the end of the experiment the bulbs were held in a chamber at 30°C and 70% relative humidity.

Fumigation conditions. Fumigations were carried out in glass desiccators with a capacity of 6.3 L. The fumigant was pure MB (without chloropicrin). The dosage was calculated on the basis of the free air space within the desiccator less the volume of the bulbs. Bulb volume was calculated on the basis of bulb weight times specific weight of 1.072. The dosage of MB was injected into the desiccators and the gas was initially mixed for a few minutes by a magnetic stirrer placed on the floor of the desiccator. Samples for measuring gas concentrations were taken at the beginning and end of the exposure period using a pressure lock syringe. These samples were injected into a gas chromatograph with the FID detector calibrated for MB. For each treatment, 20 infected bulbs were exposed to different dosages of MB for 4 h. A further sample of 20 infected bulbs, held in a desiccator without fumigant, served as a control. When the exposure period had elapsed, the bulbs were removed from the desiccators, placed in net-bags, and left in a laboratory fumigation hood overnight for aeration. Mortality counts were made 24 h after exposure.

No pupae were found during the collection from infested bulbs; consequently, the study was carried out only on larvae. Hill (1987) states that the larva leaves the bulb to pupate in the soil. Conversely, Brosh and Hadar (1977) record pupae in the soil and also sometimes in the bulbs.

The small narcissus fly

Preparation. Larvae of the small narcissus fly *Eumerus* sp. are usually found in small groups between the outer layers of rotting bulb leaves. Infestation is characteristically more superficial than with the large narcissus fly. Consequently, since gas penetration into the bulb was not considered a crucial factor in determining the sensitivity of *Eumerus* to MB, the larvae were collected and exposed directly to the gas rather than being placed inside bulbs.

Fumigation conditions. Larvae and pupae were collected separately from the rotting material of infested bulbs and 30 individuals each were placed inside cages. The cages consisted of capped plastic vials 34 mm in length and 14 mm in diameter, to the top and bottom of which wire mesh (No. 25) had been heat soldered. Flat-bottomed glass flasks with a capacity of 3 L served as fumigation chambers. The vials were suspended in the centers of the flasks with nylon thread which was attached to hooks in the ground-glass stoppers with which the flasks were sealed. The stoppers were also equipped with a device for injecting the measured dosage of MB into the flasks. This device consisted of a rubber septum set into the opening of a glass tube attached to the stopper. Initial mixing of the gas was done with a magnetic stirrer for a few minutes. Fumigations were carried out inside a fumigation hood at 28°C for an exposure period of 4 h. Larval mortality was recorded 24 h after treatment. Pupal mortality was defined as the number of pupae that failed to develop into adults.

For both species of narcissus fly mortality results were subjected to probit analysis using the program developed by Daum (1979).

RESULTS

Figure 1 shows probit mortality rates of larvae and pupae of *Eumerus* and larvae of *Merodon* plotted against log dose while mortality rates at the levels of MB required to kill 50% (LD_{50}) and 99% (LD_{99}) of the population are given in Table 1.

From the figure and table, it can be seen that a higher dosage is required to kill 99% of the population of the large narcissus fly than that required for the small narcissus fly. Clearly the necessity of the penetration of the gas into the bulb in order for it to reach the *Merodon* larva is a significant factor, causing both a delay in exposure and a reduced concentration at the target site. In addition there are possible differences in sensitivity to MB between the two species.

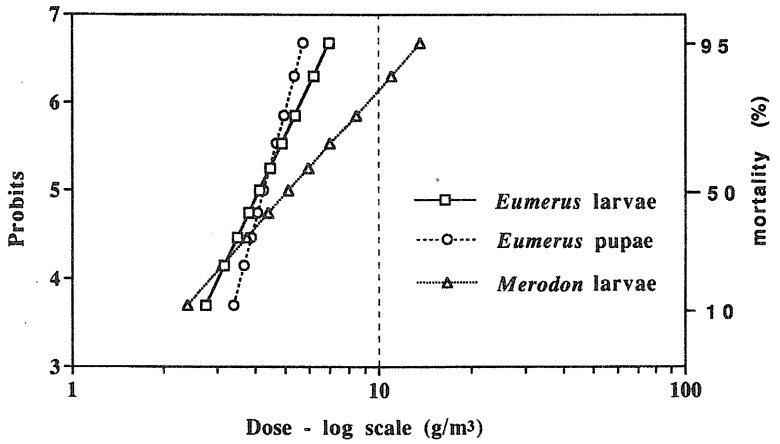


Fig. 1. Sensitivity of *Eumerus* larvae and pupae and *Merodon* larvae to MB (4-h exposure at 28°C).

TABLE 1

Mortality of *Merodon eques* and *Eumerus* sp. on exposure to methyl bromide for 4 h at 28°C

Stage	LD_{50}	Confidence limits	LD_{99}	Confidence limits	SE	Slope	Intercept
Merodon							
Larva	5.13	4.0–6.1	20.43	14.7–39.7	1.0	3.87	2.24
Eumerus							
Larva	4.13	3.1–.5–4.9	8.60	6.5–28.9	2.6	7.27	0.51
Pupa	4.28	3.8–4.6	6.49	5.9–8.0	1.0	12.86	–3.12

DISCUSSION

In spite of the recent decisions to phase out the use of MB, its status (UNEP, 1995) as a quarantine fumigant remains unchanged due to the fact that no alternative treatments exist. Therefore MB fumigations will continue to be carried out to control narcissus flies where quarantine regulations are in force. Previous fumigations were based on the fumigation schedule of Bond (1984) because no specific studies on the sensitivities of narcissus flies to MB were available. Zumreoglu and Erakay (1978) used a 2-h fumigation schedule at higher dosage rates. Though further studies are needed to confirm our findings, the results of these experiments will form the basis for the concentration levels to be achieved in future quarantine fumigations of narcissus bulbs in Israel.

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THE EFFECT OF TEMPERATURE ON SOME RESISTANT STORED-PRODUCT INSECTS EXPOSED TO PHOSPHINE

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ABSTRACT

Mixed-age cultures of susceptible strains of *Sitophilus oryzae, S. granarius, S. zeamais* and *Rhyzopertha dominica*, together with resistant strains of *S. oryzae* and *R. dominica*, were exposed to constant concentrations of 0.03, 0.05 and 0.1 mg L^{-1} phosphine (PH_3) at temperatures of 15, 25 and 35°C. All species and strains were generally more tolerant of PH_3 at 15°C than at higher temperatures. Higher temperatures reduced the required exposure time for complete mortality for susceptible strains of *Sitophilus* spp. and *R. dominica*, and for resistant strains of *S. oryzae*, at all concentrations. In contrast, the exposure time needed to achieve complete mortality of resistant *R. dominica* at concentrations of 0.03 and 0.05 mg L^{-1}, but not at 0.1 mg L^{-1}, was longer at 35°C than at 25°C. At 0.1 mg L^{-1}, complete mortality at 35°C was achieved in the same or slightly less time than at 25°C.

INTRODUCTION

Worldwide, phosphine (PH_3) fumigations are performed across a wide range of stored commodity temperatures. In Australia, and in many other parts of the world, grain is commonly stored at temperatures over 25°C. The effect of high temperatures is frequently not considered (Winks *et al.*, 1980) when disinfestation treatments are recommended since it is believed that the higher the temperature the greater the efficacy of PH_3 (Lindgren and Vincent, 1966; Hole *et al.*, 1976).

In laboratory studies, temperature has long been recognised as having complex effects on insect response to fumigants (e.g. Champ and Dyte, 1976). For this reason it is usually recommended that such studies be standardised on a single temperature that covers most species. In PH_3 toxicity studies, for example when using the FAO discriminating dosage test (Anon., 1975), the generally adopted exposure temperature has been 25°C.

Hole *et al.* (1976) found a positive correlation between temperature and dosage over the range of 10–35°C when they examined the response of immature stages of susceptible insects to PH_3. Price and Mills (1987) found a similar result when they exposed resistant

insects to PH_3 at 15 and 25°C. One of the ways temperature affects insect mortality is the rate of fumigant uptake. Price (1984) examined the effect of temperature on PH_3-uptake in susceptible and resistant adult laboratory insects exposed to PH_3 for 5-h periods. He found that increased temperature stimulates uptake in susceptible insects and enhances the active exclusion of PH_3 in resistant insects. Thus, resistant adult insects became more resistant with increased temperature.

This finding raised the question of whether resistant strains in the field would have an even greater likelihood of survival in grain at elevated temperatures (30°C or more). As part of the development of the SIROFLO® fumigation technique, studies were conducted to determine the response to PH_3 of a number of the major insect species infesting grain in Australia over a range of temperatures. SIROFLO® applies a continuous low concentration of PH_3 into a pressurised distribution system (Winks, 1992). This paper reports the results of studies in which mixed-age cultures of resistant insects were exposed to PH_3 at different temperatures and compares these results with those of Price (1984).

MATERIALS AND METHODS

The effects of temperature on PH_3 toxicity were determined by exposing mixed-age cultures to a continuous flow of PH_3 in air to determine the time to population extinction. The time to population extinction is an estimate of the minimum exposure time necessary to achieve complete mortality of all stages of the life cycle for a given concentration and temperature. In these experiments, concentration was held constant and time was the dosage variable. The methods used are generally those described in Winks and Hyne (1994). However, a more detailed description of the method using mixed-age cultures, including its rationale and repeatability, is given in Winks and Hyne (1997).

Insect material

A number of strains of four species of stored-product beetles, *Sitophilus oryzae, S. granarius, S. zeamais* and *Rhyzopertha dominica*, were used in these experiments. The strains of *S. oryzae* and *R. dominica* included PH_3-susceptible strains, PH_3-resistant field strains and laboratory-selected resistant strains (only susceptible strains of *S. granarius* and *S. zeamais* being used). Adults of all strains were diagnosed as susceptible (S) or resistant (R) by the FAO discriminating dosage test (Anon., 1975). Strain names and collection locations and dates of the insects are given in Table 1.

Wheat (var. Corella or Rosella) used as a culture medium was conditioned to 12% m.c. prior to use and sterilised by heating to 60°C for a minimum of 1 h. Mixed-age cultures were established by placing 300 adults on approximately 1,000 g of whole wheat (*Sitophilus* spp.) or layers of whole wheat and flour (*R. dominica*) in 2-L glass jars. The adults were not removed from these cultures. After 6 weeks, samples of the cultures were taken and X-rayed to verify the presence and relative abundance of larval and pupal stages. Cultures of *Sitophilus* spp. were reared at 25°C, 57% r.h., and *R. dominica* at 30°C, 70% r.h.

TABLE 1
Strains, collection dates and origins of stored-product insects used in this study

Species (Strain)	Resistant/ Susceptible	Origin
Sitophilus oryzae		
LS2	S	Queensland 1962
CSO404P10	R	Laboratory selection with PH_3 for ten generations of CSO404 (formerly FAO strain SO476, from Karnal, India), 1976
CSO421	R	Goondiwindi, Queensland, 1991
Sitophilus granarius		
CSG4	S	Queensland, 1952
CSG46	S	Tutye, Victoria, 1974
Sitophilus zeamais		
CSZM9	S	Brisbane, Queensland, 1971
Rhyzopertha dominica		
CRD2	S	Pest Infestation Control Laboratory (UK), 1962
CRD316	R	Trangie, New South Wales, 1989
CRD235P10	R	Laboratory selection with PH_3 for ten generations of RD235 (formerly FAO strain CRD484, from Borivli, India), 1976

Fumigation chambers

Mixed-age cultures were placed in 3-L perspex or stainless steel fumigation chambers (Winks and Hyne, 1994) 72 h before fumigation. Clear acrylic (perspex) chambers were made from a perspex tube fitted with two perspex screw-top lids and made gastight with neoprene O-rings. Both top and bottom lids were fitted with an inlet and an outlet. The lids were lined with stainless steel mesh to prevent insects and dust from escaping from the inlet and outlet. The bases of the stainless steel chambers were secured with six hex screws and made gastight with a neoprene O-ring.

Fumigation procedures

The PH_3 concentrations used and the temperatures to which the insects were exposed are given in Table 2. A fixed concentration of PH_3 was continuously supplied to the bases of the fumigation chambers. PH_3 in nitrogen (BOC) was diluted with air using two Brooks mass flow controllers (5850E series). Atmospheric air was filtered through charcoal to a Charles Austin diaphragm pump supplying the mass flow controllers. The diluted PH_3

TABLE 2
Summary of the insects, PH_3 concentrations and temperatures used in experiments to determine times to population extinction

	Phosphine concentration (mg L^{-1})								
	0.03			0.05			0.1		
Strain	15°C	25°C	35°C	15°C	25°C	35°C	15°C	25°C	35°C
LS2		×	×	×	×	×	×	×	×
CSG4		×	×	×	×	×	×	×	×
CSZM9		×	×		×	×		×	×
CSO421		×			×	×			
CSO404P10		×			×	×		×	
CRD2		×		×	×	×	×	×	
CRD316	×	×	×	×	×	×	×	×	×
CRD235P10	×	×	×	×	×	×	×	×	×

was humidified to 57% r.h. for the experiments at 15 and 25°C and to 60% r.h. for the experiments at 35°C. Air was humidified to saturation by passage through distilled water maintained at 7, 15 and 26.5°C and then being reheated to laboratory temperatures of 15, 25 and 35°C, respectively.

Each chamber received a flow of the PH_3 air mixture at 30–40 ml min^{-1}, obtained by dividing the source flow equally using Brooks flowtubes (A125-5).

Throughout the experiment, PH_3 concentrations were monitored using a gas chromatograph fitted with a Tracor flame photometric detector in the phosphorus mode. The flame photometric detector was calibrated using accurate dilutions made from a concentrated source of PH_3. PH_3 was prepared from aluminium phosphide according to Anon. (1975), and its concentration was determined using a Gowmac gas density balance.

Post-fumigation procedures

Samples of grain-and-insects, approximately 200 g each, were generally taken from the fumigation chambers at intervals of 2, 4, 7, 14, 21 and 28 d, although for some experiments daily samples were taken over a shorter period to obtain better estimates of time to population extinction. These samples were incubated at 25°C, 60% r.h., and assessed for the presence of live adults by sieving. Assessments were made 24 h after sampling and again after 8 weeks and 16 weeks incubation. The first sample from which no adults emerged (confirmed by subsequent samples) indicated the extinction of the population. The time of exposure of this sample was recorded as the estimate of the *time to population extinction* (Winks and Hyne, 1997). Data was presented graphically as split-column plots

according to the method described by Winks and Hyne (1997). The height of the split column shows the exposure time of the first sample from which no survivors were recorded. The split in the column shows the exposure time of the previous sample from which survival was recorded.

RESULTS

Susceptible strains

The times to population extinction for susceptible strains of all species exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C are given in Figs. 1, 2, 3 and 4. All susceptible strains were more tolerant of PH_3 at 15°C than at the higher temperatures for the concentrations examined. The *Sitophilus* species required longer exposure periods than *R. dominica* (CRD2) at all concentrations. The most tolerant strain at 15°C, LS2, required more than 22 d exposure for control at 0.05 mg L^{-1} PH_3. As the concentration increased to 0.1 mg L^{-1}, the time to population extinction decreased for the *Sitophilus* spp. but not for CRD2 (Figs. 1, 2 and 4).

At 25°C the time to population extinction for LS2 and CSG4 increased slightly while that of CSZM9 decreased slightly as concentration increased from 0.03 to 0.05 mg L^{-1}. Due to the differences in sampling frequency, for example a 7-d sample interval versus a daily sample, it was not possible to determine if these trends continued to 0.1 mg L^{-1}. The time to population extinction for CRD2 appeared to be similar

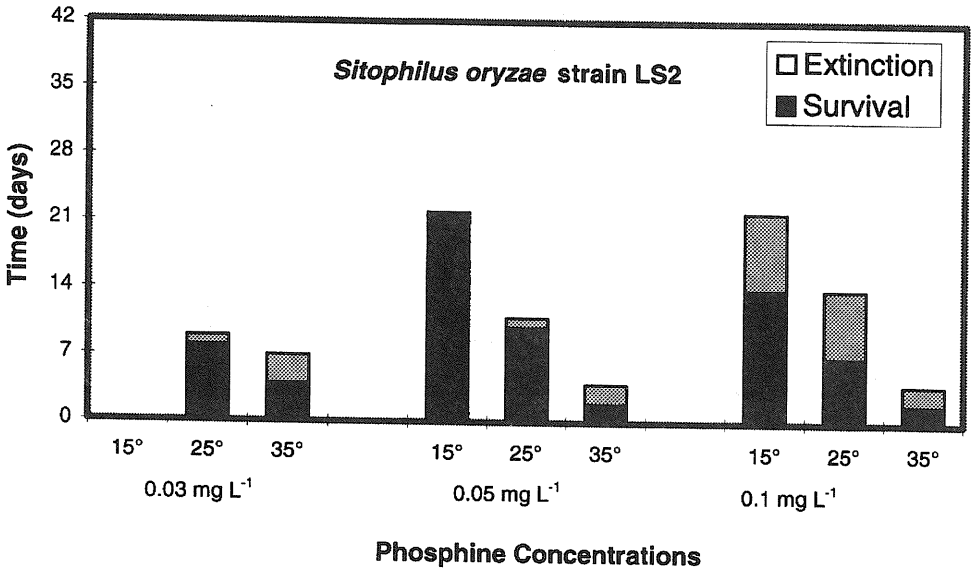


Fig. 1. Time to population extinction of mixed-age cultures of a PH_3-susceptible strain of *Sitophilus oryzae* (LS2) exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

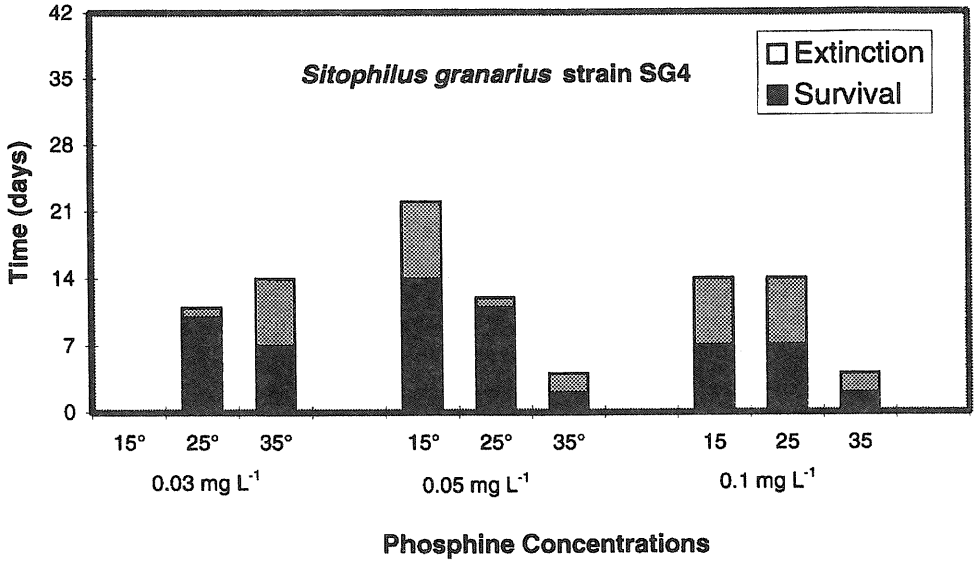


Fig. 2. Time to population extinction of mixed-age cultures of a PH_3-susceptible strain of *Sitophilus granarius* (CSG4), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

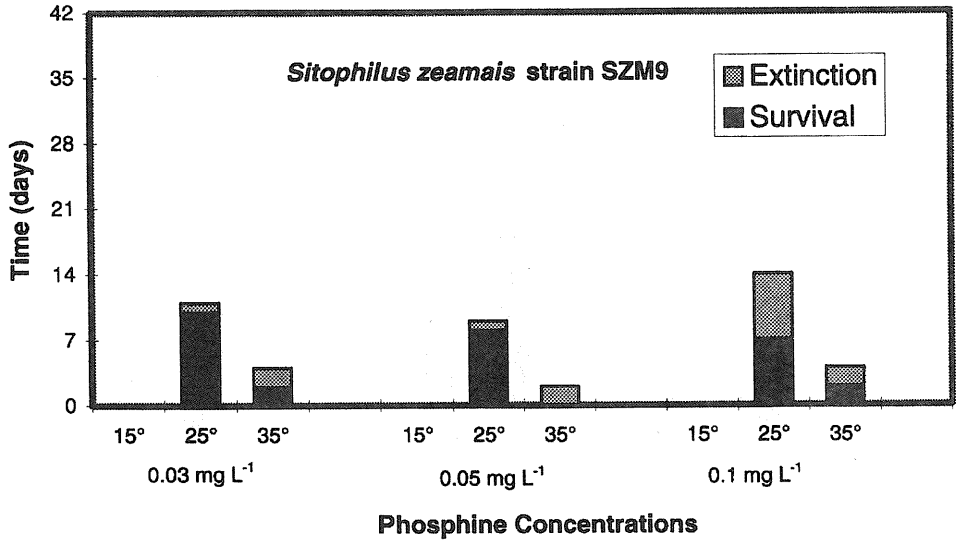


Fig. 3. Time to population extinction of mixed-age cultures of a PH_3-susceptible strain of *Sitophilus zeamais* (CSZM9), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 25 and 35°C.

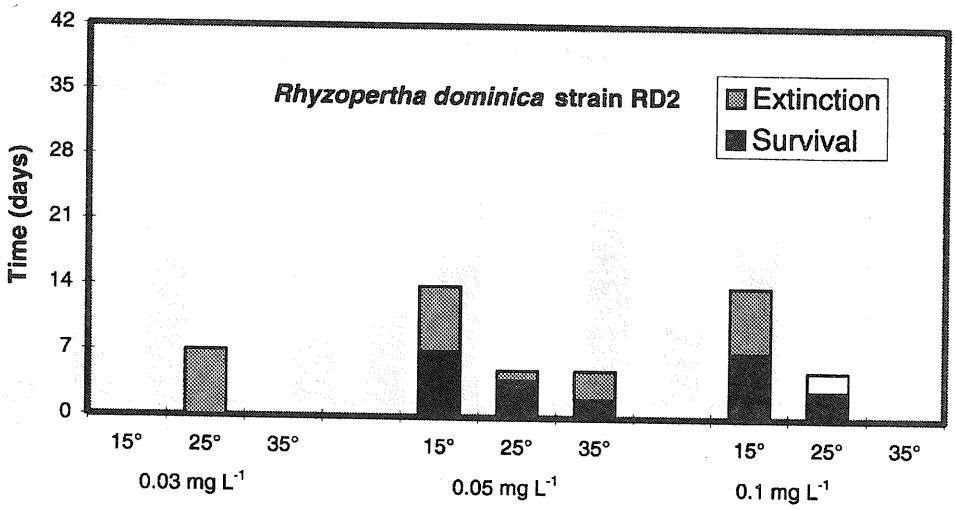


Fig. 4. Time to population extinction of mixed-age cultures of a PH_3-susceptible strain of *Rhyzopertha dominica* (CRD2), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

for all concentrations (Note: as the first sample taken at 25°C for 0.03 mg L^{-1} was at 7 d, the time to population extinction could be earlier). By contrast, at 35°C, time to population extinction decreased for LS2 and CSG4 as concentration increased from 0.03 to 0.05 mg L^{-1}.

The time to population extinction for the *Sitophilus* spp. was longest at 15° and shortest at 35°C for all concentrations except CSG4 at 0.03 mg L^{-1}, where the time to population extinction at 25°C and 35°C appeared similar (Figs. 1, 2 and 3). There appeared to be a similar relationship with CRD2, although at 0.05 mg L^{-1} the observed times to population extinction were the same at 35 and 25°C (Fig. 4).

Resistant strains of *Rhyzopertha dominica*

The times to population extinction for resistant strains of *R. dominica* exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C are given in Figs. 5 and 6.

The relationship between temperature and time to population extinction for the resistant strains produced a complex picture. The time to population extinction of CRD316 was greatest at 35 and least at 25°C for 0.03 and 0.05 mg L^{-1}. The time to population extinction for CRD235P10 followed a similar trend at 0.05 mg L^{-1} but not at 0.03 mg L^{-1}, where the time to population extinction was greater at the higher temperatures. Although not apparent from Fig. 6, the laboratory selected strain CRD235P10, at 0.03 mg L^{-1}, appeared to be close to extinction after 35 d at 25°C when two insects were found

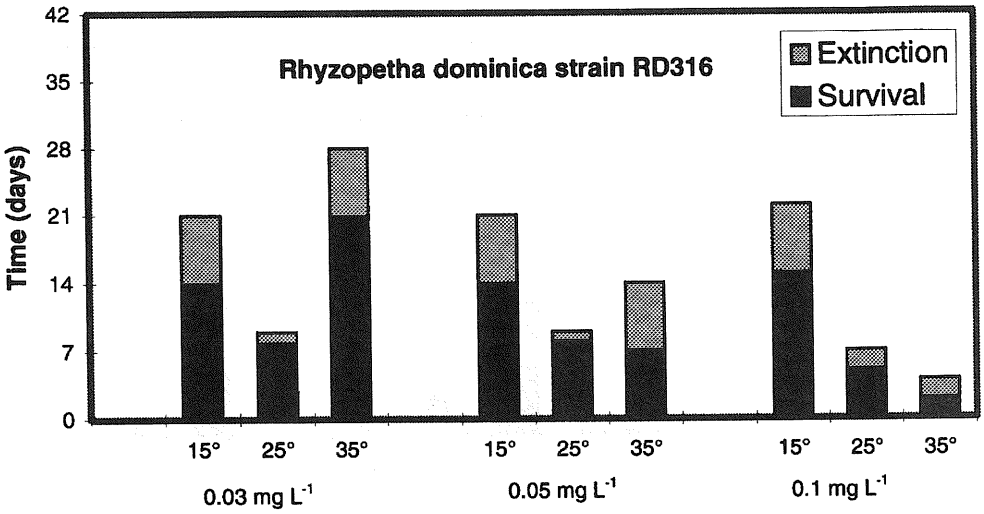


Fig. 5. Time to population extinction of mixed-age cultures of a field resistant strain of *Rhyzopertha dominica* (CRD316), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

compared with the thousands found after 28 d at 35°C. This indicated a time to population extinction well beyond 28 d at this higher temperature. In contrast, at 0.1 mg L^{-1}, the time to population extinction was lowest at 35°C in strain CRD316 and was equally short at 25

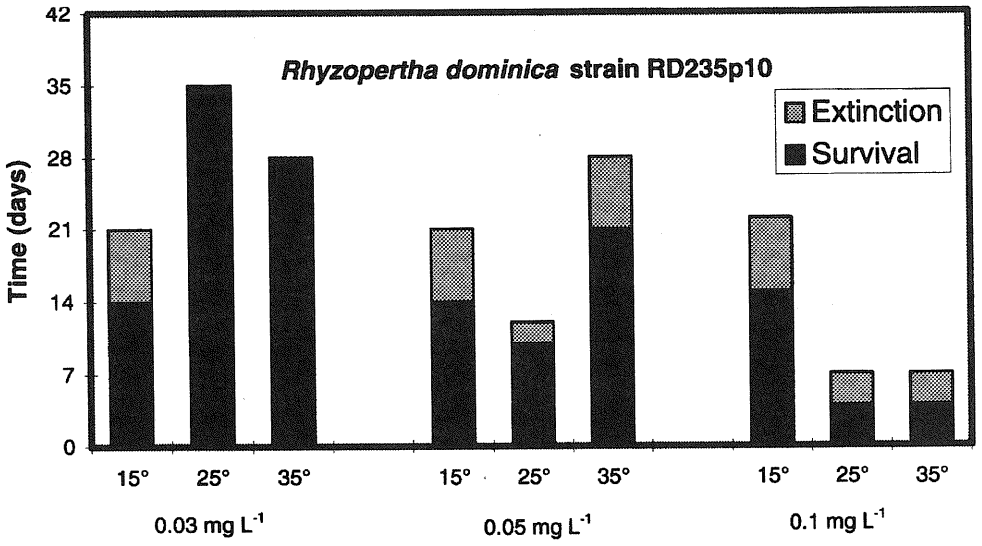


Fig. 6. Time to population extinction of mixed-age cultures of a laboratory selected resistant strain of *Rhyzopertha dominica* (CRD235P10), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

and 35°C in CRD235P10. At both 25 and 35°C the time to population extinction became progressively shorter with each increase in concentration.

At 15°C the time to population extinction of both resistant strains, CRD235P10 and CRD316, and the susceptible strain, CRD2, appeared to remain the same at each of the three concentrations (Figs. 4, 5 and 6). As the concentration increased at 25°C, the time to population extinction of CRD235P10 and CRD316 decreased to a level similar to that of CRD2. For example, with CRD235P10, the time to population extinction decreased from more than 35 d at 0.03 mg L^{-1} to 7 d at 0.1 mg L^{-1}, similar to the susceptible CRD2 strain which, at the same concentration, took 5 d.

Resistant strains of *Sitophilus oryzae*

The time to population extinction for the resistant strains of *S. oryzae* are given in Figs. 7 and 8. This data set is incomplete as the resistant insects had not been exposed to PH_3 at 15°C and had been exposed at only one concentration at 35°C. From the available data, it seems that the time to population extinction for these strains became progressively shorter with each increase in concentration at both 25 and 35°C in a manner similar to that of the susceptible strain.

The resistant strains (CSO421 and CSO404P10) are most tolerant when exposed to 0.03 mg L^{-1} at 25°C, requiring more than 35 d for control, compared with the susceptible LS2 which required 14 d. At 0.1 mg L^{-1} the effect of resistance in the laboratory-selected CSO404P10 was lost, and the time to population extinction was the same as the susceptible LS2.

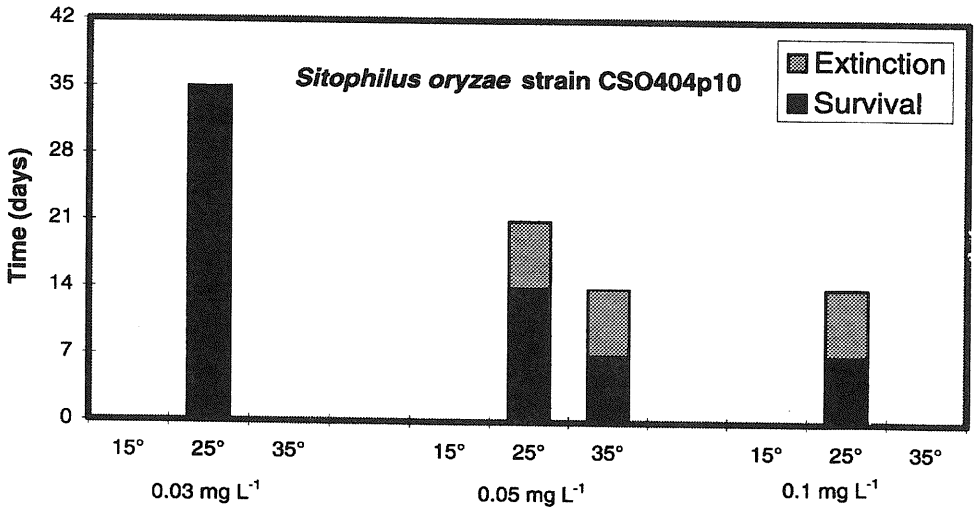


Fig. 7. Time to population extinction of mixed-age cultures of a field resistant strain of *Sitophilus oryzae* (CSO421), exposed to 0.03 and 0.05 mg L^{-1} PH_3 at 15, 25 and 35°C.

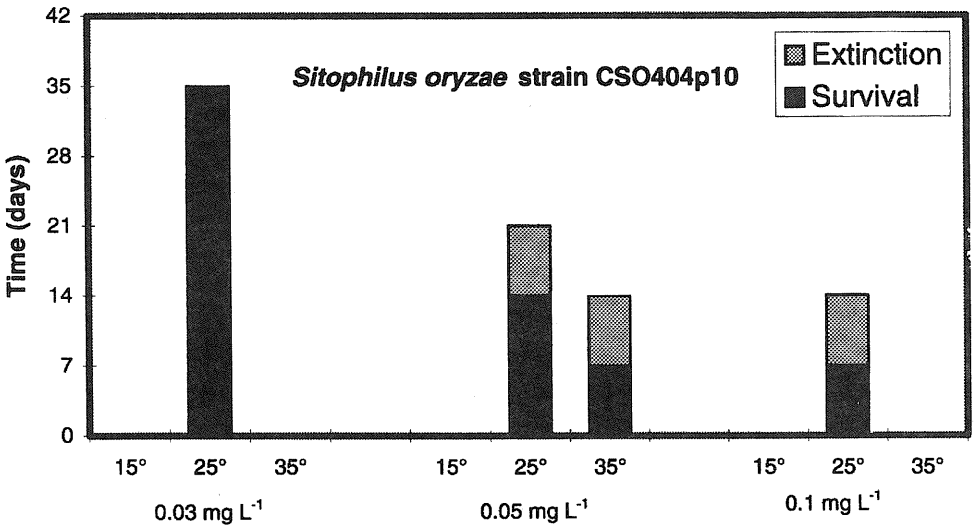


Fig. 8. Time to population extinction of mixed-age cultures of a laboratory selected strain of *Sitophilus oryzae* (CSO404P10), exposed to 0.03, 0.05 and 0.1 mg L^{-1} PH_3 at 15, 25 and 35°C.

DISCUSSION

Susceptible strains

The susceptible strains of all species tolerated PH_3 for longer periods at 15°C than at the higher temperatures. This finding is supported by the work of Hole *et al.* (1976) and Price and Mills (1987). Hole *et al.* (1976) suggested that the long exposure periods needed to kill insects at 15°C were due, in part, to the time taken for tolerant stages to develop into more susceptible stages. For many stored-product insect pests, 15°C is close to the temperature threshold at which development of immature stages can occur. For example, the developmental thresholds are 16.6°C for *R. dominica* and 11.4°C for *S. oryzae* (Beckett *et al.*, 1994). Development of the immature stages of *R. dominica* would be substantially protracted at 15°C, and this raises doubts as to whether the mortality observed in the present study was due entirely to tolerant stages developing into more susceptible stages.

With each progressive increase in temperature, the time to population extinction generally became successively shorter for all strains. There were a few exceptions however. For example, at 0.03 mg L^{-1} the time to population extinction of susceptible CSG4 at 35°C appeared greater than at 25°C, but this may have been due to the 7 d interval in the data at 35°C. These data support the finding that susceptible species become less tolerant of PH_3 with increased temperature (Price, 1984).

Resistant strains

Resistant *S. oryzae* do not demonstrate increased tolerance to PH_3 with increased temperature, as suggested by Price (1984) for *R. dominica*. *S. oryzae* was easier to control at 35°C than at 25°C for the single concentration examined. This was not the case for resistant *R. dominica* which was able to tolerate PH_3 longer at some concentrations at higher temperatures. For example, at 0.05 mg L^{-1} the laboratory-selected CRD235P10 required 28 d at 35°C to be killed, compared with 21 and 12 d at 15 and 25°C. At 0.1 mg L^{-1}, however, the insects were killed in 7 d at the higher temperatures compared with 21 d at 15°C.

The ability of resistant *R. dominica* and *S. oryzae* to tolerate PH_3 was affected by both temperature and concentration. At 15°C the time to population extinction for both strains of resistant *R. dominica* did not appear to change with each increase in concentration. At the higher temperatures the tolerance to PH_3 of the resistant strains of *R. dominica* and *S. oryzae* became shorter with each increase in concentration. For example, at 25°C resistant *S. oryzae* (CSO404P10, Fig. 8) required over 35 d to be killed at 0.03 mg L^{-1}, and 14 d at 0.1 mg L^{-1}, compared with 9 and 14 d to control LS2 at the same concentrations (Fig. 1). Similarly, resistant CRD235P10 required more than 35 d exposure at 0.03 mg L^{-1}, and only 7 d exposure at 0.1 mg L^{-1}, compared with CRD2 which required 5 to 7 d exposure at all concentrations. At 35°C, the time to population extinction of CRD235P10 was more than 28 d at 0.03 mg L^{-1} and 7 d at 0.1 mg L^{-1}.

It is possible that the differences in mortality response among the species at 35°C are due to differences in the individual species' ideal metabolic conditions. Resistance mechanisms operate more effectively in *R. dominica* at 35°C, 0.03 and 0.05 mg L^{-1}, which is close to the insects' optimum development temperature of 34°C (Birch, 1945; Beckett *et al.*, 1994). Further work, exposing resistant *S. oryzae* to PH_3 at 30°C, their optimum development temperature, would verify whether resistance in this species was or was not enhanced by temperature.

What does seem clear from this study is that, over the range of concentrations and temperatures observed for both the resistant strains, time is the critical component of dosage.

CONCLUSIONS

The temperature-dependent model proposed by Price (1984), in which susceptible insects become more susceptible to PH_3 with successive increases in temperature, appears to apply to all the susceptible insects in this study. However, the mortality response of resistant strains of *R. dominica* is not described by this model. Resistant *S. oryzae* appear, from the limited data, to follow the model for susceptible insects, while *R. dominica* is more tolerant at higher temperatures (but only at some concentrations).

The data of this report have important implications in the management of resistant *R. dominica* in countries with stored-product temperatures above 30°C. The exposure period is clearly more important than the level of concentration in determining effective dosages of PH_3 for control of insect pests of stored products. Hence, increasing exposure time rather than concentration would appear to be a better approach to the control of resistant *Sitophilus* spp. However, a concentration of at least 0.1 mg L^{-1} would seem to be essential for practical control of strains of *R. dominica* which are as resistant as the laboratory-selected strain CRD235P10.

ACKNOWLEDGEMENTS

Elizabeth Hyne would like to thank the Ian McLennan Achievement for Industry Award which enabled her to attend the 1996 CAF Conference in Cyprus.

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THE POTENTIAL OF METHYLPHOSPHINE AS A FUMIGANT FOR THE CONTROL OF PHOSPHINE-RESISTANT STRAINS OF FOUR SPECIES OF STORED-PRODUCT INSECTS

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ABSTRACT

The use of phosphine (PH_3) as the major fumigant of stored foodstuffs is threatened by the emergence of widespread resistance in stored-product insects in many countries. Studies have indicated that the mechanism of PH_3-resistance probably involves respiratory exclusion as well as detoxification of PH_3 in resistant insects. The search for a suitable alternative for control of PH_3-resistant insects led us to evaluate methylphosphine, a close analogue of PH_3, as a potential fumigant of stored products. Results indicated that exposure to methylphosphine produced much greater mortality in PH_3-resistant insects of four species of stored-product beetles compared to the corresponding susceptible conspecifics. It is possible that the presence of the methyl group in methylphosphine prevents exclusion by resistance mechanisms, and metabolism by the PH_3-detoxification process produces reactive products which lead to a higher mortality in resistant insects. The possibilities of using methylphosphine as a fumigant to control PH_3-resistant insects are discussed.

INTRODUCTION

Phosphine gas (PH_3) has been extensively used over the past four decades to control insect pests in grains and other stored commodities throughout the world (Bond, 1984). Many features, such as the lack of toxic residues after fumigation (Bruce *et al.*, 1962; Scudamore and Goodship, 1986) and the availability of solid phosphide formulations that generate the gas *in situ*, have made PH_3 the fumigant of choice. With recent restrictions on the production of the alternative, methyl bromide, due to alleged effects on the ozone layer (Taylor, 1994), PH_3 might soon become the only safe fumigant available for disinfesting stored commodities. The use of PH_3 as a fumigant is especially important for developing countries where most grain storage is in bags and the technology for the application of non-gaseous grain-protectants is not available. The future efficacy of this indispensable fumigant is, however, threatened by the emergence of widespread resistance in several species of stored-product insects in many countries (Champ and Dyte, 1976; Mills, 1983;

Tyler *et al.*, 1983; Champ, 1985; Taylor and Halliday, 1986; Taylor, 1989; Zettler *et al.*, 1989; Pacheco *et al.*, 1990; Udeaan, 1990; Chaudhry, 1991; Taylor, 1991; Zettler, 1991; Irshad *et al.*, 1992; Rajendran and Narasimhan, 1994; Zettler and Keever, 1994; Bell and Wilson, 1995). PH_3-resistance has been linked to poor fumigation practices that could lead to sub-lethal exposure and consequent selection of resistant insect populations (Halliday *et al.*, 1983; Mills, 1983; Tyler *et al.*, 1983). At least 11 species of stored-product insects, 10 species of beetles and 1 species of moth (Mills, unpublished) are now known to have developed resistance to PH_3.

Chemically, PH_3 is a strong reducing agent. Biological redox systems, especially the components of the mitochondrial electron transport chain, are its likely target sites in insects. However, the action of PH_3 differs from other known inhibitors of the respiratory chain, such as anoxia and hydrogen cyanide (Price, 1980; Price and Bell, 1981; Price and Dance, 1983; Price and Walter, 1987). The toxicity of PH_3 appears to arise from oxidation *in vivo* which could produce phosphorylating electrophilic species (Lam *et al.*, 1991), and it has been shown to cause generation of reactive oxyradicals in insects (Bolter and Chefurka, 1990; Chaudhry, 1991; Chaudhry and Price, 1992).

Compared to their susceptible counterparts, PH_3-resistant strains of several species of stored-product insects have been shown to absorb very small amounts of PH_3 (Price, 1981; Price and Dance, 1983; Price, 1984; Nakakita and Kuroda, 1986; Chaudhry and Price, 1989, 1990; Chaudhry, 1991; Reichmuth, 1994). The fact that some of the strains tested originated in different countries indicates a common mechanism of PH_3 resistance in insects. The amount of PH_3 absorbed by live resistant insects was even lower than that passively absorbed by dead insects, and Price (1984) suggests that there is active exclusion of PH_3 in the lesser grain borer *Rhyzopertha dominica*. This was supported by the findings that resistant strains of *R. dominica* excluded much greater amounts of gaseous [^{32}P] after treatment with [^{32}P]-PH_3 compared to a similarly treated susceptible strain (Chaudhry and Price, 1992). Other studies indicated that reduced uptake of PH_3 was not the only underlying mechanism of resistance in insects; a detoxification process was also involved (Chaudhry and Price, 1990). This was supported by conventional genetic studies on different species of stored-product insects which indicate that two or more genes are involved in PH_3 resistance (Ansell *et al.*, 1990; Li and Li, 1994).

Due to the importance of PH_3 in post-harvest protection of grains and other commodities, many improvements in fumigation practices have been suggested to increase efficacy against insects and control resistant pest populations. This includes application of PH_3 in multiple doses (Friendship *et al.*, 1986), use of formulations that release PH_3 at a slower rate (Halliday, 1986), fumigation under gas-proof sheets to minimise leakage (Taylor and Harris, 1994) and maintaining a low level of PH_3 by continuous supply from cylinders (Anderson, 1989). Increasing the concentration of CO_2 in the air to 14% has been reported to increase respiratory activity and enhance the uptake and efficacy of PH_3 in insects (Kashi and Bond, 1975). A mixture of PH_3 in CO_2 has therefore been used to increase the efficacy of PH_3 against insects (Desmarchelier, 1984; Desmarchelier *et al.*, 1984; El-Lakwah *et al.*, 1991). The usefulness of most of these methods in completely controlling

PH_3-resistant insects in a field situation is, however, doubtful. Resistance, at least in some instances, has reached levels where it can lead to control failures unless the best fumigation practices are applied in conditions of acceptable gastightness.

There is therefore a strong need to develop new fumigants as alternatives to PH_3, but the choice of suitable chemicals that exist as gases at normal temperature and pressure is very limited. Recently, the fumigant properties of carbonyl sulphide (COS) gas have been reported (Banks *et al.*, 1993). The insecticidal action of COS is thought to be due to hydrolysis by carbonic anhydrase to produce H_2S and CO_2 inside the insects. However, high aqueous solubility of COS and breakdown of the solubilised gas to H_2S might lead to sulphurous residues in the fumigated commodities. We previously carried out studies on arsine (AsH_3) and stibine (SbH_3), the group Vb analogues of PH_3. We found a negative correlation between resistance to PH_3 and tolerance to both of these gases which indicated that the PH_3-exclusion mechanism probably failed to exclude AsH_3 and SbH_3, and the oxidative breakdown by the PH_3-detoxification process produced toxic products (Chaudhry and Price, 1991). These findings suggested the possibility that the PH_3-resistance mechanism could be manipulated to selectively kill resistant insects using substances which are chemically similar to PH_3 but, unlike PH_3, produce reactive metabolites in oxidative breakdown. Unfortunately, AsH_3 and SbH_3 could not be used as fumigants because of the probability of toxic residues. We also tested two silicone hydride gases, silane (SiH_4) and methylsilane (CH_3SiH_3), but their insecticidal action was not comparable to the hydride gases of group Vb elements, i.e. PH_3, AsH_3 and SbH_3 (Chaudhry, unpublished). These studies led us to evaluate methylphosphine (CH_3PH_2), a close analogue of PH_3, as a potential fumigant against PH_3-resistant and PH_3-susceptible insects. The results of these tests are presented in this paper.

MATERIALS AND METHODS

The four species of stored-product insects used in the toxicity tests were the lesser grain borer, *R. dominica*; the rust-red flour beetle, *Tribolium castaneum*; the rice weevil, *Sitophilus oryzae*; and the flat grain beetle, *Cryptolestes ferrugineus.* All insects were cultured at 25°C and 70% r.h. except *C. ferrugineus* which was cultured at 30°C and 60% r.h. The strains of *R. dominica* and *S. oryzae* were cultured on whole wheat, *T. castaneum* on wheat flour containing 5% yeast and *C. ferrugineus* on food containing oats, flour and yeast.

Three strains of *R. dominica* used in the tests comprised a susceptible (reference), a laboratory selected PH_3-resistant (306sel) and a highly PH_3-resistant field strain (BR2) which originated from a population sampled in Bangladesh. The *T. castaneum* strains tested comprised a susceptible (lab) and a PH_3-resistant strain collected from Bangladesh (BT1vs). The strains of *S. oryzae* included a susceptible (reference) and a PH_3-resistant (476s) strain, and *C. ferrugineus* strains comprised a susceptible (reference) and a PH_3-resistant (BC12s) strain.

Methylphosphine was prepared by the reaction of dimethyl-methylphosphonate and lithium-aluminium-hydride ($LiAlH_4$) in ethylene-glycol-dimethyl-ether (monoglyme),

using a slight modification of the method described by Crosbie and Sheldrick (1969). In a typical synthesis, a pellet of $LiAlH_4$ weighing about 2.5 g (Aldrich Chemical Co., UK) was added to 15 ml of monoglyme, in a three-neck flask fitted with a rubber septum, and maintained under a flow of nitrogen (N_2) gas. The hydrogen gas produced by the reaction of $LiAlH_4$ with any trace of moisture was discarded through a bubbler containing 2% mercuric chloride ($HgCl_2$) solution while the pellet was stirred in monoglyme to form a slurry. About 1.60 g of dimethyl methylphosphonate (Aldrich Chemical Co., UK) were very slowly stirred into the slurry. Methylphosphine generated by the reaction was collected in a stream of N_2 gas over the surface of water in a gas burette fitted with a rubber septum. The identity of methylphosphine was confirmed by mass spectrometric analysis which was consistent with its previously reported mass spectrum (Wada and Kiser, 1964).

The concentration of methylphosphine was measured by Gas Chromatography (GC) using a Shimadzu-GC-9A which was fitted with a glass-lined packed column (1 m long, 1/8" O.D., Poropak-QS 80–100 mesh packing), flame photometric detector and automatic gas sampling loop. The column temperature was maintained at 200°C with injector and detector temperatures at 150°C. Methylphosphine was eluted from the column immediately after PH_3; retention times were 0.255 and 0.397 min, respectively, for PH_3 and methylphosphine, and the amounts were estimated by comparison to standard concentrations of PH_3 in N_2 gas. A correction factor was used to account for the difference in molecular weights of PH_3 and methylphosphine. In all syntheses, a trace of PH_3 (1–2% of methylphosphine) was present, presumably arising from phosphate contamination of the glassware and/or from breakdown of methylphosphine. In some cases, concentrations of methylphosphine measured by GC were verified by chemical analysis. This was carried out by reacting a known volume of methylphosphine with standard $HgCl_2$ solution and titrating the amount of HCl, produced in the resulting reaction, with NaOH solution: $CH_3PH_2 + 2HgCl_2 \rightarrow CH_3P(HgCl)_2 + 2HCl$.

We also carried out investigations into the nature of the oxidation products of methylphosphine by bubbling the gas through 2% $HgCl_2$ and oxidising the resulting precipitate of $CH_3P(HgCl)_2$ by boiling in bromine-water. The supernatant was isolated and used for paper chromatography together with ortho-phosphate, phosphite, hypophosphite and methylphosphonic acid standards. The paper chromatogram was developed in 1-butanol saturated with 2N HNO_3 (Robinson and Bond, 1970), dried in air, sprayed with Harrap's reagent (Harrap, 1960) and visualised under UV light to record *Rf* values for each compound.

Treatments of insects with methylphosphine gas were carried out in gastight 6.25-L desiccators, each fitted with a rubber septum. In all the tests, 50 or more adult insects aged 4 to 8 weeks were used. Each treatment with methylphosphine was replicated at least twice. Appropriate control insects were also sealed in similar desiccators to estimate mortality in untreated insects during the exposure period. After exposing insects to the fumigant for 24 h, the desiccators were opened and aired for 15–20 min. Both the untreated control insects and the treated insects were transferred to labelled glass jars containing appropriate food and kept there for 1 week before estimating mortalities.

RESULTS AND DISCUSSION

Initial tests to evaluate the efficacy of methylphosphine against insects were carried out before a suitable analytical method for methylphosphine was available. In these tests, a calculated amount of methylphosphine (based on theoretical yield) was applied to produce a nominal concentration of 0.2 mg/L in the desiccators. Subsequent information indicated that the actual concentration was, however, much lower than 0.2 mg/L. The results of these tests (shown in Fig. 1 and Fig. 2) should, therefore, be seen only as a preliminary assessment of the efficacy of methylphosphine against PH_3-resistant and PH_3-susceptible insects. The tests nevertheless indicated that methylphosphine had a much greater toxic effect on PH_3-resistant insects than it did on PH_3-susceptible insects of all four species of beetles tested over treatment periods of 24 h and 48 h.

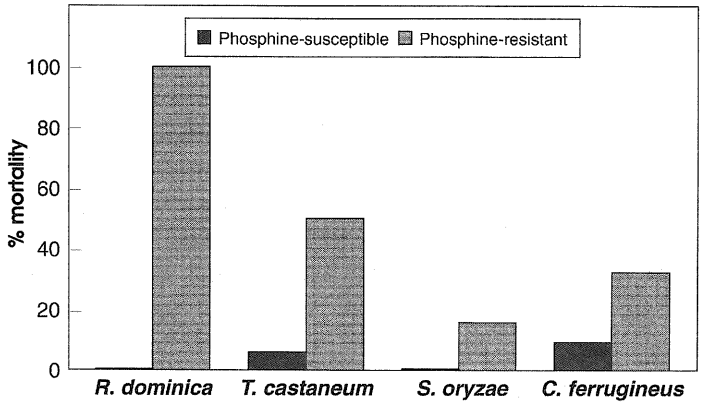


Fig. 1. Percentage of insect mortality after a 24-h exposure to a nominal concentration of 0.2 mg/L of methylphosphine (Chaudhry *et al.*, 1995).

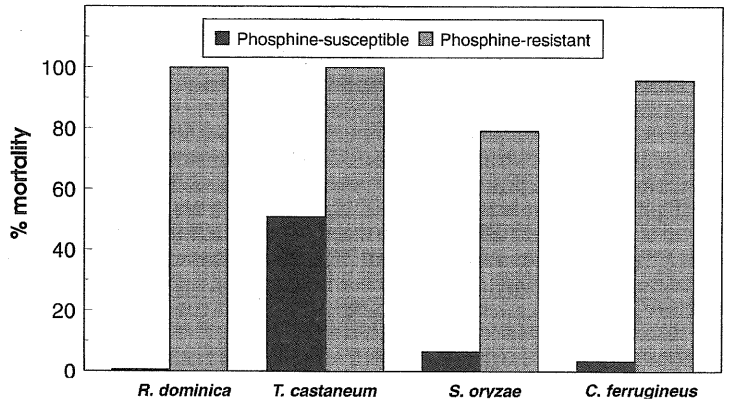


Fig. 2. Percentage of insect mortality after a 48-h exposure to a nominal concentration of 0.2 mg/L of methylphosphine (Chaudhry *et al.*, 1995).

This led us to carry out further toxicity tests, using different doses, to characterise dose-response relationships using methylphosphine on several species of stored-product insects. The results shown in Table 1 are, in some cases, preliminary, and further results are needed to obtain more reliable data. However, these results confirmed that methylphosphine is more toxic to resistant strains than to the corresponding susceptible insects in all the beetle species tested.

In the case of *R. dominica*, a comparison of LC_{50} values indicates that almost 4–8 times more methylphosphine was needed for PH_3-susceptible insects than for the two PH_3-resistant strains. It is also interesting to note that, in terms of molarity, the concentration of methylphosphine that produced 99.9% mortality of PH_3-resistant insects is comparable to the discriminating dose of PH_3 required to kill all PH_3-susceptible insects (Table 1).

Similar effects of methylphosphine on PH_3-resistant and PH_3-susceptible insects of *S. oryzae* were observed. Whereas a 24-h exposure to 0.0162 mg/L of methylphosphine would have killed 50% of the PH_3-resistant (476s) strain, a concentration more than seven times higher (0.1196 mg/L) was required to kill 50% of the PH_3-susceptible insects. A similar difference in the toxicity of methylphosphine to PH_3-resistant and PH_3-susceptible insects was observed at the $LC_{99.9}$ level. The concentration of methylphosphine which produced 99.9% mortality in PH_3-resistant insects is comparable to the discriminating concentration of PH_3 which kills all susceptible insects of this species (Table 1).

TABLE 1

Toxicity of methylphosphine (mg/L) to adults of phosphine-resistant and phosphine-susceptible strains of four species of stored-product beetles over a 24-h exposure at 25°C

Species/strain	LC_{50}	95% FL	$LC_{99.9}$	95% FL	Slope	p-value
Rhyzopertha dominica						
Susceptible	0.1016				*	0.0105
306-sel	0.0129	0.0118–0.0140	0.0468	0.0389–0.0615	5.54	0.9705
BR2	0.0229	0.0218–0.0242	0.0473	0.0417–0.0561	9.83	0.4276
Sitophilus oryzae						
Susceptible	0.1196	0.1084–0.1287	0.4607	0.3734–0.6393	5.28	0.1048
476s	0.0162	0.0147–0.0174	0.0590	0.0469–0.0857	5.51	0.4805
Tribolium castaneum						
Susceptible	0.0113	0.0017–0.0167	0.0860	0.0538–0.8594	3.50	0.9020
BT1vs	0.0168	0.0063–0.0215	0.0497	0.0303–0.0440	6.55	0.0050
Cryptolestes ferrugineus						
Susceptible	0.0747				*	0.8931
BC12s	0.0348	0.0277–0.0405	0.1102	0.0795–0.2427	6.17	<0.001

FL = fiducial limits; * = insignificant slope.

Discriminating dose of phosphine (mg/L, 20-h exposure): *R. dominica* = 0.03 (Anon., 1975); *T. castaneum* = 0.04 (Anon., 1975); *S. oryzae* = 0.04 (Anon., 1975); *C. ferrugineus* = 0.06 (Mills, 1983).

The dose-response line for the PH_3-resistant (BT1vs) strain of *T. castaneum* was not statistically significant, and the LC_{50} values indicate that a similar concentration of methylphosphine is required to kill PH_3-resistant and PH_3-susceptible insects. At the $LC_{99.9}$ level, however, about twice the concentration of methylphosphine was required to kill PH_3-susceptible insects than that required for the PH_3-resistant ones. Also, as with *R. dominica* and *S. oryzae*, the $LC_{99.9}$ for the BT1vs strain was comparable to the discriminating dose of PH_3 which kills all PH_3-susceptible insects of this species.

The PH_3-resistant and PH_3-susceptible strains of *C. ferrugineus* also responded similarly to methylphosphine treatment. At the LC_{50} level, almost twice the concentration of methylphosphine was required to kill PH_3-susceptible insects than that for PH_3-resistant insects. Because of the insignificant slope of the probit line for susceptible insects, a comparison of $LC_{99.9}$ values was not possible. However, on a molar basis, the $LC_{99.9}$ of methylphosphine for the PH_3-resistant (BC12s) strain was very close to the discriminating dose of PH_3 for this species.

Some physical and chemical properties of methylphosphine are presented in Table 2. As with PH_3, environmental breakdown of methylphosphine is expected to produce non-toxic products. The *Rf* value of the only visible oxidation product of methylphosphine (*Rf* = 0.73) on paper chromatograms was similar to that for methylphosphonic acid. The *Rf* values recorded for ortho-phosphate, phosphite and hypophosphite were 0.58, 0.75 and 0.70, respectively. This indicates that the main oxidation product of methylphosphine appears to be methylphosphonate which is non-toxic. However, the oxidation of the methylphosphine-$HgCl_2$ complex was more difficult than that of PH_3, requiring extensive boiling in bromine-water. In conjunction with observations on stability in air, this indicates that oxidative breakdown of methylphosphine may be a slow process.

In these preliminary studies there were indications that the concentration of methylphosphine in the test desiccators fell during the exposure period. This drop in concentration was considerable at low levels, and the cause of this phenomenon, which might be sorption into the nylon material used to retain insects in glass-dishes, is currently being investigated.

Although more tests are needed to improve the reliability of LC_{50} and $LC_{99.9}$ values, the results presented here clearly indicate that this newly discovered fumigant has greater toxic effects on PH_3-resistant insects than on PH_3-susceptible ones. The results also indicate that control of PH_3-resistant insects could be achieved by application of doses of methylphosphine that are comparable to the discriminating doses of PH_3 that kill PH_3-susceptible insects. A similar trend in the strains of all four insect species tested is consistent with earlier findings which indicate that there is a common mechanism of PH_3-resistance in insects. It is possible that the presence of the methyl group in the CH_3PH_2 molecule prevents exclusion by the resistance mechanism. The much greater mortality of PH_3-resistant insects further indicates that oxidative breakdown of methylphosphine by the PH_3-detoxification mechanism produces reactive products in insect tissues. Our earlier work with two other analogues of PH_3, arsine (AsH_3) and stibine

TABLE 2
Some physical and chemical properties of methylphosphine

Chemical formula	CH_3PH_2
Molecular weight	48
Physical state at STP	Colourless gas with garlic-like odour
Boiling temperature	–14°C (Kosolapoff and Maier, 1972)
Vapour pressure	28.1 mm Hg at –78.5°C, 72.5 mm Hg at –63.5°C (Crosbie and Sheldrick, 1969)
Flammability	Likely to be flammable
Method of detection	GC/chemical analysis. Concentration in air can be monitored by PH_3/AsH_3 detector tubes
Toxicity	Classified as very toxic although no data is available in the literature (Van Wazer, 1958). Toxic to insects
Mode of toxic action	Not known. Likely to act on mitochondrial respiratory chain in insects
Solubility in water	Not known
Stability in air	Not known. Appears to be reasonably stable in initial tests
Stability when mixed with foodstuffs	Not known
Breakdown products	Main oxidation product may be methylphosphonic acid
Residues in treated grain	Not known. Possibly methylphosphonate and other oxidation products (phosphite, phosphate, etc.)
Toxicity of residues	Not known. Oxidation products are likely to be non-toxic
Method of synthesis	By reaction of $LiAlH_4$ with dimethylmethylphosphonate (Crosbie and Sheldrick, 1969)
Likely formulation	Either as compressed gas mixture with an inert gas in cylinders or in solid formulation as HCl salt to release the gas *in situ* on reaction with H_2O (Groenweghe, 1965)
Corrosion of metals	Not known. Oxidation products may be corrosive to metals

(SbH_3), also showed that they had more effect on PH_3-resistant insects than on PH_3-susceptible ones (Chaudhry and Price, 1991). This was also probably due to the conversion of arsine and stibine into toxic metabolites by the PH_3-detoxification process which converts PH_3 to non-toxic products in resistant insects.

The more effective insecticidal action of methylphosphine on PH_3-resistant insects, therefore, appears to be due to a chemical structure which, avoiding the mechanism of active exclusion in resistant insects, is instead activated by the PH_3-detoxification mechanism. Methylphosphine could therefore be used to selectively control PH_3-resistant

insects, although either higher doses of this gas or longer exposure periods, or a combination of both, can be utilised to kill susceptible as well as resistant insects. The gaseous nature of methylphosphine means that its use as a fumigant, alone or in combination with PH_3, would be possible. Our preliminary tests also showed that the addition of 0.05 mg/L of PH_3 to methylphosphine at a nominal concentration of 0.2 mg/L for a 24-h exposure period killed all susceptible and resistant insects of *R. dominica* and *T. castaneum* species (data not shown). More work is needed to establish whether the toxic action of these two gases is cumulative or synergistic.

The discovery of the fumigant potential of methylphosphine and its greater efficacy against PH_3-resistant insects presents an extraordinary opportunity to use this gas in the management of PH_3-resistance. The fumigant use of methylphosphine, alone or in combination with PH_3, is expected to exert a negative selection pressure on PH_3-resistance genes, thus preventing further selection of resistance. This will enhance the useful life of PH_3 as a fumigant. Its effectiveness would ensure that any possible development of methylphosphine resistance in insects could be managed by alternating the use of these two gases.

The alkylphosphines' property of forming volatile phosphonium-halide salts has been reported (Groenweghe, 1965), and this represents a very useful way of generating methylphosphine gas by exposing a solid formulation to moist air. Unlike the residues left after decomposition of aluminium phosphide to generate PH_3 gas, those left after the generation of methylphosphine from phosphonium-halide salt may be acidic. This problem could, however, be overcome by using specially designed containers for the solid preparation which can be collected after the fumigation operation. Alternatively, appropriate solid formulations could contain other ingredients (such as carbonate salts) which would simultaneously neutralise the acid produced and generate carbon-dioxide gas, minimising the potential hazard of flammability associated with pure methylphosphine.

The results presented here have shown that methylphosphine has considerable potential as a new fumigant for the protection of stored products, especially as a complement to the use of PH_3 (threatened by the development of resistance). However, further studies will be needed to investigate such various aspects of the use of methylphosphine as the extent and nature of resulting residues, stability at higher relative humidity and other properties which might influence its use as a fumigant.

ACKNOWLEDGEMENTS

We gratefully acknowledge funding from the Chief Scientist's Group of the Ministry of Agriculture, Fisheries and Food, UK. The authors are also grateful to Mr Tim Wonter-Smith and Mr Simon Cardwell for their help in the analysis of methylphosphine.

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EFFECTS OF CARBONYL SULPHIDE ON *SITOPHILUS GRANARIUS* (L.) (COLEOPTERA: CURCULIONIDAE), *FUSARIUM CULMORUM* AND *FUSARIUM AVENACEUM* (SACC.) (DEUTEROMYCOTINA: HYPHOMYCETES), AND CORROSION ON COPPER

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ABSTRACT

All life stages of *Sitophilus granarius* (L.) were tested for their susceptibility to different concentrations and exposure times of carbonyl sulphide (COS) at 20°C and 70% r.h. Complete kill of *S. granarius* occurred at concentrations of 18 g m^{-3} COS for 120 h or 32 g m^{-3} COS for 72 h. The eggs were most tolerant to the toxic gas, followed by pupae and adults. Larval stages were most susceptible to the fumigant. Sublethal dosages prolonged the developmental periods of the immature life stages. Lethal dosages on *S. granarius* also caused growth inhibition in *Fusarium culmorum* and *F. avenaceum*, though the fungi recovered fully after treatment. In the presence of high relative humidity, COS is degraded partly to H_2S, which causes corrosion on copper. Carbonyl sulphide is discussed as being a possible alternative to methyl bromide.

INTRODUCTION

The treatment of stored products or storage facilities with fumigants is a common and effective method to control stored-product pests. In comparison with contact insecticides applied either as dusts, wettable powders or emulsifiable concentrates, fumigants have the advantage of penetrating the treated commodities completely. This becomes extremely important when grain, legumes or nuts are infested internally (Reichmuth, 1990). In general, fumigations leave either very low residues or none at all in the treated products and are also very time-effective. Therefore, fumigants impact heavily on import and export quarantine procedures. Nevertheless, the hazardous properties of fumigants, their carcino-

genic potential and concerns about worker safety, as well as environmental threats, have led in recent years to the recession of licensed and registered formulations. Because methyl bromide (MB) potentially contributes to the depletion of the ozone layer, its use will be heavily regulated even though it is one of the most commonly applied fumigants worldwide. Although the actual impact of anthropogenic produced MB is still vigorously debated (Detmers, 1993), in the near future its use will be restricted, and eventually it will be banned (Anon., 1994a). Either existing fumigation techniques must be modified in order to reduce emission of fumigants into the environment after aerating a treated facility (Reichmuth, 1990; 1993; Schreiner, 1993), or new alternative active agents and techniques for their safe application must be developed.

Carbonyl sulphide (COS) is regarded as one future alternative to MB (Catley, 1993). The Commonwealth Scientific and Industrial Research Organization in Canberra, Australia, has already filed a worldwide patent covering the use of COS as a fumigant in pest control (CSIRO, 1993). Although COS is not a novel chemical, its additional uses as a fumigant to protect stored products such as grain, in treating empty storage facilities, for soil fumigation and as a quarantine fumigant must be regarded as new fields of application (Anon., 1994b). COS showed toxic effects on such pest insects as stored-product Coleoptera and Lepidoptera, as well as on aphids, fruitflies and termites. It has been shown to be lethal to mites, nematodes and fungi. No residues have been recorded on wheat, rice or barley after treatment with COS. Nor does COS negatively affect germination (Desmarchelier, 1994a). Possible control of such rodents as rats and mice with COS has also been mentioned (Falbe and Reglitz, 1989).

COS appears naturally in the atmosphere at a concentration of approximately 1.5 $\mu g\ m^{-3}$, and it is the most common form of sulphur in the stratosphere. It is emitted from soil, marshes, manures, compost and most combustible products (Catley, 1993; Desmarchelier, 1994a). Furthermore, COS is found in a variety of industrial and natural gases (Ferm, 1957). The physical and chemical properties of COS have been described in detail by Stock and Kuss (1917), as well as by Ferm (1957) and Hommel (1993). It boils at –50.2°C and melts at –138°C. The vapor pressure is 1.1 MPa (11 bar) at 20°C. The specific weight is 124 kg m^{-3}, and the molecular weight is 60.07. Purified COS is a colorless, odorless and tasteless gas. It is highly flammable, burning with a slightly luminous blue flame ($2COS + 3O_2 \rightarrow 2SO_2 + 2CO_2$). In the absence of humidity, COS is a very stable compound. Thermal decomposition occurs at either 600°C ($2COS \rightarrow CO_2 + CS_2$) or 900°C ($COS \rightarrow CO + S$). With water or in the presence of water vapor, COS slowly reacts to form carbon dioxide and hydrogen sulphide ($COS + H_2O \rightarrow CO_2 + H_2S$).

In its pure form, COS does not corrode polished copper. However, contamination by as little as 1 ppm of elemental sulphur or hydrogen sulphide causes it to discolor copper. Information about its action on other metals is not available, but it is said to be corrosive toward concrete. The molecular structure of COS has been a matter of controversy. Nonlinear and linear configurations have been proposed, with the latter being favored. Intramolecular bonds vary between three possible structures: O=C=S; $^{+}O{\equiv}CS^{-}$; and $^{-}O{\equiv}CS^{+}$. The third structure, containing the triple carbon–sulphur bond, is the least impor-

tant; the first two structures predominate. The best method for making COS in the laboratory is the hydrolysis of metallic thiocyanates with mineral acids ($KCNS + 2H_2SO_4 + H_2O \rightarrow COS + KHSO_4 + NH_4HSO_4$).

Lethal effects of COS on insects and mites, including stored-product pests, have been reported by Desmarchelier (1994b). The most important results are those on *Rhyzopertha dominica* (the lesser grain borer) and *Sitophilus oryzae* (the rice weevil) because the immature life stages of these insects develop inside grain kernels. At 25°C, COS concentrations of 20 mg L^{-1} for an exposure time of 168 h, a concentration of 30 mg L^{-1} for 72 h and a concentration of 40 mg L^{-1} for 48 h all resulted in a complete kill of all the rice weevil's life stages. Damage caused by *R. dominica* can be ignored in temperate climatic conditions where it is negligeable (Chittenden, 1911; Weidner, 1983); although the occurrence of *S. oryzae* is increasing (Reuter and Bahr, 1988; Hallas, 1992; Stengård Hansen, 1994), economically the most important pest of stored grain in Central Europe is still the granary weevil *S. granarius*. The effect of COS on this weevil has not been investigated.

In a stationary fumigation chamber located at the Institute for Stored-Product Protection of the Federal Biological Research Centre for Agriculture and Forestry in Berlin, seven developmental stages including eggs, larvae, pupae and adult weevils of *S. granarius* were tested for their susceptibility to different concentrations and exposure times of COS at 20°C. Also of interest were the potential fungicidal and growth inhibition fungistatic effects of COS on mycotoxin-producing storage fungi. Short exposure times with high gas concentrations and long exposure times with low gas concentrations, both at dosages lethal to the granary weevil, were tested for their effects on two *Fusarium* species, *F. culmorum* and *F. avenaceum*. The possible corrosion effects of COS on copper, under European climatic conditions with high relative humidities, were also investigated.

MATERIALS AND METHODS

To obtain the necessary developmental stages of *S. granarius*, a quantity of 2.0 cm^3 of adult weevils were transferred weekly on to 600 cm^3 wheat substrate at 25°C and 70% relative humidity (r.h.). Adult weevils were sifted out of the culture after 3–4 d. Cultures 1–3 d old contained eggs; cultures aged 1 week, 2 weeks, 3 weeks and 4 weeks contained first, second, third and fourth instar larvae, respectively. Pupae (just prior to adult emergence) were expected after 5 weeks. Mesh-wire 8.8-cm^3 tubes were filled with approximately 3 g wheat kernels infested with the designated life stages. Fifty adult weevils, together with approximately 3 g uninfested wheat kernels, were caged in the same way. Seven tubes, one for each developmental stage, were stacked in perforated metal probes which could be inserted into a stationary airtight fumigation chamber of approximately 0.5 m^3 (Reichmuth, 1981).

The fumigant COS with a quality of N17 (97%) was procured from Air-Liquide, Branch Alphagaz.

The COS from a filled gas sampling tube was released into the fumigation chamber by a pump-driven circulation system. To obtain effective gas concentrations, the evacuated

gas-sampling tube was filled with a precalculated weight of COS using a pressure system of approximately 200 kPa (2 bar). The actual effective gas concentration inside the fumigation system was measured by a Miniature Infra Red Analyzer (MIRAN) [1A General Purpose Gas Analyzer, Foxboro Analytical (WILKS)] through absorption of COS at 4850 nm. For this purpose three 5.0-ml samples were taken with a gas syringe from the fumigation volume and then injected into the MIRAN analyzer (see Table 1). After the designated exposure times (see Table 1), two metal probes, each containing all seven life stages of *S. granarius*, were taken out of the system without opening the chamber and/or disturbing the effective gas concentration.

TABLE 1
Effective COS-concentrations and exposure times for treatment of *Sitophilus granarius*

COS concentration ($g\ m^{-3}$)	Exposure times (h)
15.12 ± 0.47	12/24/48/72
18.11 ± 0.41	120
18.27 ± 0.59	18/24/48/72
18.93 ± 0.64	96
28.39 ± 0.34	15/24/48/72
32.31 ± 1.08	19/24/48/72

All treatments were carried out at a temperature of 20 ± 1°C and a r.h. of 70 ± 10%. After the probes were taken from the fumigation chamber and the mesh-wire tubes emptied, the infested wheat samples were kept in an incubator for another 8 weeks at 25°C and 70% r.h. An equal number of infested samples was prepared in the same manner and held for the same times at 20°C and 70% r.h., but not exposed to COS. These untreated control samples were also transferred to the incubator as described. The number of emerging adult weevils was recorded weekly.

Mortality resulting from COS was calculated by correcting the observed mortality in the treated samples by the naturally occurring mortality in the untreated control samples (Abbott, 1925). Mortality of treated adult weevils was recorded immediately after exposure and again 48 h later. Uninfested wheat kernels were exposed together with adult weevils during fumigation, and they were incubated as described in order to check both for possible egg deposition during treatment and for the possible hatch of an F_1-generation.

The possible corrosion effect on copper caused by COS degradation under high relative humidities was tested on polished copper pennies which were exposed to the gas during fumigation. Changes in weight before and after exposure, together with discolorations, were compared to those in untreated pennies.

The *Fusarium* species *F. culmorum* and *F. avenaceum* were exposed to COS in 6300-ml desiccators. Strains No. 65219 and 64218 of *F. culmorum* and No. 64211 and

64854 of *F. avenaceum* were obtained from the Institute for Microbiology of the Federal Biological Research Centre for Agriculture and Forestry in Berlin. Each strain was cultured on a Bacto Potato Dextrose Agar (PDA from DIFCO) and on a Synthetic low Nutrition Agar (SNA) (Table 2).

TABLE 2
Nutritional compositions of PDA and SNA

PDA	Potato extract from 200 g; 20 g Bacto Dextrose; 15 g Bacto Agar in 1000 ml H_2O
SNA*	1 g KH_2PO_4; 1 g KNO_3; 0.5 g $MgSO_4 \times 7H_2O$; 0.6 ml NaOH; 0.2 g glucose; 0.2 g saccharose; 22 g agar-agar in 1000 ml H_2O

*Developed in the Institute for Microbiology of the Federal Biological Research Centre for Agriculture and Forestry.

The fungal cultures were transferred into the desiccators 2 d after an initial growth period and exposed at 20°C and 70% r.h. to COS concentrations of either 30 g m^{-3} for 72 h or 20 g m^{-3} for 120 h. These COS concentrations and exposure times were those necessary for complete kill of *S. granarius* as ascertained earlier. The actual effective gas concentrations in the desiccators were again measured in the MIRAN, as described above (Table 3).

TABLE 3
Effective COS-concentrations and exposure times for treatment of *Fusarium* strains

Species	Strain	Medium	COS (g m^{-3})	Exposure time (h)
F. avenaceum	64211; 64854	PDA; SNA	20.64 ± 1.78	120
F. culmorum	65219; 64218	PDA; SNA	23.28 ± 0.17	120
F. avenaceum	64211; 64854	PDA; SNA	30.06 ± 1.77	72
F. culmorum	65219; 64218	PDA; SNA	26.97 ± 3.73	72

The areas covered by fungal mycelia on the respective media were recorded prior to the fumigation (0 h), directly after the fumigation (72 or 120 h) and again after an additional incubation period (240 h) at 20°C and 70% r.h. Possible fungicidal or growth inhibition effects of COS could be determined by comparison to *Fusarium* cultures identically cultivated without any gas exposure.

RESULTS

The mean number (with standard deviation) of emerging weevils and the survival of adults in the untreated control samples are shown in Table 4. Because 3 g of wheat infested with the designated developmental stages were used for experiments, the increasing number of emerging weevils from egg to pupal stage was easy to follow. The older the immature

TABLE 4
Mean emergence and survival with standard deviation of granary weevils in untreated control samples

Developmental stage	Number of weevils (mean ± SD)
Eggs	43.0 ± 10.6
Larvae 1	46.5 ± 6.3
Larvae 2	47.5 ± 7.3
Larvae 3	54.1 ± 7.8
Larvae 4	63.1 ± 8.5
Pupae	74.9 ± 5.3
Adults	49.8 ± 0.3
Eggs*	53.7 ± 7.5

*Deposited during fumigation.

instar, the more kernel substance material metabolized by the insect and the greater resulting weight loss of the grain. Therefore, the larger number of infested kernels compensated for the weight loss per kernel with increasing larval age.

The effects of the four different COS concentrations for different exposure times are plotted in Fig. 1, in which given mortality is corrected with the natural mortality of the untreated control samples. The first to third instar larvae were most susceptible to COS. A mortality of 90% occurred at a concentration of ca. 15 g m^{-3} for an exposure time of 72 h. COS-concentrations of ca. 18 and 28 g m^{-3} for 48 h caused mortality of over 95 and 100%, respectively. At ca. 32 g m^{-3} COS, a 95% kill occurred after 19 h of exposure.

The stage most tolerant to COS was the egg of *S. granarius*, oviposited into the kernel prior to fumigation. Low COS concentrations of approximately 18 g m^{-3} required exposure of at least 120 h for a complete kill. For lower exposure times, the COS-concentration required to achieve 100% mortality after 72 h had to be over 32 g m^{-3}. Eggs were more susceptible when they were laid during the exposure to the fumigant. This was determined by incubating the originally uninfested wheat kernels placed together with adult weevils during the exposure to COS. In these cases ca. 18 g m^{-3} for 72 h, and over 32 g m^{-3} for 19 h, were sufficient to achieve 95% mortality.

Adult weevils were completely controlled at concentrations of ca. 18 g m^{-3} for 72 h or 28 g m^{-3} for 48 h. Over 95% mortality was achieved at a concentration of ca. 15 g m^{-3} for 72 h.

Fourth instar larvae and pupae of the granary weevil were more tolerant to the tested COS concentrations and exposure times than were the adults. The fourth instar larvae seemed to be slightly more susceptible to lower COS concentrations than were the pupae. A 95% kill of fourth instar larvae occurred at ca. 18 g m^{-3} COS for 72 h, and an exposure time of 96 h resulted in 100% mortality. A complete kill of pupae required at least 120 h of exposure at this COS concentration. Above a concentration of ca. 28 g m^{-3}, differences in mortality between fourth instar larvae and pupae disappeared.

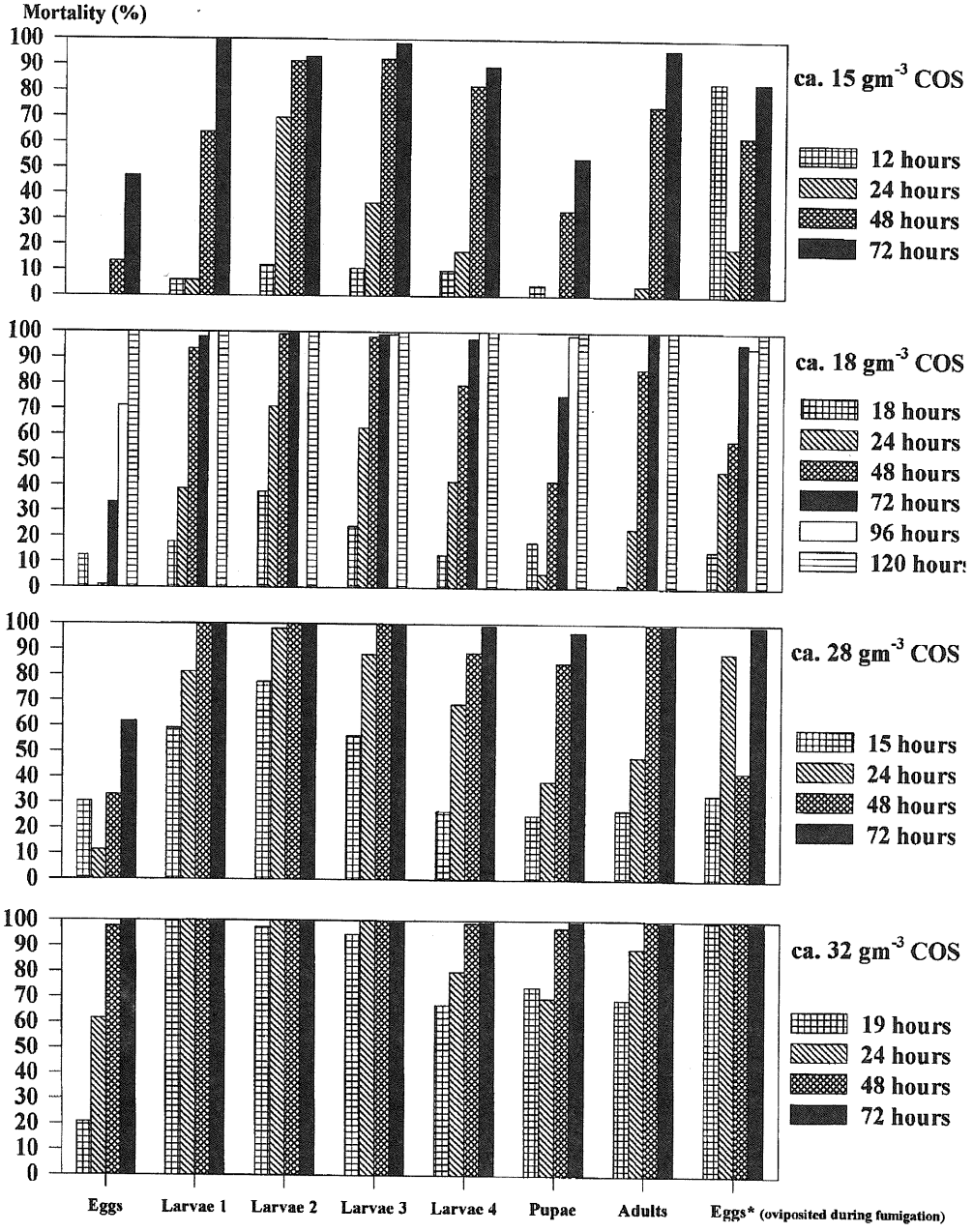


Fig. 1. Effects of four concentrations of carbonyl sulphide on life stages of the granary weevil *Sitophilus granarius* at different exposure times at 20°C and 70% r.h.

The following series shows the increasing susceptibility to COS: egg → pupae → 4th instar larvae → adult → 2nd, 1st and 3rd instar larvae.

Table 5 shows the necessary exposure times required to achieve LT_{50}, LT_{95}, LT_{99} and $LT_{99.9}$ for the corresponding COS concentrations, as calculated using the computer software "Table Curve".

Sublethal concentrations and exposure times of the fumigant prolonged the developmental periods in the juvenile stages of *S. granarius*. Onset and duration of weevil hatch from kernels infested with the designated stage and exposed to the fumigant were compared to those of untreated control samples. In all cases, especially after long exposure times but also at the higher concentrations, the onset of adult emergence was delayed. The prehatching duration of eggs increased with increasing sublethal dosages. The pupal stage was least affected; prolongation of development was most marked in the larval stages.

No fungicidal effect of COS was observed at concentrations and exposure times which were lethal to all stages of the granary weevil. Growth of both *Fusarium* species on both media was inhibited during exposure to COS. The higher concentrations of approximately 27 g m^{-3} or 30 g m^{-3} for a shorter exposure time of 72 h suppressed mycelial growth more than did the lower concentrations of ca. 23 g m^{-3} or 21 g m^{-3} for a longer exposure time of 120 h (Fig. 2). Additionally, a discoloration of the mycelia (to a whitish yellow) was

TABLE 5

Calculated exposure times in hours to obtain LT_{50}, LT_{95}, LT_{99}, and $LT_{99.9}$ at COS-concentrations of ca. 15, 18, 28 and 32 g m^{-3} for different developmental stages of the granary weevil

COS (g m^{-3})	LT	Eggs	L1	L2	L3	L4	Pupae	Adults	Eggs*
15	LT_{50}		44.14	20.19	28.02	34.86	54.91	53.44	40.94
15	LT_{95}		62.42		51.49			63.38	
15	LT_{99}		68.80						
15	$LT_{99.9}$		71.56						
18	LT_{50}	79.84	27.44	20.07	22.03	30.99	51.76	34.02	32.81
18	LT_{95}	116.55	48.84	35.65	40.85	65.08	95.93	55.63	89.69
18	LT_{99}	119.11	73.25	48.00	50.44	86.63	105.19	68.44	107.52
18	$LT_{99.9}$	119.68		64.88			108.01	84.92	114.57
28	LT_{50}	62.45	12.44	10.03	14.21	17.26	28.14	23.90	17.22
28	LT_{95}		36.24	21.27	30.65	59.87	64.36	44.90	27.24
28	LT_{99}		47.43	26.07	40.36	76.08		50.70	39.29
28	$LT_{99.9}$		53.88	31.48	46.06			52.94	
32	LT_{50}	21.65		02.84	03.59	09.87	19.42	13.32	
32	LT_{95}	43.45		12.19	15.29	39.86	40.27	32.78	
32	LT_{99}	48.38		18.49	22.67	54.92	56.05	48.75	
32	$LT_{99.9}$	50.04		25.75	29.11	62.96	69.25	56.64	

*Deposited during fumigation. L1, L2, L3 and L4 = first, second, third and fourth instar larvae.

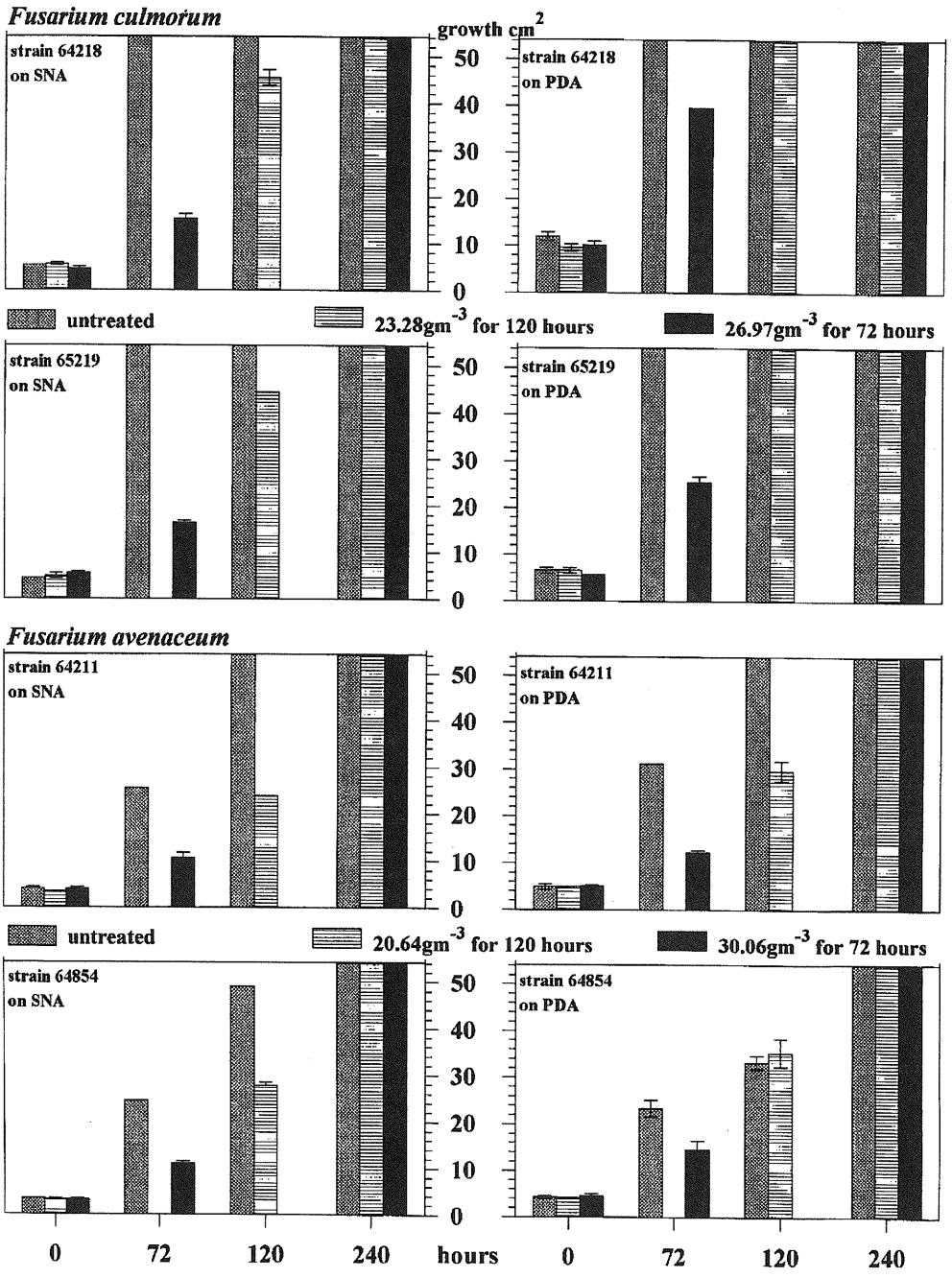


Fig. 2. Growth inhibition by carbonyl sulphide on *Fusarium culmorum* and *F. avenaceum.*

observed. The untreated control cultures did not show a change in color. An analysis of the treated and untreated culture media for changes in the pH-value and sulphide concentration showed no changes. After COS treatment the fungi recovered fully and, as with the untreated control samples, the growth pattern reached the boundaries of the petri dishes within a few days. The discoloration was retained.

At high r.h., COS is partly degraded to hydrogen sulphide, which causes corrosion on copper. This was indicated by the discoloration of copper pennies (which altered to a bluish color) and by a gain in weight of the magnitude of 10^{-4} g.

DISCUSSION

The possibility of controlling the granary weevil, including all of its immature stages, with atmospheres enriched with COS was demonstrated. Depending on the COS concentration, at 20°C exposure times of 120 h at ca. 18 g m^{-3} or 72 h at over 32 g m^{-3} were necessary to achieve complete kill of *S. granarius*. Comparable COS concentrations of 30 g m^{-3} or 20 g m^{-3}, for similar exposure times of 72 or 168 h, respectively, were needed to control the rice weevil *S. oryzae*, including all its immature stages (Desmarchelier, 1994b). Because these findings were recorded at 25°C, the granary weevil might be regarded as more susceptible to COS than the rice weevil. This remains to be verified. It can be assumed, though, that insect metabolism increases with a rise in temperature which results in a higher respiratory activity and a faster intake of the toxic gas.

The egg stage of *S. granarius* was most tolerant to COS when eggs were oviposited prior to treatment. Eggs deposited into grain kernels while females were exposed to the fumigant were far more susceptible. Several explanations are possible. During oviposition, or while still in the female's body, the egg could be more exposed to the toxic fumigant than when already inside the grain kernel. The sealed kernel might provide excellent protection for the immobile egg of relatively low metabolic activity. As long as the egg-laying channel has not been sealed by the female, direct contact between COS and the egg is a possibility, and this could also result in a higher mortality.

The other relatively immobile stage of the granary weevil, the pupa, not only showed a high level of tolerance to COS but its development time was also least affected by sublethal dosages. The tolerance of the pupal stage of *S. granarius* for other toxic fumigants, such as MB, phosphine (PH_3) and nitrogen-enriched atmospheres, has also been reported (Howe and Hole, 1966; Howe, 1973; Adler, 1992, respectively). The other immature stages of the granary weevil were more or less affected by sublethal dosages of COS, and their developmental times were prolonged. This might be a direct effect of the fumigant, but it is also possible that the larval stages actively reduced respiration to minimize exposure to the toxic gas. This is likely to be correlated with less metabolic activity and longer growth periods.

When fumigating with MB, where acting concentrations of the fumigant and exposure times are equally important to determine a certain lethal dosage, the product of concentration and exposure time (Ct-product) is constant. Applying the formula $c^n \times t = k$ (Zettler,

1993, according to Haber, 1924, and Winks, 1984) with c = concentration, t = exposure time, k = constant and $n < 1$, and using the calculated LT-values from Table 5, it can be shown that for COS the Ct-product is not constant. When varying the exposure time, the concentration necessary to achieve a certain lethal dosage needs to increase or decrease in proportion. Extending the exposure time results in a reduction of the necessary concentration, in multiples of the time, for a certain lethal dosage. In this regard COS acts very similarly to PH_3. This may have economic implications, such as in flour mill fumigation where the application of higher gas concentrations and shorter exposure times is desirable since a shut-down of the facility would result in greater financial losses than would the additional expense for the fumigant. Increasing gas concentration also carries the risk of higher gas losses when the treated commodities are improperly sealed. Applying sublethal dosages causes a delay in development, but it does not sufficiently control the very tolerant egg stage, and it also has potential risks. In order to avoid the propagation of tolerant strains, a complete control of the pest must be obtained.

F. culmorum and *F. avenaceum* are known as potential producers of mycotoxins on wheat in moderate climate zones (Miller, 1995). The application of COS concentrations and exposure times lethal to the granary weevil resulted in only temporary growth inhibition of the fungi. Similar findings have been reported for *Aspergillus flavus*, *A. parasiticum* and *Eurotium chevalieri* when treated with insecticidal dosages of PH_3 (Hocking and Banks, 1991). The discoloration of the mycelia can be regarded as a direct reaction of the fungi to the presence of the fumigant. An increase in sulphurous compounds or a change in pH-values of the treated culture media, which could also have been responsible for discoloration, were not detected. Exposure of fungi to such stress factors as fumigation can result in an increase of toxin formation. Unfortunately, analyses of toxin compounds and amounts were not possible. Nevertheless, fungal growth can be prevented by controlling insect pests which tend to form hotspots where higher temperatures and relative humidities favor fungi; dryer and colder grain storage also helps to prevent fungal growth.

Degradation of COS, already under high relative humidity, to hydrogen sulphide might cause severe problems of corrosion. This is of major concern. Because of it, COS does not appear to be a practical substitute for MB in moderate or temperate climatic regions such as Central Europe. In Australia, where the general climate is less humid and this new fumigant is highly regarded, COS might have a potential in pest control.

ACKNOWLEDGEMENTS

This research was supported through a research grant from the Federal Biological Research Centre for Agriculture and Forestry. The authors would like to thank Mrs Karacoglu and Mr Schmidt of the Institute for Stored-Product Protection for providing test insects and for excellent chemical analyses. We would also like to thank Dr Nirenberg of the Institute for Microbiology for providing *Fusarium* cultures and for very helpful suggestions and discussions. Carbonyl sulphide was obtained with the kind financial support of Binker

Company. We also thank W.E. Burkholder, J.K. Phillips and J.L. Zettler for carefully reviewing the manuscript.

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THE EFFECT OF MODIFIED ATMOSPHERES ON THE JUVENILE STAGES OF SIX GRAIN BEETLES

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ABSTRACT

The adult emergence of six species of grain beetle, *Cryptolestes ferrugineus*, *Oryzaephilus surinamensis*, *Rhyzopertha dominica*, *Sitophilus granarius*, *S. oryzae* and *Tribolium castaneum*, was assessed after the exposure of the juvenile stages to three different modified atmospheres (MA's) at 15°C and 70% r.h. The use of this low temperature was important as it is typical of the conditions prevalent in grain stores at the start of the UK storage season.

A range of juvenile stages was exposed to three MA's, one based on nitrogen (N_2), one on simulated burner gas and one on carbon dioxide (CO_2), with 0.5% oxygen. The three internal grain feeders, *R. dominica*, *S. granarius* and *S. oryzae*, were capable of emerging even after 30 d exposure to CO_2, the most effective of the MA's. N_2 was the least effective of the MA's. *S. granarius*, the most tolerant species, required a 60-d exposure for complete control of all its stages. Of the other species tested, *T. castaneum* was the most tolerant; its pupae required 9 or 10 d exposure to prevent emergence in all three atmospheres.

INTRODUCTION

Modified atmospheres (MA's) provide an alternative method, particularly with pesticide admixture, for insect control in stored grain. The technology involves the alteration of the ratio of atmospheric gases, nitrogen (N_2), oxygen (O_2) and carbon dioxide (CO_2), to produce conditions lethal to pests in stores (Banks and Fields, 1995). MA's are now used in both agriculture and food industries in order to disinfest and protect raw materials from the harvest through to the packaging of finished products (Bell and Armitage, 1992). Easily combined with the present strategies of drying and cooling, MA's could play an important role in integrated systems for the protection of grain, particularly where residue-free *in situ* treatments are desired (Banks *et al.*, 1991). Sophisticated markets expect pesticide-free foods, and with the development of resistance by target pests the position of insecticides as the main tool of pest control is becoming ever more problematical (Banks and Fields, 1995). Legislative restrictions also make other alter-

native treatments, such as fumigation, much more expensive (Banks *et al.*, 1991). Adoption of a N_2-based MA treatment at the main grain export terminal in Australia was motivated by such problems (Banks, 1994).

The use of MA's does not adversely affect the quality of the stored grain. The use of N_2 for continuous storage for up to a year did not have a detrimental effect on the germination or end-use properties of wheat, rice or barley (Ouye, 1984). Germination potential, so important for the maltsters, is much more likely to be lost because of such climatic conditions of storage as high temperature than because of MA storage (Fleurat-Lessard *et al.*, 1994). MA's may also be advantageous as they can extend the storage life of grain at levels of moisture which are normally considered marginal for safe storage and do not allow fungi to multiply at lower water activities (Banks *et al.*, 1991).

Various methods are available for generating MA's from gas or liquid sources (Banks *et al.*, 1980; Annis, 1990; Bell *et al.*, 1993a). On-site generation of gas using a separating mechanism which removes O_2 from air, leaving pure N_2, has an obvious advantage over bulk liquid supplies of CO_2 or N_2. An alternative system produces an O_2-deficient atmosphere by burning propane in air (Storey, 1973; Fleurat-Lessard and Le Torc'h, 1987; Bell *et al.*, 1993b). However, in order to evaluate the suitability of each system, it is important to test the efficacy of the MA's in the laboratory. Many such tests have been carried out (Annis, 1987; Reichmuth, 1987; Jayas *et al.*, 1991); however, the results do not cover all the environmental conditions required.

The current work was carried out in order to assess the relative efficacy of different MA's on various stored-grain pests. There were two ways to reduce the time which would have been needed to cover each stage separately: using the most tolerant juvenile stage and using a mixed culture with a known age range, as described by Annis and Dowsett (1993). The juvenile stages of *Cryptolestes ferrugineus* (Stephens) (rust-red grain beetle), *Oryzaephilus surinamensis* (L.) (saw-toothed grain beetle), *Rhyzopertha dominica* (F.) (lesser grain borer), *S. granarius* (granary weevil), *S. oryzae* (L.) (rice weevil) and *Tribolium castaneum* (Herbst) (rust-red flour beetle) were treated with three MA's: CO_2, simulated burner gas and N_2, at the temperature usual at the beginning of the UK grain storage season.

MATERIALS AND METHODS

Insect rearing

The six beetle species were obtained from stock cultures set up by placing 100 adults of mixed ages in a glass culturing jar (diameter: 7.5 cm, height: 14 cm) about one-third full of food. *R. dominica*, *S. granarius* and *S. oryzae* were raised on whole wheat, *C. ferrugineus* on a mixture of rolled oats, wholemeal flour and 5% brewer's yeast, *O. surinamensis* on rolled oats and *T. castaneum* on a mixture of wholemeal wheat flour and 5% brewer's yeast. The stock cultures, excepting *C. ferrugineus* which was reared at 30°C, were maintained at 25°C and 70% r.h. Two different strains of each species were used to determine if there were any differences in their tolerance of the MA's. Standard

laboratory strains and malathion-resistant strains of *R. dominica*, *S. granarius* and *S. oryzae*, and a phosphine-resistant strain of each of the other species, were used.

For *R. dominica*, *S. granarius* and *S. oryzae*, where juveniles develop within the wheat grains, the cultures were divided among the containers used for the exposures. For the other species, either 50 pupae or 50 late fourth instar larvae were counted into each container. These containers were one-third full of fresh culture medium. Three containers were used for each exposure period and another six (three kept in the same room as the exposure apparatus and three remaining in the rearing conditions) were used as controls. The exposures took place in glass tubes (diameter: 2.5 cm, height: 7.5 cm) sealed with squares of nylon mesh held in place by sections of rubber pipe which were inserted in the openings of the tubes.

All the insects, including the controls, were lowered to the exposure temperature of 15°C in daily 5°C stages (so that they could acclimatise) while humidity was held constant at 70% r.h. At the completion of the longest exposure period, they were again raised to their rearing temperatures, the duration and temperature stages being parallel. Then they were checked regularly for adult emergence. The results were used to calculate the mean emergence for each exposure time as a percentage of the emergence from the control samples for *S. granarius*, *S. oryzae* and *R. dominica*. For the other species correction for other sources of mortality was made from the controls. The percentage emergence of each treated sample was calculated as a proportion of the emergence of the controls.

MA's

The three gas mixtures (0.5% O_2 and 99.5% N_2; 0.5% O_2, 10% CO_2 and 89.5% N_2 — burner gas; and 0.5% O_2 and 99.5% CO_2) were produced using a three-channel gas blender (Signal Instrument Co. Ltd., Camberley, Surrey) supplied with high purity gases from cylinders. After the gases were mixed to the required proportions, the gas stream was split into eight, each gas flow being limited to 100 ml/min by means of flow control valves. The eight streams were then humidified to 70% by passing the gas over solutions of potassium chloride (Winston and Bates, 1960). The humidified gas stream then passed via a tube into the bottom of the exposure chamber, a 5-L desiccator. The exposure containers were then placed on a layer of wire mesh supported above the bottom of the desiccator.

A vent in the top effected a flow-through system. This set-up ensured a constant gas mixture, with no change through leakage or respiration, within the exposure chamber. The apparatus was kept in a controlled-environment room at 15°C and 70% r.h. O_2, CO_2 and humidity levels were monitored every day, using a Model 570A paramagnetic O_2 analyser (Servomex Ltd., Crowborough, Sussex), a Model PA 404 infra-red CO_2 analyser (Servomex Ltd.) and a Protimeter DP680 (Protimeter Ltd., Marlow, Bucks.), respectively, and were adjusted if required.

At the end of the longest exposure period for each species the exposure containers and the controls were moved back to their culturing temperatures by reversing the steps used

to acclimatise the insects before exposure. The containers were then checked once a week for any adult emergence. This procedure was continued until all possible emergence had taken place. A comparison was then made with the mean exposure results for each time period for the experimental insects and for the controls. Thus, the level of mortality produced by the MA's was determined.

RESULTS

Nitrogen

From previous experience it was expected that *S. granarius* would be the species most tolerant to this mixture. A 55-d exposure period killed most of the juveniles, indicating that there was no difference in strain tolerance. A 60-d exposure period was required to kill all stages (Table 1).

There was little difference in mortality between the strains of *S. oryzae*. An exposure period much longer than any tested would be required for complete control; this was also true for *R. dominica*, the cultures of which were at a younger developmental stage. It appears that a complete kill of the more tolerant pupal stage of this beetle would require a longer exposure than that required for *S. oryzae*.

In this test the resistant strain (Res) of *R. dominica* appeared more tolerant of the atmosphere than the laboratory strain (Lab). This may be notable only because the longest exposure times used gave survivals of approximately 50%.

The remaining three species, and particularly *T. castaneum* Lab, had poor emergence due to the low temperature in some of the control replicates. These results call into

TABLE 1

A comparison of the mean percentage emergence of resistant (Res) and laboratory (Lab) strains of six species of grain beetle after varying lengths of juvenile exposure to 0.5% O_2 and 99.5% N_2 at 15°C and 70% r.h.

Species	*S. granarius*		*S. oryzae*		*R. dominica*		*T. castaneum*		*C. ferrugineus*		*O. surinamensis*	
Strain	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res
Age (wks)	0–5	0–5	0–3	0–3	0–3	0–2	5–6	5–6	3–4	3–4	4–5	4–5
Time (d)												
3									92	52	6	9
5									52	42	3	0
6							56	28				
8							17	28				
9							0	0				
38			39	32	63	70						
44			34	34	49	66						
55	0	2										
60	0	0										

question the accuracy of the emergence results from the exposed juveniles. There was little difference between the strains of *T. castaneum*, which was more tolerant than the remaining two species. *O. surinamensis* was the least tolerant of the three species; only a short extension of the 5-d exposure period would be required for complete control, whereas a period closer to the longest exposure of 9 d used for *T. castaneum* would be needed for *C. ferrugineus*.

Burner gas

There was little difference between the strains of *S. granarius* in the tolerance to burner gas (Table 2). Complete control was achieved in 65 d, which was not significantly longer than the period required using the N_2-based atmosphere. For *S. oryzae* and *R. dominica* the results were better than those achieved with N_2, and a far higher level of control was achieved in a shorter time period. This occurred even though the *R. dominica* cultures were much older than those used for the N_2 test cultures and contained pupae, which are more tolerant of these atmospheres than are larvae. There was some evidence that the Res strain of *S. oryzae* was more tolerant than the Lab strain.

Results of the burner gas and N_2-based atmospheres were generally similar for *T. castaneum* and *O. surinamensis*.

TABLE 2
A comparison of the mean percentage emergence of six species of grain beetle after varying lengths of juvenile exposure to 0.5% O_2, 10% CO_2 and 89.5% N_2 at 15°C and 70% r.h.

Species	*S. granarius*		*S. oryzae*		*R. dominica*		*T. castaneum*		*C. ferrugineus*		*O. surinamensis*	
Strain	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res
Age (wks)	3–4	3–4	0–3	0–3	7–9	0–9	5–6	5–6	3–4	3–4	4–5	4–5
Time (d)												
4												11
5											0	0
9							0	0	0	0		
28			3	19	6	3						
36			3	11	4	1						
55	2	0										
65	0	0										

Carbon dioxide

For *S. granarius* 38 d was required for complete mortality (Table 3). This is a much shorter time than that required for both N_2 and burner gas. A similar pattern was seen with *S. oryzae* and *R. dominica* although the difference was much less in comparison with the results obtained from burner gas. However, the *R. dominica* stages used for the burner gas

TABLE 3

A comparison of the mean percentage emergence of six species of grain beetle after varying lengths of juvenile exposure to 0.5% O_2 and 99.5% CO_2 at 15°C and 70% r.h.

Species	*S. granarius*		*S. oryzae*		*R. dominica*		*T. castaneum*		*C. ferrugineus*		*O. surinamensis*	
Strain	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res	Lab	Res
Age (wks)	0–5	0–5	0–3	0–3	0–3	0–2	5–6	5–6	3–4	3–4	4–5	4–5
Time (d)												
3											25	31
5									33	82	3	0
6							22	28				
8							0	3				
10							0	0				
28			1	1	0	1						
32			2	1	0	0						
34	0	1										
38	0	0										

MA test were much older than those used for the CO_2 test and the time required to control the tolerant pupal stage with CO_2 was sometimes much longer than the 32 d needed to kill the younger stages (Table 3).

With the other three species there was little variance from the previous results. A strain difference was evident for *C. ferrugineus* where Res was the more tolerant. An extension of the range of exposure times tested to achieve complete control of this species is required. Such additional tests are needed for both the CO_2 and the N_2-based atmospheres.

DISCUSSION

For MA treatments to become established, it is essential that they effect complete control of any insects present, whatever their stage. In general, results have indicated that CO_2 atmospheres are more toxic than O_2-deficient ones (Navarro and Donahaye, 1990). This is because CO_2 relies not on anoxia only to be lethal but also on acidification of body fluids and inhibition of glycolysis (Adler, 1994). For *S. granarius*, one of the most tolerant insects, 60 d were required to kill the most tolerant pupal stage with N_2 but only 38 d were required with CO_2. Reichmuth (1990) achieved an LT_{95} with 99% N_2 in 45 d and a similar result with 90% CO_2 in only 35 d. Adler (1994) gave 50 d for 97–100% N_2 and these differences demonstrate the importance of monitoring tolerances of pest populations in order to determine the correct time period needed for control. Differences in tolerance to MA's were demonstrated by Adler (1991), working with many strains of the same species.

The juvenile stages of beetles showed a marked difference between the internal grain feeders, *R. dominica*, *S. granarius* and *S. oryzae*, and the other three, the external feeders,

in their tolerance of all MA's, the latter being much more susceptible. Ten days was the maximum exposure required for the external feeders for all three MA's, whereas *R. dominica* and *S. oryzae* required 32 d exposure with CO_2, at least 36 d with burner gas, and more than 44 d with N_2. The tolerance of MA's occurs when consumption of O_2 is lowest, often during the egg and pupal stages (Reichmuth, 1987). Clearly, these insects must be controlled before their development has reached the most tolerant stage. However, there are differences in response among other strains of these species (Jay, 1984); Jay used a slightly higher temperature of 16°C and a lower 55–60% r.h. that produced 100% mortality with 60% CO_2, and 91% and 86% mortality with 99% N_2, with *R. dominica* and *S. oryzae*, respectively, after exposures of only 2 weeks.

Similar differences were seen among the external feeders. White and Jayas (1993), using mixed populations of *C. ferrugineus* and *T. castaneum*, achieved control in 14 d with 34% CO_2 and the temperature decreasing from 18 to 10°C. A 100% CO_2 atmosphere controlled *T. castaneum* pupae in 14 d at 15.6°C and 38% r.h. (Aliniazee, 1971), and 100% N_2 controlled the same stage under the same conditions in only 5 d (Aliniazee, 1972). This species was also studied in one of the few published investigations using burner gas. Storey (1977) used a mixture of <1% O_2 and 9–9.5% CO_2 in N_2 at 18°C, effecting an LT_{95} of 4.5 d for pupae. The last species, *O. surinamensis,* was the least tolerant of all; it succumbed within 5 d to all MA's. Jay (1984) used 98% CO_2 at 16°C and 50% r.h., effecting 100% mortality in 3 d for all stages. Any differences in the environmental conditions would have significantly influenced the results in all these comparisons.

In conclusion, in this brief survey CO_2 has proven to be the most effective MA, followed by burner gas and N_2. Pupae of *Sitophilus* spp. showed the highest tolerance to these atmospheres, followed by the other internal grain feeder *R. dominica*. The other three species, the external grain feeders, were much less tolerant.

ACKNOWLEDGEMENTS

This work was funded by the Home-Grown Cereals Authority, London, UK.

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CONTROL OF THE DERMESTID BEETLE *DERMESTES MACULATUS* DE GEER WITH CONTROLLED ATMOSPHERES

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ABSTRACT

Various hypoxic controlled atmospheres were tested against all stages of *Dermestes maculatus* under laboratory conditions at 25°C and 30°C, both at 75% r.h. Using atmospheres of pure nitrogen (N_2) and pure carbon dioxide (CO_2), respectively, complete control of all stages was achieved within 48 h. The same effect occurred with an atmosphere consisting of 98% N_2 and 2% O_2 by volume. At 30°C with a gas mixture of either 40% CO_2, 44% N_2 and 16% O_2, or 60% CO_2 in air, an exposure time of 96 h controlled all stages. Pupae and larvae were shown to be more tolerant than other stages.

INTRODUCTION

Proper pest management strategies are necessary to minimise economic losses. Problems associated with the use of chemical pesticides, such as worker safety, development of insect resistance and product contamination, draw attention to the development of new strategies and the improvement of existing pest control methods.

The leather or hide beetle *Dermestes maculatus* is one of the most important pests of dried food commodities of animal origin as well as of leather, furs and other materials. Adults and larvae of *D. maculatus* feed on a wide variety of animal products, including dry animal corpses, feathers and fur. In their search for food or a place for pupation, the larvae penetrate into baled tobacco, the woodwork of boxes, fibre-board, vegetable fibre, cork, books, cardboard, linen, cotton, plaster moulds, the lead of fuses and cables, mortar and the stonework of walls (Cline, 1978; Hinton, 1945; Levinson and Levinson, 1978; Nair, 1986). *D. maculatus* damages insect collections and mummies in museums as well as other valuable goods. However, Dermestidae are also appreciated as useful insects in cleaning the skeletons of small to medium sized animals (Hinton, 1945).

Among their natural habitats are bird nests, wasp nests, and sometimes even bee hives. Because they are good flyers, they can also penetrate houses, infesting stored products and materials of animal origin. During dry summers larvae and adult beetles may infest

urban structures in great numbers (Engelbrecht, 1989). Wildey and Wayman (1981) and Binns and Pemberton (1981) reported that three out of every four poultry houses in England and Wales were infested.

In addition to eating chicken feed and other organic matter, the adults and larvae of *D. maculatus* prey on smaller arthropods. They also attack dead or moribund chicks. They have been recorded as injuring, and even killing, young pigeons in pigeon lofts by boring into their wings (Hinton, 1945).

In storage structures and households, *D. maculatus* has been recorded as feeding on bacon, ham, sausages, dry cheese, noodles, dried fish and stuffed animals. *D. maculatus* can cause considerable damage in the processing and storage of dried fish (Taylor and Evans, 1982). In Nigeria 71% of the infestations of dried fish were by *D. maculatus* (Osuji, 1975).

Occasionally *D. maculatus* may have minor deleterious effects on human health, acting as an intermediate host for parasites or as a vector of such pathogenic organisms as nematodes (Hinton, 1945) and enterobacteriaceae (Julseth *et al.*, 1969). Allergic symptoms, including dermal itching, conjunctivitis, irritation of the respiratory tract and nausea, can result from contact with the detached hairs of the larvae.

Laboratory tests have shown that *D. maculatus* is highly tolerant of many insecticides (Pasalu *et al.*, 1974; Taylor and Evans, 1982), and the larvae are less susceptible to a wide range of insecticides than are many other stages of stored-product pests (Lloyd and Dyte, 1965; Ellis, 1964). Insect growth regulators and juvenile hormone analogues have been effective in laboratory tests but their efficacy has not yet been demonstrated in the field (Axtell and Arends, 1990). Such controlled atmospheres as mixtures of carbon dioxide (CO_2) or nitrogen (N_2) with low residual oxygen (O_2) concentrations offer two main advantages for pest control in stored-product protection: the disinfested commodity is residue free and the application is relatively safe. On the other hand, both fairly long exposure periods and gastight enclosures are required. The present study was undertaken to determine the efficacy of various controlled atmospheres at 25°C and 30°C against all stages of *D. maculatus*.

MATERIALS AND METHODS

All test insects originated from cultures at the Institute for Stored-Product Protection in Berlin and were kept in continuously monitored climatized chambers at 25 ± 1°C and 30 ± 1°C, both at 70 ± 5% r.h. The rearing substrate consisted of fish flour and dried pig bones.

In the experiments, four different stages of *D. maculatus* were tested: 24-h-old eggs, 10-d-old larvae, young pupae and young adults.

To obtain eggs, 200 adult beetles were placed on the rearing substrate for 24 h at 25°C and another 200 at 30°C. The eggs were counted and placed in exposure cages, together with some substrate. The substrate served to both maintain relative humidity and prevent friction with the walls of the cage.

Ten-day-old larvae were produced by placing 24-h-old eggs on the food substrate for a period of 10 d after the hatch of the first larvae. Fifty young larvae were counted and placed in the exposure cage with some food substrate.

Two stainless steel wire mesh cages (length 8 cm, diameter 1.5 cm) with 50 insects at each stage were exposed to the experimental conditions, and two other cages with the same population served as control.

Each of the stages was exposed at 25°C and 30°C to the gas mixtures (Table 1).

TABLE 1
Gas mixtures (%) used for the experiments

Mixture no.	CO_2	N_2	O_2
1	100	0	0
2	0	100	0
3	0	98	2
4	40	44	16
5	60	32	8

The experimental exposure apparatus is illustrated in Fig. 1. The procedure of exposure to the gas mixtures was as follows: the exposure cages (y), each containing about 50 individuals of each instar, were introduced into 500 ml Dressel flasks (e) and the flasks were sealed with Vaseline and locked with metal clamps. They were then connected to each other by plastic tubing in the following order: (a) empty safety flask to prevent any backflush of the humidifying mixture from the following bottle; (b) flask with saturated sodium chloride solution to obtain 76 ± 5% r.h.; (c) one or more empty

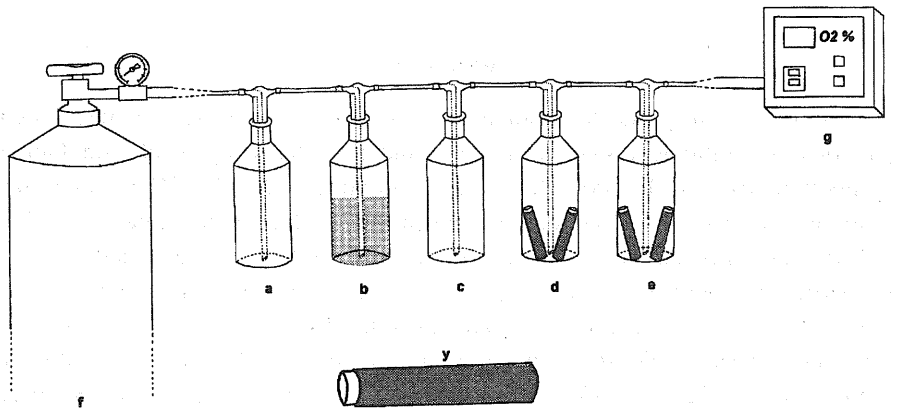


Fig. 1. Set-up of the fumigation experiments: (a) safety bottle; (b) bottle with the saturated sodium chloride solution; (c) empty safety bottle; (d) and (e) bottles with the test insect cages; (f) gas cylinder with the tested mixture; (g) an oxygen analyser; (y) fine metal gauze cage.

safety flasks to prevent accidental overflow of salt solution; (d) and (e) flasks with the test insect cages; and (g) an O_2 analyser. This battery of Dressel flasks was connected to a gas cylinder containing the test mixture (f). The gas was purged through the bottles at a flow rate of 10 ml/h and at a room temperature of 18–23°C for about 1 h. When the required O_2 content had been obtained, the Dressel flasks with the test insect cages were sealed, disconnected and held at the two experimental temperatures. After exposure times of 24 h, 48 h and 96 h, respectively, flasks and cages were opened and the surviving individuals were counted and placed in petri dishes (diameter 10 cm). Mortality rates in each dish were checked weekly for 5 weeks. Each experiment was repeated three times.

The following parameters were checked to determine mortality rates in treated and untreated samples:

1. The eggs, exposed with the substrate, were examined immediately after treatment for colour, size and possible internal development of larvae. Shrunk or darkened eggs were counted as dead but left in the petri dishes for further observation. Dead larvae were removed from the petri dishes. The same procedure was carried out with the untreated controls.

2. The larvae, exposed with the substrate, were examined for mobility and size immediately after exposure. Survivors were counted and left in the petri dishes. Dead larvae were removed. The same procedure was carried out with the untreated controls.

3. The pupae were checked for size, colour and signs of vitality immediately after treatment and subsequently at weekly intervals for 5 weeks. Adults emerging from pupae were counted as survivors, as were pupae that were neither shrunk nor darkened. Eggs and larvae produced after the treatment were not removed.

4. The adults were checked for size and mobility immediately after treatment. During the weekly bioassay surviving and dead adults were counted separately and the dead adults removed. Eggs and larvae produced after the treatment were not removed.

RESULTS

A high rate of mortality due to cannibalism was noted in the experiments. This made comparison of mortality levels between treated and untreated samples difficult. Only in the treatments with 100% CO_2, 100% N_2 and a gas mixture of N_2 plus 2% O_2 were there no survivors of any stage to be found after exposure for 48 h at both temperatures. In these cases, 100% mortality, clearly attributable to the effect of the gas, was noted.

In experiments where larvae and/or adults survived, cannibalism masked the effect of the treatment. This was also true for larvae and adults developing from treated eggs, untreated eggs and pupae during the post-treatment observation process. In untreated controls maximum mortality in 5 weeks was 15% in eggs, 88% in larvae, 65% in pupae and 78% in adults. This high post-mortality rate of both treated and untreated insects will be discussed below.

All egg stages failed to survive exposure to gas mixtures 1, 2, 3 and 5 (Table 1) at 25°C for 24 h. After treatment with mixture 4 the mortality level was less than 100%.

Eggs did not become deformed within 48 h of treatment with any of the gases. Egg mortality, as evidenced by shrinking and colour change, could only be noted within a few days following treatment. Adults survived treatment 4 for the 24 h exposure period.

CO_2 mixtures with 8% and 16% O_2 (treatments 4 and 5) resulted in significantly higher insect survival levels at the higher CO_2 concentration levels than at the lower ones. Larvae were more tolerant than pupae.

Only a few treatments were carried out with gas mixtures 1, 2, 3 and 5 at 25°C and 30°C using an exposure time of 96 h. In all these experiments no insects survived.

DISCUSSION

Usually control of *D. maculatus* is effected by spraying all surfaces with a residual insecticide (Wildey and Wayman, 1981), but this application is problematic in animal houses or museums due to the resulting contamination of walls with persistant poisons.

Neem seed powder has been recommended for protecting dried fish. It controls 93% of the *D. maculatus* larvae within 30 d of exposure (Okorie *et al.*, 1990). However, the disadvantage of neem is its bitter taste, which makes dried fish palatable only after all neem residues are thoroughly washed off.

The use of controlled atmosphere treatments could in theory be successful in controlling *D. maculatus*. In practice, however, there is a problem: a hermetic chamber or enclosure is needed to obtain and maintain the very low O_2 content needed to control the insect in all its stages.

One hundred percent mortality directly after treatment was observed only for 100% CO_2, 100% N_2 and the mixture of N_2 with 2% O_2.

The development of surviving eggs, larvae and pupae was retarded. Treated individuals were often smaller and less active than non-treated ones. Bioassay was difficult to perform due to cannibalism; therefore, smaller numbers of insects per exposure cage, larger post-treatment chambers than those used and ample amounts of food substrate are recommended for this species.

The elytrae and abdomens of some emerging adults were deformed. These deformed adults were more frequently cannibalised than were normally developed ones. Despite the presence of food substrate, most of the dead adults were attacked and devoured but the darkened (dead) pupae were not.

These preliminary experiments indicate that complete control of all stages of *D. maculatus* requires less than 4 d exposure at 25°C and 30°C when the O_2 content is under 2%.

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RAPID DISINFESTATION THROUGH THE COMBINATION OF CONTROLLED ATMOSPHERES AND HEAT

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ABSTRACT

The suggested phaseout of methyl bromide production and use in industrialised countries by the year 2010 increases the need for alternative rapid disinfestation methods. High temperatures increase the metabolic rates of insect pests and thus enhance the lethal action of both fumigants and Controlled Atmospheres (CA's). Experiments with the granary weevil *Sitophilus granarius* indicated that treatment with carbon dioxide (60% CO_2 and 40% air or 90% CO_2 and 10% air) or nitrogen (98% N_2 and 2% O_2) over 46 d or 77 d, respectively, provided complete control at 10°C. This could be reduced to 8 d at 35°C and 1.5 d at 40°C for all tested atmospheres. Therefore, such valuable products as nuts, herbs, tobacco or spices, which can tolerate such temperatures without loss of quality, could be rapidly disinfested by either of these two CA's. The results at 40°C also indicate that empty room treatments are feasible by a combination of CA's and heat in sufficiently gastight structures. Moreover, the data indicate that CA's hold special promise in dry warm climates where the stored-product temperatures are naturally high.

INTRODUCTION

During its meeting in Vienna in November 1995, the United Nations committee on ozone depletion agreed on a phase-out of the production and use of methyl bromide (MB) in industrialised countries by the year 2010 (Mueller, 1996). The implementation of this decision will further reduce the number of fumigants available for pest control. The rapid toxic action and cost efficiency are two of the reasons why the loss of MB would leave a gap not readily filled by other fumigants. For each MB usage the most suitable alternative treatment needs to be found. If such stored products as grain, herbs, tobacco, nuts or cocoa beans must be regularly disinfested within short periods of time in order to meet quarantine regulations, treatment with carbon dioxide under high pressure (10–40 bars) may be one possible alternative (Prozell and Reichmuth, 1991). However, such a treatment necessitates moving the infested goods, thus spreading the risk of infestation to all transportation

equipment used; furthermore, the pressure-tight steel chambers needed for such treatments are quite costly. Another possible alternative is the acceleration of the lethal action of phosphine (PH_3) by the use of quick-releasing formulations, the application of a ready-made gas supply, or combination with carbon dioxide (CO_2) and/or increased temperatures (Desmarchelier and Wohlgemuth, 1984; Mueller, 1994).

CA's of low oxygen (O_2) and/or high CO_2 concentrations are only slowly gaining importance because of both the high standards of gastightness required and the relatively long exposure times needed at ambient pressure and temperature.

In this paper the effects of grain temperatures from 10°C to 40°C on the efficacy of three different CA's are presented. The laboratory study was carried out to determine the effects of various ambient temperatures on the time needed for disinfestation, as well as to determine how much it would be possible to reduce exposure periods lethal to the rather tolerant grain pest *Sitophilus granarius* by using a combination of CA's and heat.

MATERIALS AND METHODS

To study the effects of gas mixtures on developmental stages of defined age, the following insect culture method was used: at 25 ± 1°C, 75 ± 5% r.h., approximately 2500 young adult granary weevils were placed on 142 g of fresh uninfested wheat for an oviposition period of 3 d. This culture technique was repeated weekly to obtain the following five juvenile stages for use at the start of each experiment: stage 1: eggs 1–4 d after oviposition; stage 2: young larvae 8–11 d after oviposition; stage 3: larvae 15–18 d after oviposition; stage 4: old larvae 22–25 d after oviposition; stage 5: mainly prepupae and pupae 29–32 d after oviposition.

Details of this procedure are given in Adler (1991). For each juvenile group, 70 kernels of infested wheat were placed in a wire mesh cage.

These samples plus an additional sample with 50 adult weevils on uninfested grains were all transferred into a 2.3-L Dressel flask. Then the efficacy of the following gas mixtures was tested at 10, 15, 20, 25, 30, 35 and 40°C using five different exposure times for each experiment: (1) 98% N_2, 2% O_2; (2) 60% CO_2, 32% N_2, 8% O_2; and (3) 90% CO_2, 8% N_2, 2% O_2 (% by volume).

The gas mixtures were prepared manometrically prior to the experiment (details in Adler and Reichmuth, 1988). Five Dressel flasks (to be held for five different exposure times) were connected by PVC-tubing in a climatised chamber and flushed with the experimental gas mixture until the measured O_2 content matched that of the particular experimental gas mixture. The experimental gases were humidified to 75% r.h. using a saturated sodium chloride solution (Winston and Bates, 1960) before being flushed through the experimental vessels. After purging, the flasks were closed for the desired exposure period. When the flasks were opened, O_2 content was again determined. The insects were held at 25°C, 75% r.h., and they were checked weekly for adult emergence from the juvenile stages and adult mortality, respectively.

RESULTS AND DISCUSSION

The experiments revealed the paramount influence of temperature on treatment time. Exposure times needed to kill all stages of the granary weevil are given in Fig. 1. At all tested temperatures, 60% CO_2 and 90% CO_2 produced almost identical results.

The susceptibility of stages decreased in the following order: (1) 98% N_2, 2% O_2 mixture: adults, eggs, young larvae, old larvae, pupae; (2) mixtures containing CO_2: adults, young larvae, old larvae, eggs, pupae.

As the temperature increased the difference in susceptibility of the different developmental stages was reduced. At exposure times of more than 1 week the residual O_2 content dropped, especially in the flasks treated with the N_2 gas mixture. This can be attributed to O_2 consumption by the insects. At 25°C and below, significantly shorter exposure times were needed for complete control using atmospheres containing CO_2; above 25°C the difference between anoxic and hypercarbic gas mixtures was negligible.

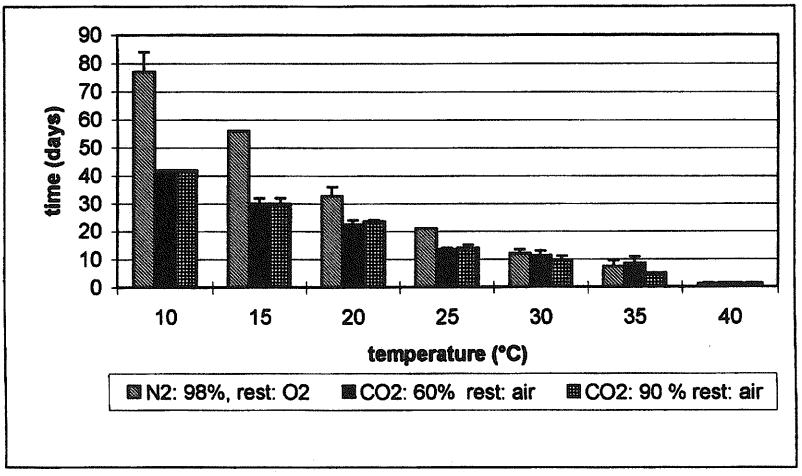


Fig. 1. Exposure times needed with various CA's at different temperatures for complete control of adult and 100% reduction in emergence of juvenile *S. granarius*.

In warm climates, commodities could be effectively treated at temperatures around 30°C within 10 d or less, depending on the pest species present. Under these conditions, increased respiration rates of all living organisms present might even allow for continuous hermetic storage under hypoxic conditions, provided that stored-product moisture contents are low, thereby minimising the risk of condensation. Moreover, shorter exposure times at increased commodity temperatures may render CA's feasible for in-transit treatment on ships sailing from warm climates. In some cases it may even be economically feasible to heat, treat, and then cool down valuable products before long-term storage or transportation.

At 40°C an exposure time of 36 h was sufficient for complete control with all three tested gas mixtures, whereas pupal stages of untreated samples survived exposure to 40°C for 48 h. This corresponds to the data of Jay (1987), who exposed mixed ages of *S. oryzae* to an atmosphere of air and 78% CO_2 or 91% CO_2, respectively, at 55% r.h. At 38°C, 48 h were required for complete control with both atmospheres, and at 43°C similar results could be achieved within 16 h. Thus, given a sufficiently gastight structure, high summer temperatures with some additional heating could be used for empty-room treatment with CA's, in exposure times comparable to those required for fumigation with such toxic fumigants as MB.

The treatment of grain storages with CA's is always more expensive than a MB fumigation (and often more expensive than a PH_3 fumigation). However, under certain conditions, the use of CA's may be more economic and more convenient than the use of a toxic fumigant. In the Berliner Hafen und Lagerhausgesellschaft (BeHaLa), Berlin Westhafen, about 20 concrete silo-bins are located in a building dating from the 1940's. Although these silo-bins are generally not very gastight, two of them were constructed for fumigations with cartox (CO_2 and ethylene oxide). They achieve a pressure half-life of 60–120 sec. According to the storage keeper, in these silo-bins grain treatment with 70% CO_2 for 3 weeks at 20°C costs approximately 3 DM per t, whereas PH_3 fumigation would cost 5 DM per t. Moreover, during CA treatment work can proceed in the vicinity of the treated silo-bin, whereas during fumigation an entire section of the building would have to be shut down. In Germany the local authorities responsible for workers' safety and emission control must be notified before treatment with toxic fumigants, but such notice is not necessary if CA's are used.

In conclusion, a multitude of factors may influence the decision for or against the use of CA's. Today, however, it seems that this technique will be used more frequently in future and that the combination of CA's and heat may be one way to achieve shorter treatment times.

ACKNOWLEDGEMENTS

The author is indebted to Silvia Krause for excellent technical assistance. He also thanks Christoph Reichmuth for reviewing the manuscript.

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A COMPARATIVE STUDY OF PHOSPHINE RESISTANCE LEVELS IN STORED-GRAIN BEETLES COLLECTED FROM SEALED AND UNSEALED FARM STORAGES IN WESTERN AUSTRALIA

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ABSTRACT

Western Australia has established a reputation as an exporter of residue-free grain despite having conditions that are more suitable to the development of grain-insect pests than many other countries. This has been brought about by the extensive use of sealed storage and phosphine (PH_3) fumigation both in the central handling system and on-farm.

The Western Australian grain industry has been concerned for some time that PH_3 resistance could result in control failures due to the inefficient use of PH_3 in unsealed and poorly maintained sealed storage units. To monitor this threat a survey was conducted in 1991 to study the frequency of PH_3 resistance in the rust-red flour beetle, *Tribolium castaneum*; the rice weevil, *Sitophilus oryzae*; the granary weevil, *S. granarius*; the lesser grain borer, *Rhyzopertha dominica*; and the sawtoothed grain beetle, *Oryzaephilus surinamensis*. This study, which used a discriminating dose test, concluded that there was no significant difference between sealed and unsealed storage units with respect to the *frequency* of resistant populations.

There is, however, a need to determine whether sealed or unsealed storage units give rise to either significantly different *levels* of PH_3 resistance or to a different frequency of resistant individuals within a population. This paper reports on a frequency distribution analysis of data collated from an earlier PH_3 resistance survey and on progress toward the establishment of resistance levels of grain-insect strains collected during the same survey.

INTRODUCTION

Since 1990 all Western Australian grain exports have been effected without the use of contact insecticides. This represents 27 million t of residue-free grain. Strategic planning, resistance management and close cooperation between industry and government over the last 20 years has enabled the use of insecticides to be phased out both on-farm and in the central handling system (Dean, 1994).

Cooperative Bulk Handling (WA) has sealed over 65% (7 Mt) of its permanent storage

capacity and is currently using phosphine (PH_3) for grain-insect control, although other controlled atmosphere alternatives could be employed if required.

Sealed storage with PH_3 fumigation is also widely used on Western Australian farms. A recent survey (Newman, 1994) has shown that over 60% of farms have at least one sealed silo on the property.

There is a danger that the indiscriminate use of contact insecticides on-farm and the subsequent delivery of treated grain to the central handling system could jeopardise the residue-free status of Western Australian grain. To protect its marketing advantage, the use of contact insecticides on farms in Western Australia is highly regulated. There are no insecticides registered for application to farm-stored grain, and seed treatments which contain insecticides must be applied in conjunction with a dye to ensure that late deliveries of treated grain will be detected and rejected before the grain bulk is contaminated.

Clearly there is heavy reliance on PH_3 fumigation and sealed storage in the Western Australian grain industry for which protection is needed from the development of resistant grain-insect pests. A major concern is that the type of storage might be influencing resistance; for example, using PH_3 in unsealed storage units could be selecting for high frequencies of low level resistance, whereas sealed storage units could be selecting for low frequency, but high level, resistance.

Monitoring for resistant grain-insects has underpinned the state's PH_3 resistance management strategy and will continue to do so. Emery (1994) reports the results of a PH_3 resistance survey of 4,547 farms in 1991. This survey also looked at the resistance frequency in grain-insect samples collected from sealed and unsealed farm storage units, but it did not consider either the resistance level of strains or the frequency of resistance within a strain.

This paper compares the frequency of resistant individuals in strains of *Tribolium castaneum* (Herbst), *Sitophilus oryzae* (L.), *S. granarius* (L.), *Rhyzopertha dominica* (F.) and *Oryzaephilus surinamensis* (L.) collected from both sealed and unsealed storage units and reports on progress towards establishing resistance levels for these strains.

MATERIALS AND METHODS

Over 4,500 farms were inspected for grain-insect infestation during the 1991/92 financial year. Samples of grain insects were collected, where possible, from infested sealed and unsealed storage units on each property, resulting in over 2,000 discriminating dose-resistance tests. Test methods followed the procedures described by the FAO (Anon., 1975) and are detailed in Emery (1994) along with sampling methods. Discriminating dosages, taken from the FAO method, but with the *T. castaneum* dosage increased by 0.008 mg/L, are shown in Table 1. Susceptible control insects were included in every test to ensure that there were no protocol failures, such as blocked syringes or broken seals, and tests were repeated if any control insects survived. Mortality was assessed 14 d after treatment, and insects were classified as dead if incapable of coordinated movement.

TABLE 1
Discriminating dosages used in phosphine resistance testing

Species	Dose (mg/L)	Exposure period (h)
Oryzaephilus surinamensis	0.050	20
Rhyzopertha dominica	0.030	20
Sitophilus granarius	0.070	20
Sitophilus oryzae	0.040	20
Tribolium castaneum	0.048	20
Tribolium confusum	0.050	20

Strains were classified as resistant in the 1991 survey if two or more insects of at least 50 test individuals survived the discriminating dose. This survey tested 2,238 samples and identified 349 resistant strains, 39 from sealed storage and 310 from unsealed storage. A random subset of 126 farms (63 using sealed and 63 unsealed storage) has been chosen for a follow-up survey to establish the resistance level of these strains. A subset was necessary due to the more extensive resistance testing required in order to determine resistance levels. Sealed and unsealed storage on these farms will be inspected for grain-insect infestations and the specimens collected will be retested using the discriminating dose procedure described above. Storages were classified as sealed in this study if they were originally manufactured as sealed storages. If the strain has retained its PH_3 resistance, the survivors of the test will be cultured until sufficient insects are available to test with five graded concentrations and a control. Three groups of at least 50 insects will be exposed at each concentration and probit regressions fitted using the method of Finney (1971).

RESULTS AND DISCUSSION

Emery (1994) showed that there was no significant difference in the frequency of resistant populations collected from sealed and unsealed storages — 16% and 17%, respectively. The frequency of resistance in individuals within a population was not considered in this paper. To determine the frequency of PH_3-resistant individuals in a population, the 1991 data were re-analysed to show the frequency distribution of resistance scores (expressed as the percentage of insects surviving the discriminating dose) for sealed and unsealed storage.

Table 2 shows 21 class limits for resistance frequency, ranging from 0 to 95.1–100% surviving the discriminating dose, the number of scores which occurred in that class limit, and the scores expressed as a percentage of the total scores for that storage type. The latter is required to compensate for the large difference in the number of strains collected from sealed and unsealed storage facilities (246 and 1,861, respectively).

The data from Table 2 are presented as a cumulative frequency polygon (ogive) in Fig. 1 and indicate that there is no significant difference ($p > 0.05$) in the frequency of resistance within grain-insect populations collected from sealed and unsealed farm storage in Western Australia. The error bars have been calculated as standard error for proportions (Zar, 1984).

TABLE 2

Frequency of resistance in grain insects collected from sealed and unsealed storage facilities

Upper limit of class (% insects surviving discriminating dose)	Sealed storage		Unsealed storage	
	Frequency	% of total	Frequency	% of total
0	186	75.61	1392	74.80
5	43	17.48	295	15.85
10	6	2.44	72	3.87
15	3	1.22	33	1.77
20	4	1.63	22	1.18
25	1	0.41	15	0.81
30	0	0.00	12	0.64
35	1	0.41	10	0.54
40	1	0.41	5	0.27
45	1	0.41	2	0.11
50	0	0.00	3	0.16
55	0	0.00	0	0.00
60	0	0.00	0	0.00
65	0	0.00	0	0.00
70	0	0.00	0	0.00
75	0	0.00	0	0.00
80	0	0.00	0	0.00
85	0	0.00	0	0.00
90	0	0.00	0	0.00
95	0	0.00	0	0.00
100	0	0.00	0	0.00
Total	246	100.00	1861	100.00

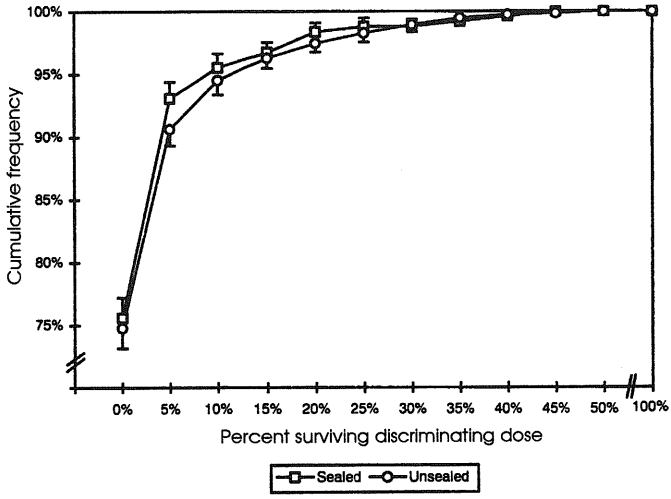


Fig. 1. Cumulative frequency polygon of resistance in grain insects collected from sealed and unsealed storage. Error bars are standard errors for proportions.

Newman (1994) found that 73% of sealed farm storage units were not well maintained and failed the standard pressure decay test for gastightness (a half-life of 5 min from a 25-mm water column). Most of the failures were due to leaking seals. This may have caused some sealed storage units in this survey to behave like unsealed ones in gas holding ability. However, it is unlikely that this could account for the very close correlation between storage types.

Having established that neither sealed nor unsealed storage selects for higher frequency of either resistant populations or individuals, there is a need to study the level of PH_3 resistance, i.e. comparing the resistance factors of strains collected from sealed and unsealed storage. This graded concentration work is in progress. As well as establishing resistance factors, it will measure the gastightness of sealed silos and compare the resulting data with resistance levels. Results of this work are incomplete at the time of writing and will be reported elsewhere.

ACKNOWLEDGEMENTS

The skilled technical expertise of Ms H. Collie, Ms D. Hutchinson, Mr G. McDonald and Mr A. Szito is appreciated, as is the enthusiastic collection of samples by the Agriculture Protection Board field staff. Mr K. Dean and Mr C. Newman provided assistance with the farm selection. The financial support by the Australian Grains Research and Development Corporation is also gratefully acknowledged.

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SESSION 2

BIOLOGICAL RESPONSES OF MICROFLORA TO TREATMENT WITH CONTROLLED ATMOSPHERES AND/OR FUMIGATION

Chairperson: Ailsa Hocking, *Australia*

Donanaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 103–111

GROWTH OF FUNGI IN HIGH CARBON DIOXIDE AND LOW-OXYGEN ATMOSPHERES

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ABSTRACT

Food and grain spoilage fungi are traditionally regarded as aerobic organisms, but some species are efficient oxygen (O_2) scavengers capable of near normal growth in very low concentrations of O_2. The extent to which fungi are able to develop in low-O_2 atmospheres often depends on the concentration of carbon dioxide (CO_2) that is present as atmospheres high in CO_2 are often more effective in controlling fungal growth. Although atmospheres of 20% CO_2 inhibit the growth of many fungi, >80% CO_2 may be required to prevent fungal deterioration in high-moisture commodities. Growth and mycotoxin production by seven species of spoilage fungi was studied in atmospheres where residual O_2 was controlled at less than 0.5%, and the CO_2 concentrations were 20, 40 or 60%, with the balance being the inert filler gas, nitrogen. The potential for mycotoxin production was also assessed. Residual O_2 was a much more critical factor than CO_2 concentrations in reducing growth of *Penicillium* and *Aspergillus* species. *Mucor plumbeus, Fusarium oxysporum* and two *Byssochlamys* species were able to grow in all atmospheres tested although growth was reduced as CO_2 concentration increased. All species tested were able to grow in an atmosphere of 80% CO_2 with 20% O_2, but growth was slower than in air, particularly for the *Penicillium* species and *Aspergillus flavus.* Gas composition had a more pronounced effect on mycotoxin production than on growth.

INTRODUCTION

The important parameters controlling fungal growth in modified or controlled atmospheres are the minimum amount of oxygen (O_2) needed for growth to occur, the inhibitory effects of carbon dioxide (CO_2) and any interactions or synergism between these two gases in mixture. Nitrogen (N_2) has no inherent inhibitory effects; atmospheres high in N_2 may be inhibitory simply because of the lack of available O_2.

Fungi are normally considered to be obligate aerobes, requiring some molecular O_2 for germination, sporulation and growth. However, many species are able to grow in the presence of very small amounts of O_2 (Gunner and Alexander, 1964; Curtis, 1969; King *et al.*, 1969; Wells and Uota, 1970; Gibb and Walsh, 1980; Hesseltine *et al.*, 1985; Hocking, 1988; Taniwaki, 1995), and many fungi have been demonstrated to be efficient O_2 scavengers; thus the total amount of O_2 available, rather than O_2 tension, may determine the extent of growth (Pitt and Hocking, 1985).

Many field and storage fungi important in the spoilage of grains and processed foods are able to grow quite well in atmospheres containing 1% O_2 or less. Many *Fusarium* species, which can be classed as field fungi, are well adapted to growth in low-O_2 environments: *Fusarium moniliforme, F. oxysporum, F. culmorum* and *F. solani* all grow strongly in atmospheres containing 1.0–0.1% O_2 or even less (Gunner and Alexander, 1964; Tabak and Cooke, 1968; Walsh, 1972; Gibb and Walsh, 1980; Magan and Lacey, 1984) if other growth conditions (e.g. temperature and water activity) are favourable. Many *Mucor* and some *Rhizopus* species grow well at low-O_2 tensions (Wells and Uota, 1970; Gibb and Walsh, 1980; Yanai *et al.*, 1980) or even anaerobically (Hesseltine *et al.*, 1985) and can proliferate in high-moisture commodities even when stored under low-O_2 atmospheres (Bottomley *et al.*, 1950; Wilson *et al.*, 1975). Other field fungi such as *Alternaria* species and *Cladosporium herbarum* are more sensitive to reduced O_2 tensions (Magan and Lacey, 1984), and gradually die off during storage.

Storage and food spoilage fungi such as *Penicillium* and *Aspergillus* species generally are more sensitive to low levels of O_2 than the more tolerant field fungi. With the exception of *P. roqueforti*, the growth rates of many *Penicillium* species are reduced by more than 50% in atmospheres of 1% O_2 or less (Yanai *et al.*, 1980; Magan and Lacey, 1984). Of the Aspergilli, *A. candidus* is the most tolerant of reduced O_2 conditions (Magan and Lacey, 1984) and thus can proliferate in controlled-atmosphere stored grains (Di Maggio *et al.*, 1976). Some *Eurotium* species are also reasonably tolerant of low O_2 (Peterson *et al.*, 1956; Yanai *et al.*, 1980).

While CO_2 is known to be inhibitory to growth of many microorganisms, including fungi (Jones and Greenfield, 1982; Daniels *et al.*, 1985), the inhibitory concentration varies markedly among fungal species. Levels of CO_2 from 4 to 20% can be stimulatory to growth of many fungi in atmospheres containing low levels of O_2 (Wells and Uota, 1970; Gibb and Walsh, 1980), but atmospheres containing >50% CO_2 will substantially inhibit growth of most spoilage fungi (Petersen *et al.*, 1956; Wells and Uota, 1970). Some fungi, e.g. *Rhizopus, Mucor* and *Fusarium* species, are very tolerant of high levels of CO_2 and can grow in atmospheres containing 95–100% CO_2 (Stotzky and Goos, 1965).

The interactions between low-O_2 and high-CO_2 atmospheres and their effects on fungal growth are not well understood. The work described here investigated the effects on the growth of several food spoilage fungi of low-O_2 (≤0.5%) atmospheres in combination with elevated (20, 40 and 60%) CO_2 compared with both air and an atmosphere containing 80% CO_2 and 20% O_2.

METHODS

Fungi

Seven species of fungi were chosen for this study primarily because of their recognised ability to grow at low O_2 tension. Most were isolated from spoiled packaged processed foods containing low levels of O_2.

The species studied were *Mucor plumbeus* FRR 2414, isolated from spoiled, fermenting apple juice, Sydney, Australia; *Fusarium oxysporum* FRR 3414, from spoiled, fermenting orange juice, Sydney, Australia; *Byssochlamys fulva* FRR 3792, from fermenting strawberry puree, Sydney, Australia; *B. nivea* FRR 4421 from strawberries, Brazil, capable of producing patulin; *Penicillium roqueforti* FRR 2162, from spoiled Cheddar cheese, Lincoln, Nebraska, USA, capable of producing roquefortine C and reported to be tolerant of low O_2 and high CO_2; *P. commune* FRR 3932, from spoilage of Cheddar cheese packed in low-O_2 atmospheres and capable of producing cyclopiazonic acid; and *Aspergillus flavus* FRR 2757, from peanuts, Kingaroy, Queensland, Australia, a producer of aflatoxin B_1. *A. flavus* is not recognised as being particularly tolerant to modified atmospheres but was included because it is an important mycotoxin producer in many stored products.

Gas systems

The fungi were grown in anaerobe jars (HP11, Oxoid, Basingstoke, UK), evacuated and flushed several times with the appropriate gas mixture. O_2 scavengers ("Ageless"; Mitsubishi Gas Chemical Company, Japan, supplied by W.R. Grace, Melbourne, Australia) were added to jars to maintain residual O_2 at less than 0.5%. Gas concentrations were measured using a gas chromatograph (Model 8AIT, Shimadzu Corporation, Japan) as described by Taniwaki (1995). Atmospheres were checked daily, and the jars were flushed again when CO_2 varied by more than 2% or O_2 exceeded 0.5%.

Media

Cultures were grown on Czapek Yeast extract Agar (CYA; Pitt and Hocking, 1985), a mineral salts medium containing sucrose (3%) and yeast extract (0.5%), and Potato Dextrose Agar (PDA), as being more representative of a natural substrate.

Incubation

The anaerobe jars were incubated at 25°C for up to 30 d. At each sampling time, one jar containing plates of each fungus was opened.

Estimation of fungal growth

The extent of growth was measured using several methods. The simplest of these was measuring colony diameters as an indication of the extent of growth. Fungal biomass was estimated by hyphal length, following the method of Schnürer (1993), or, where growth was not filamentous, by using mycelia dry weight determined by a method based on Paster *et al.* (1983) and Zill *et al.* (1988). Ergosterol content of colonies was also

measured, using a method based on Zill *et al.* (1988). These methods are described in detail in Taniwaki (1995).

Mycotoxin analyses

Mycotoxins were analysed by HPLC. Aflatoxins, after derivatisation with trifluoracetic acid, were analysed according to the method of Beebe (1978). Cyclopiazonic acid was analysed using the method of Urano *et al.* (1992), roquefortine C using the method of Ware *et al.* (1980) and patulin using the method of Burda *et al.* (1992). These assay methods are described in detail in Taniwaki (1995).

RESULTS

Growth at low O_2 in various concentrations of CO_2

P. commune failed to grow in any of the low-O_2 atmospheres tested. *P. roqueforti* grew quite well in 20% CO_2, developing colonies of 25–30 mm on both media, but failed to grow at the higher concentrations of CO_2.

A. flavus showed very weak growth after 30 d in an atmosphere of 20% CO_2 with <0.5% O_2, with colony diameters of 11.0 and 9.5 mm on CYA and PDA. It did not grow at the higher CO_2 concentrations and consequently was used as a biological indicator of O_2 content in these experiments; if the "control" plate of *A. flavus* showed growth when a jar was opened, the plates of other fungi from that jar were not analysed as it was assumed that the O_2 had risen above 0.5%.

The other four fungi, *M. plumbeus, F. oxysporum, B. fulva* and *B. nivea*, were able to grow in all three concentrations (20, 40 and 60%) of CO_2 with <0.5% O_2.

M. plumbeus grew more strongly on CYA than on PDA, and growth responses for this species are shown in Fig. 1. Very thin colonies were produced, and at the higher CO_2

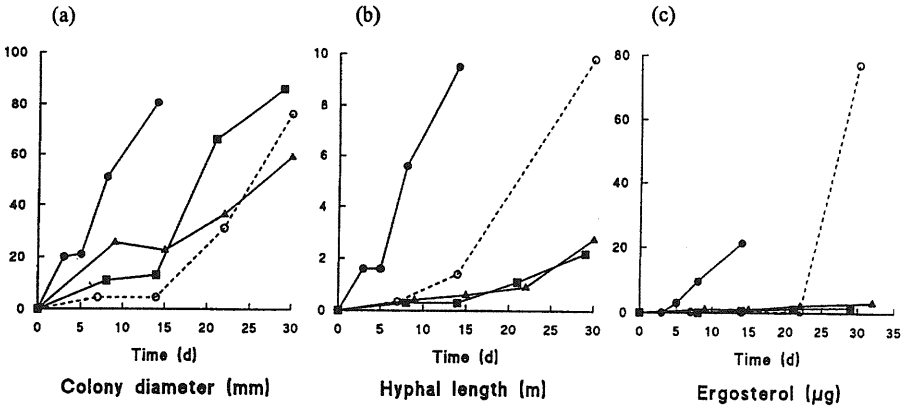


Fig. 1. Growth of *Mucor plumbeus* on CYA in four concentrations of CO_2 as measured by (a) colony diameter, (b) hyphal length and (c) ergosterol content (on the ordinates). full circles = 20% CO_2/<0.5% O_2; squares = 40% CO_2/<0.5 O_2; triangles = 60% CO_2/<0.5% O_2; empty circles = 80% CO_2/20% O_2.

concentrations individual hyphae could be seen at the colony margins. Ergosterol production in 40 and 60% CO_2 was very low compared with colony diameter, as was hyphal length, reflecting the sparseness of the colonies in these atmospheres.

F. oxysporum exhibited similar growth responses on CYA and PDA. Data for growth on CYA are shown in Fig. 2. A distinct difference was observed in response between 20% CO_2 and the two higher concentrations at which much greater (but similar) inhibition occurred. This difference was more obvious in the hyphal length and ergosterol data than in the colony diameter data.

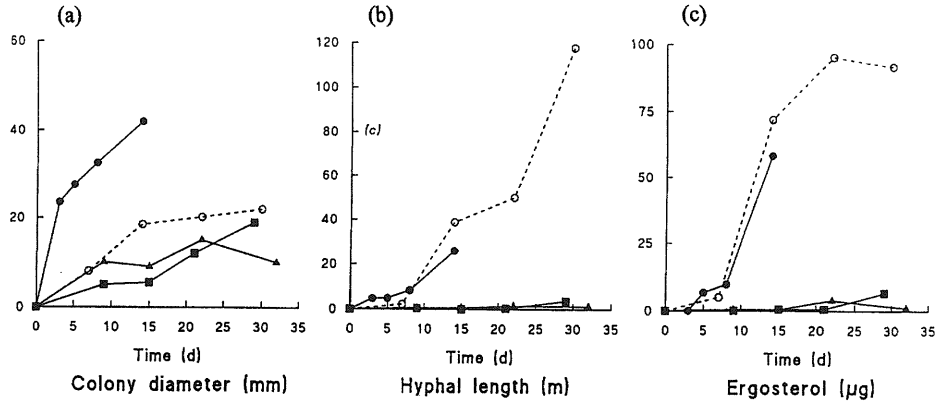


Fig 2. Growth of *Fusarium oxysporum* on CYA in four concentrations of CO_2 as measured by (a) colony diameter, (b) hyphal length and (c) ergosterol content (on the ordinates). full circles = 20% CO_2/<0.5% O_2; squares = 40% CO_2/<0.5% O_2; triangles = 60% CO_2/<0.5% O_2; empty circles = 80% CO_2/20% O_2.

B. fulva and *B. nivea* grew better on PDA than CYA. Both were capable of growth in the three concentrations of CO_2, but inhibition increased with increasing CO_2 concentration. Growth responses for *B. fulva* on PDA are shown in Fig. 3. *B. nivea* reacted similarly but produced lower concentrations of ergosterol than did *B. fulva* at 40 and 60% CO_2.

Growth in 80% CO_2, 20% O_2

P. commune was able to grow in this atmosphere, producing compact wrinkled colonies (approx 15 mm after 30 d) with some sporulation (data not shown). *P. roqueforti* grew well with abundant sporulation in this atmosphere (but better on PDA than on CYA), producing 20–30 mm sporulating colonies in 2–3 weeks. However, ergosterol production was very low on both media. *A. flavus* grew very slowly on both media, with white, non-sporulation colonies only reaching 10 mm after 30 d. As with the *Penicillium* species, ergosterol production was very low.

M. plumbeus produced colonies that were smaller, but more dense, than those produced in 40% and 60% CO_2 with <0.5% O_2 (Fig. 1). This was reflected in the hyphal length and ergosterol figures.

F. oxysporum showed little inhibition in the 80% CO_2/20% O_2 atmosphere, and although its growth rate as measured by colony diameter was less, the hyphal length and ergosterol production were both much greater than in any of the low-O_2 atmospheres (Fig. 2).

B. fulva produced smaller colonies in the high-CO_2 atmosphere, but, as with *F. oxysporum,* hyphal length and ergosterol concentration both exceeded those produced in any of the CO_2 atmospheres with <0.5% O_2 (Fig. 3).

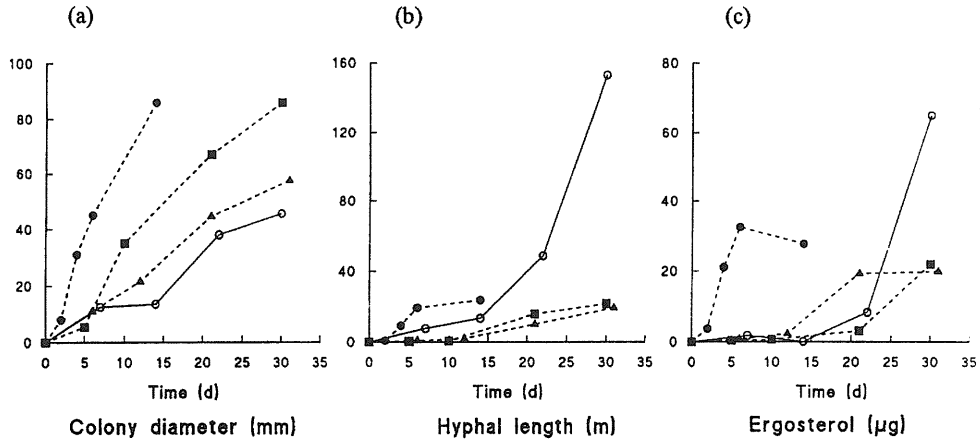


Fig 3. Growth of *Byssochlamys fulva* on PDA in four concentrations of CO_2 as measured by (a) colony diameter, (b) hyphal length and (c) ergosterol content (on the ordinates). full circles = 20% CO_2/<0.5% O_2; squares = 40% CO_2/<0.5% O_2; triangles = 60% CO_2/<0.5% O_2; empty circles = 80% CO_2/20% O_2.

Mycotoxin production

Mycotoxin production was generally inhibited by the atmospheres tested. Aflatoxins were not produced by *A. flavus* in either of the atmospheres in which growth was observed, i.e. 20% CO_2/<0.5% O_2 or 80% CO_2/20% O_2. This is in agreement with other reports in the literature which observed that increased CO_2 levels inhibited aflatoxin production to a much greater degree than it did the growth of *A. flavus* in natural substrates (Landers *et al.*, 1967; Sanders *et al.*, 1968; Wilson and Jay, 1975).

Patulin was produced by *B. nivea* in 20, 40 and 60% CO_2 on PDA, but at very low levels. Paster and Lisker (1985) observed similar inhibition of patulin production by *P. griseofulvum* (synonym *P. patulum*) in low-O_2 atmospheres (1% and 5% O_2) or in 20% CO_2 with 20% O_2.

Roquefortine C was produced by *P. roqueforti* in 20% CO_2, peaking in 10–14 d (0.2–0.3 µg per agar plate) but not at higher CO_2 concentrations in the absence of O_2. Roquefortine C production was also observed in 80% CO_2/20% O_2 (0.9 µg/plate) after 30 d. *P. commune* produced low concentrations of cyclopiazonic acid (0.5 µg/plate) in 80% CO_2/20% O_2 after 30 d.

CONCLUSIONS

The work described here shows that there are important interactive effects between low-O_2 and high-CO_2 atmospheres with respect to their inhibitory effects on fungal growth and metabolism. Some fungi, for example *Fusarium* species and mucoraceous fungi (represented in this study by *M. plumbeus*) are well adapted to growth in atmospheres low in O_2 and rich in CO_2, whereas the species more likely to be found in stored grains (*Aspergillus* and *Penicillium*) are substantially inhibited under these conditions. Mycotoxin production is almost totally suppressed under all the conditions studied.

There is no single satisfactory method for estimating fungal growth under modified atmospheres. Extension of colony radius or diameter has been used to measure fungal growth in many physiological studies (Pitt and Hocking, 1977; Hocking and Pitt, 1979; Wheeler and Hocking, 1988, and others) but this does not take colony density into account and thus is not a good measure of fungal biomass. Hyphal length and colony dry weight are better measures of fungal biomass, but these measurements, together with colony diameter, are unsuitable for estimating fungal growth in foods and stored commodities. Ergosterol content has been used to estimate the extent of fungal invasion in grains and grain products (Seitz *et al.*, 1977; Schnürer, 1991), but since the formation of sterols usually proceeds via aerobic biochemical pathways, the amount of ergosterol in the membranes of fungi growing under O_2 stress may not reflect the true extent of fungal growth.

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RESPIRATION OF WHEAT GRAIN STORED IN DIFFERENT ENVIRONMENTS

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ABSTRACT

Respiratory activity in grain is usually measured by the the release of carbon dioxide (CO_2) or the uptake of oxygen (O_2) in a closed system. An automatic electrolytic respirometer, which constantly monitors O_2 uptake and allows overall measurement of CO_2 production, has allowed replicate determinations of respiration rates in 25-g samples of grain at different constant temperatures (15–35°C) and water activities (0.65–0.95 a_w). O_2 uptake increased linearly with temperature up to 35°C and with time at water activities above 0.90 a_w, but not at lower water activities. With a_w and high germinability, most respiration could be attributed to respiration by the grains themselves. However, with 0.90 a_w, germinability decreased and microbial respiration predominated. Autoclaved grain inoculated with either *Eurotium amstelodami* or *Penicillium aurantiogriseum* respired at similar rates at 0.85 a_w and 20°C, but O_2 uptake by *P. aurantiogriseum*-inoculated grain was more than ten times that of *E. amstelodami*-inoculated grain at 0.90 a_w. Comparisons of O_2 consumption and CO_2 production generally yielded respiratory quotients less than 1.0 except at 15°C. No visible mould developed after 7 d at 15°C but the amount of visible mould increased from 20 to 35°C. Up to 0.13% of the dry matter was lost before the grain was visibly mouldy whereas 0.13–1.24% of the dry matter was lost from visibly mouldy grain, the exact percentage depending on the temperature and water content.

INTRODUCTION

Respiration is a fundamental process, common to all living organisms, which provides energy for metabolism and growth. By aerobic respiration, carbohydrates are oxidised into carbon dioxide (CO_2) and water with the release of energy as per the equation: $C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 2835$ kJ. A 1% loss of carbohydrate is thus accompanied by the production of 14.7 g CO_2 kg^{-1} dry matter. Respiration over time can be measured by the uptake of oxygen (O_2), or the production of CO_2, or even as the loss of dry matter or

temperature change. The grain itself, its microflora and its insect infestations all contribute to the total respiration of stored grain, and the extent of respiration is a measure of the total metabolic activity of the system. The intensity of the process is governed by the following physical parameters: water availability, water temperature, and, to a lesser extent, O_2 concentration, the degree of microbial contamination, mechanical damage and the conditions and length of previous storage.

Microorganisms, especially fungi, are important factors in the deterioration of stored grain. They contribute greatly to total respiration at water activities (a_w) which are insufficient to support germination. However, the relative contribution of grain and microorganisms to total respiration remains controversial. Respiration of maize with 22–27% water content was reported to be considerably greater than that of its microflora (Seitz *et al.*, 1982). By contrast, relatively low and constant respiration rates were reported from mould-free wheat with 12–35% water content (Larmour *et al.*, 1935; Hummel *et al.*, 1954). However, the sterilants used might have affected physiological processes in the seed. Woodstock and Coombs (1965) found that respiration decreased by 10% when fungi were eliminated from up to 80% of the seeds by using combinations of sodium hypochlorite, phenacridine chloride and gamma irradiation.

Respiration of naturally colonised barley and maize grains with high water content has been utilised to preserve the grain for animal feed during its storage in sealed steel bins and in unsealed concrete staved silos and also during storage in underground pits (Lacey, 1971, 1972, 1988; Clarke and Hill, 1981; Hill *et al.*, 1983).

METHODS USED TO MEASURE RESPIRATION

Respiration has usually been measured by titration after absorbing CO_2 in ascarite or alkali, by using infra-red gas analysers or by monitoring O_2 production. Milner and Geddes (1945) drew 150–2000 ml of air at a constant rate over 23.5 h into a respirometer. The air was freed of CO_2 and humidified with soda lime in sulphuric acid and saturated salt solutions before being passed through grain with an appropriate water content which had been placed in a jar standing in a constant-temperature water bath. Afterwards, a gas sample was withdrawn for analysis using Haldane-Henderson gas analysis. Essentially similar methods were used by Al-Yahya *et al.* (1993) and Aljinovic *et al.* (1995), except that the CO_2 was first absorbed with KOH, the grain was stored in plexiglass tubes, the air was subsequently dried with anhydrous $CuSO_4$ and $Mg(ClO_4)_2$ (Mg perchlorate) and CO_2 was absorbed onto Sulaimanite, a mixture of KOH solution and vermiculite.

Wilcke *et al.* (1993) used a more complex apparatus to continuously monitor CO_2 evolution from fungicide-treated maize. Sample bottles, containing maize grain conditioned to the required water content and standing in temperature-controlled water baths, were connected to a compressed air supply and an infra-red spectrometer by airlines with computer-controlled valves. Airlines and bottles were individually purged with compressed air conditioned to the appropriate relative humidity by being passed through glycerol solutions for 4 min every 20 min, to prevent CO_2 from accumulating and inhibit-

ing fungal growth. The current rate of CO_2 production in each bottle was calculated from three measurements of CO_2 concentration taken for 3 min every 6 h. The resulting rates of CO_2 production were then integrated to give a rate for cumulative CO_2 production and to allow the calculation of dry-matter loss.

We used an electrolytic respirometer (Tribe and Maynard, 1989) to continuously monitor O_2 uptake by the respiring grain. When CO_2 from grain respiration was absorbed into the alkali, there was a decrease in the air pressure within the glass leaching tube mounted in a temperature-controlled water bath. This caused a saturated $CuSO_4$ solution to rise in a U-tube until the solution came into contact with a platinum anode, thus closing a circuit with a copper cathode immersed in the solution. O_2 was produced at the anode until pressure was equalised, breaking the contact with the cathode. Periods of operation were recorded electronically and converted into volumes of O_2 produced. At the end of the experiment, the amounts of CO_2 absorbed into the alkali were determined by titration, and the respiratory quotient (CO_2 evolved/O_2 uptake) and dry matter loss were calculated. In theory, the apparatus and software allowed up to 128 individual treatments to be monitored simultaneously, although only a maximum of 32 tubes was found to be manageable in practice (one or two racks of 16 tubes each per water bath). The data obtained by this method, using winter wheat grain, is reported in this paper.

RESPIRATION OF GRAIN AT DIFFERENT TEMPERATURES AND WATER ACTIVITIES

Respiration was measured during the incubation of naturally contaminated winter wheat grain, cv Avalon, at water activities ranging from 0.70 to 0.95 a_w, in 0.05 a_w steps, and at temperatures ranging from 15 to 35°C, in 5°C steps, over 160–165 h. All experiments, except those at 35°C, were repeated at least twice. The results are shown in Figs. 1 and 2.

Cumulative O_2 consumption generally increased with both a_w and temperature. However, there was an initial lag in O_2 consumption at 15–25°C and a_w followed by a period of increased activity until the end of the experiment. Otherwise, O_2 consumption increased linearly with time. Respiration was most rapid at 0.95 a_w and 25–35°C and least rapid at 0.80 a_w and 15°C. However, there was little difference in O_2 consumption between 20 and 25°C. About 100 ml O_2 were utilised by 25 g wheat grain over 160 h at 0.95 a_w/25°C and at 0.90 a_w/35°C. Measurement of CO_2 production enabled respiratory quotients to be calculated (Table 1). Except at 15°C, respiratory quotients (*RQ*) were generally in the range 0.5–1.5, especially at low a_w where *RQ* up to 5.13 were found. The mean *RQ* from all treatments was 1.11 ± 0.228, agreeing closely with other published data.

Assuming an *RQ* of 1.0, dry matter losses, calculated using O_2 uptake data, generally increased with increasing a_w and temperature (Table 2). They were greatest at 0.95 a_w/25–35°C and least at 0.80 a_w/15°C. In many of the treatments (marked in bold in Table 2), more than 1% of the grains were visibly mouldy, but this did not necessarily indicate large dry matter losses. For instance, only 0.13% of the dry matter was lost from visibly

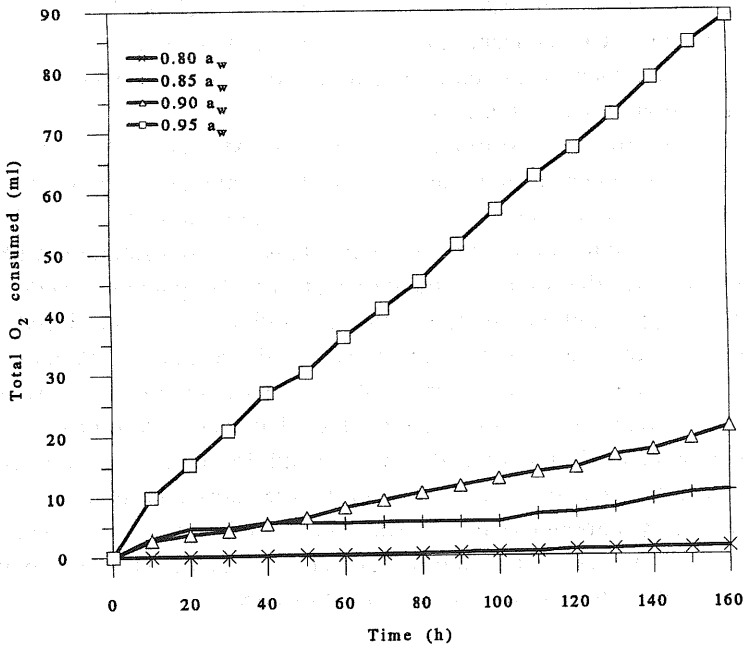


Fig. 1. Respiration of wheat grain cv Avalon at different water activities (a_w) at 20°C.

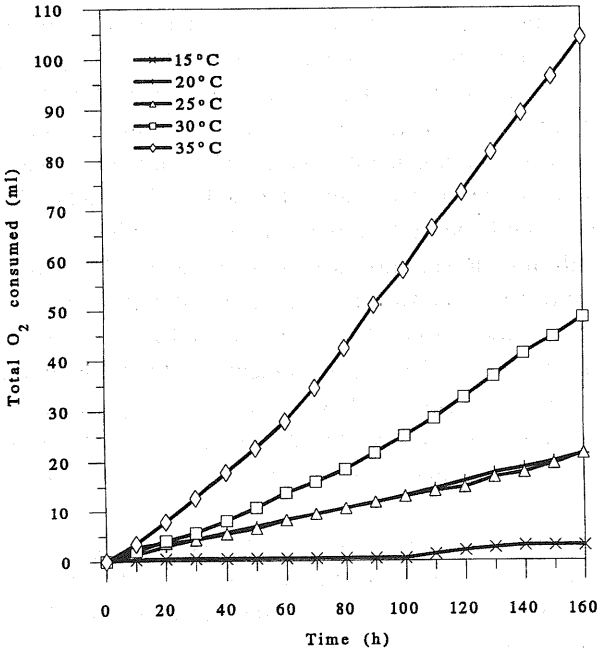


Fig. 2. Respiration of wheat grain cv Avalon at different temperatures at 0.9 a_w.

TABLE 1
Respiratory quotients calculated from the respiration of wheat cv Avalon over 160 h incubation at different a_w and temperatures

	Mean respiratory quotient ± SEM at stated temperature (°C)				
a_w	15	20	25	30	35
0.80	5.13 ± 0.91	0.68 ± 0.13	0.45 ± 0.04	0.61 ± 0.04	0.84 ± 0.04
0.85	2.50 ± 0.70	2.50 ± 0.70	0.54 ± 0.21	0.87 ± 0.07	0.67 ± 0.11
0.90	1.81 ± 0.39	0.75 ± 0.11	0.54 ± 0.17	0.90 ± 0.01	0.82 ± 0.03
0.95	1.18 ± 0.24	0.73 ± 0.02	0.59 ± 0.09	0.90 ± 0.07	1.02 ± 0.09

TABLE 2
Calculated dry matter losses in wheat cv Avalon over 160 h incubation at different a_w and temperatures

	Calculated dry matter loss (%) at stated temperature (°C)				
a_w	15	20	25	30	35
0.80	0.007	0.020	0.039	0.061	**0.133**
0.85	0.018	0.027	**0.130**	0.161	**0.372**
0.90	0.085	**0.226**	0.436	**0.347**	**0.774**
0.95	**0.517**	0.762	**1.210**	**1.187**	**1.239**

Bold text indicates visible moulding after 160 h incubation.

moulded grain at 0.85 a_w/25°C. The range of a_w supporting visible moulding increased with increasing temperature and, conversely, the range of temperatures supporting moulding increased with increasing a_w.

FUNGAL RESPIRATION

Respiration of autoclaved grain inoculated with *E. amstelodami* and *P. aurantiogriseum*, either separately or in combination, was compared at 0.85 and 0.90 a_w and at 20°C. Respiration over 350 h followed a sigmoid pattern for the fastest respiring treatments (*P. aurantiogriseum*/0.90 a_w and mixed inoculum/0.90 a_w) but failed to reach the plateau phase for others. The least O_2 was consumed by *E. amstelodami* alone at 0.90 a_w and the most by the mixed culture at 0.90 a_w, although there was no significant difference from *P. aurantiogriseum* alone. Respiration of *E. amstelodami* over 350 h incubation at 0.85 a_w was more than twice that at 0.90 a_w, although there was little difference up to 250 h. Respiration of *P. aurantiogriseum*-inoculated grain differed little from that with *E. amstelodami* at 0.85 a_w but increased more than tenfold at 0.90 a_w. Respiratory quotients

ranged from 0.75 to 0.89 and calculated dry matter losses after 14 d ranged from 0.06% (*E. amstelodami*/0.90 a_w) to 0.80% (mixture/0.90 a_w).

DISCUSSION AND CONCLUSIONS

Respiration has frequently been used to measure grain deterioration (Paster *et al.*, 1992) and the effectiveness of physical and chemical methods of mould prevention (Al-Yahya *et al.*, 1993; Wilcke *et al.*, 1993; Aljinovic *et al.* (1995). It has also been used to assess dry matter losses during storage. Only 0.5% dry matter loss in maize is sufficient to indicate that the grain is unfit for use (Saul and Lind, 1958; Saul and Steele, 1969), and such losses may occur before moulding is visible (Seitz *et al.*, 1982). Storage life before quality loss becomes unacceptable is a function of kernel damage, water content and temperature, expressed by the following formula: $\theta = \theta_R \times M_T \times M_W \times M_D$, where θ is the allowable storage time before 0.5% dry matter loss, θ_R is the elapsed time for maize grain with 25% water content and 30% of the kernels mechanically damaged to lose 0.5% dry matter at 15.5°C. M_T, M_W and M_D are multipliers used to correct for actual temperature, water content and mechanical damage determined experimentally. Storage life decreases as temperature, water content and mechanical damage increase. Such multipliers and the respiration data on which they are based have been incorporated into models of ambient air drying of cereal grains (Thompson, 1972; Stroshine and Yang, 1990). Allowable dry matter losses for wheat have ranged from 0.1 to 2% (Kreyger, 1972; Hall and Dean, 1978; White *et al.*, 1982). However, although White *et al.* (1982) predicted that wheat with 18.4% water content could be stored safely for 55 d, visible moulding appeared after only 23 d, suggesting that 0.04% dry matter loss was the limit for acceptability. Bailey (1940) suggested that respiration rate might be proportional to kernel size. Brook (1987) calculated that 0.085% dry matter loss in wheat was equivalent to 0.5% loss in maize. Our results tend to support the conclusions of Brook (1987).

The results obtained with the automatic electrolytic respirometer agree well with those reported previously by different authors (Fig. 3). Although respiration rates described by Milner *et al.* (1947), Scholz (1962) and Kittock and Law (1967) were much faster than those found in our experiments, they were also outside the range of other data. Kittock and Law (1967) were studying seed germination, which may account for the faster respiration in their experiments, but the reasons for other deviations are not known. Respiration rates determined by White *et al.* (1982) were slower than those in our experiments, but they sampled only three to five times per week, depending on temperature, and it is possible that CO_2 concentrations became inhibitory to respiration. The mean repiratory quotient agrees well with previous reports, but the high value at low temperatures needs to be explained. Allowance may need to be made for the absorption of CO_2 by the grain (Cofie-Agblor *et al.*, 1995).

In a personal communication, Nellist and White have taken our respiration data and calculated the results as mg O_2 d^{-1} kg^{-1} dry grain (R) divided by incubation temperature (θ) to give R/θ with units of mg O_2 d^{-1} kg^{-1} dry matter $°C^{-1}$. There was a linear relationship

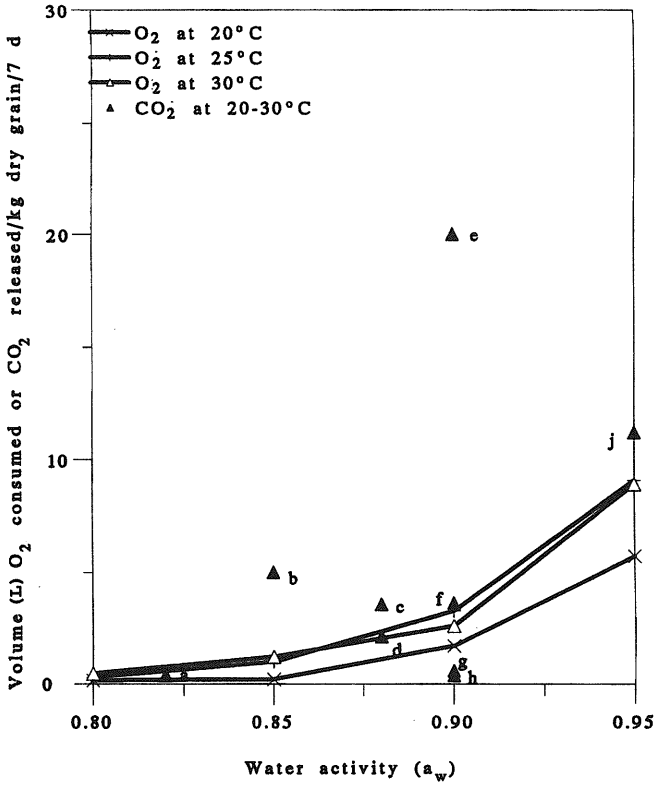


Fig. 3. Respiration of wheat grain cv Avalon measured in electrolytic respirometer over 7 d compared to published data on wheat grain respiration: a, Bailey (1940); b and e, Scholz (1962); c and f, Milner *et al.* (1947); d, Larmour *et al.* (1935); g and h, White *et al.* (1982); j, Woodstock and Justice (1967).

(r^2 = 0.9594) between R/θ and a_w (Fig. 4), corrected for the effects of temperature following Chen and Morey (1989), even though the calculated a_w at 0.90 and 0.95 a_w and 25°C was greater than that indicated by the water content/water activity isotherms determined experimentally for the grain used. This relationship was then expressed in the following equation, which is to be inserted into models of ambient air drying of wheat grain in predicting storage life and dry matter losses:

$$C = \frac{a_1 + a_2}{Y\,(1 + \exp(-(a_5 + a_6 t + a_7\theta)\,(w - a_8)))}$$

where C = cumulative O_2 consumption (mg O_2 kg^{-1} dry matter), t = time (h), w = water content (% wet basis), θ = temperature (°C), $Y = 1 + \exp(a_3(a_4 - \theta))$, $a_1 = 345.83$, $a_2 = 125.2$, $a_3 = 0.1737$, $a_4 = 20.33$, $a_5 = 0.9143$, $a_6 = -0.001036$, $a_7 = -0.013634$, $a_8 = 24.38$.

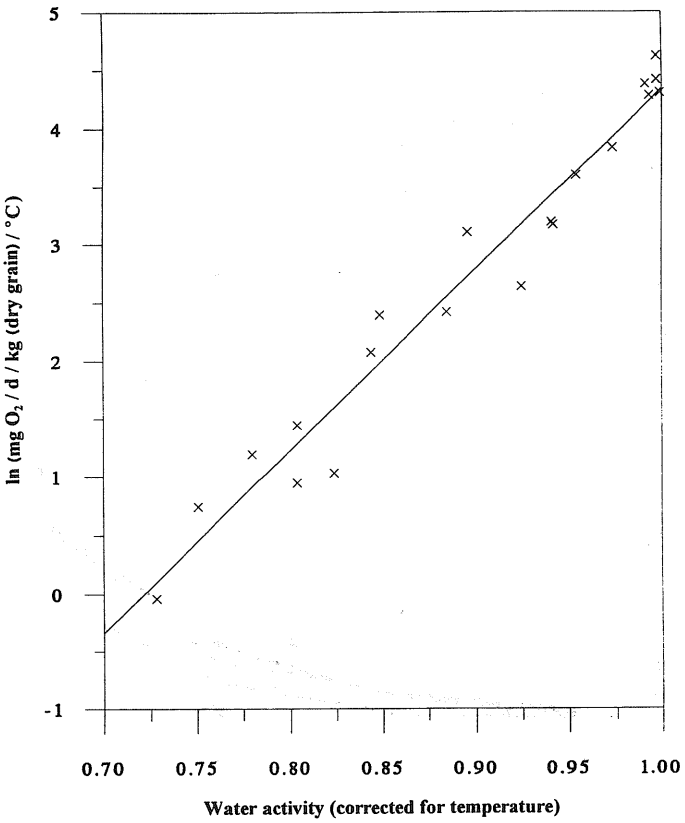


Fig. 4. The ratio of O_2 consumption rate to temperature at different a_w, corrected for temperature, in wheat grain cv Avalon after 7 d incubation.

This data could also be used in controlled atmosphere storage to predict how much the grain contributes to overall CO_2 concentrations and the time needed to produce inhibitory atmospheres in high moisture grains stored for animal feeds. However, further work is necessary to determine the concentrations of CO_2 at which respiration is inhibited and the effects of diurnal temperature fluctuations.

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EFFECT OF CONTROLLED ATMOSPHERES AND FUMIGANTS ON STORAGE FUNGI — A REVIEW OF RESEARCH ACTIVITIES AT SEAMEO BIOTROP

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ABSTRACT

Controlled atmospheres and fumigation are primary techniques of insect control, but little research has been done on the effects of carbon dioxide (CO_2) and fumigants on the development of fungi in stored products. Research has been carried out on the effects on storage fungi of CO_2 in maize, of phosphine (PH_3) in milled rice, maize and soybean meal, and of methyl bromide (MB) in milled rice and soybeans. The effects of CO_2 and PH_3 on the mycelial growth of *Aspergillus flavus* in pure culture, and on aflatoxin production in stored commodities, have also been studied. These studies yielded the information detailed below.

CO_2 had no significant effect on either the total fungal population or the population of individual fungal species infecting maize stored under warehouse conditions excepting *Eurotium chevalieri*, whose population was reduced during storage. PH_3 reduced the population of *A. wentii* but increased the population of *E. chevalieri* on maize after fumigation. PH_3 fumigation of soybean meal reduced fungal population; however, its effect was not persistent. Although fungal population was reduced immediately after fumigation, it then increased again after a certain period in storage. PH_3 retarded the growth of *A. penicillioides*, a predominant fungal species of milled rice stored in jute and polypropylene bags. MB reduced both the total fungal population and the individual populations of *E. chevalieri* and *E. rubrum* in milled rice 2 d after fumigation. However, populations increased again 45 d after fumigation. The fumigant also reduced both the total fungal population and the individual populations of *A. sydowii* and *E. chevalieri* on soybeans 2 d after fumigation. A mixture of 20% CO_2 and 0.5 mg/L PH_3 began to inhibit mycelial growth of *A. flavus* in pure culture. At 80% CO_2 and 3.5 mg/L PH_3, mycelial growth was almost totally inhibited.

INTRODUCTION

During storage, products may be infested by insects, mites, microorganisms and rodents. Among microorganisms, fungi are the most important cause of the deterioration of stored products.

In the tropics, *Eurotium* species and other *Aspergilli* are dominant, and *Penicillium* species play only a minor role (Pitt and Hocking, 1991). They can cause weight loss, seed discoloration, heating, mustiness and the production of mycotoxins, the most important of which are aflatoxins produced by *Aspergillus flavus* and *A. parasiticus*.

Controlled atmospheres and fumigation are primary techniques of insect control, but little is known about the effects of carbon dioxide (CO_2) and fumigants on either the development of fungi or aflatoxin production in stored products. CO_2 is more often used for controlling insects during long-term storage of milled rice; the fumigants phosphine (PH_3) and methyl bromide (MB) are effective for controlling insects during long-term storage of products.

This paper describes the research results on the effects of CO_2, PH_3 and MB on the development of fungi in milled rice, maize, soybeans and soybean meal. The effects of CO_2 and PH_3 on aflatoxin production are also described.

REVIEW OF PUBLISHED STUDIES

The effects of CO_2 on mycelial growth and aflatoxin production of *A. flavus* in pure culture

Dharmaputra *et al.* (1990a) studied the effects of CO_2 concentrations of 20, 40, 60 and 80% on mycelial growth and aflatoxin B_1 production of three *A. flavus* isolates (BIG-16, BIG-17 and BIG-18). As a control, these fungal isolates were maintained in air. The CO_2 concentrations significantly affected both mycelial growth and aflatoxin production of the isolates of *A. flavus*. CO_2 at 20% started to inhibit the two parameters (Figs. 1 and 2), and all parameters decreased with increased CO_2 concentration. At 80% CO_2 mycelial growth of isolate BIG-17 was almost totally inhibited (Fig. 1). Aflatoxin production of the three isolates was also reduced with increased CO_2 concentration (Fig. 2).

The effects of PH_3 on mycelial growth and aflatoxin production of *A. flavus* in pure culture

An investigation was carried out by Dharmaputra *et al.* (1991) on the effects of PH_3 on both mycelial growth and aflatoxin B_1 production of two *A. flavus* isolates (BIG-17 and BIG-18). The concentrations of PH_3 used were 0.5, 1.5, 2.5 and 3.5 mg/L. As a control, these fungal isolates were maintained in air.

The PH_3 concentrations significantly affected both the mycelial growth and aflatoxin production of the isolates of *A. flavus*. Isolate BIG-17 was more sensitive than isolate BIG-18 (Fig. 3). Mycelial growth decreased with increasing PH_3 concentrations. Inhibition of mycelial growth commenced at 0.5 mg/L, and isolate BIG-17 was almost totally inhibited at 3.5 mg/L (Fig. 3).

Aflatoxin production decreased with increasing PH_3 concentration (Fig. 4). Although the two isolates were still able to produce aflatoxin after treatment with 3.5 mg/L, the amounts were low.

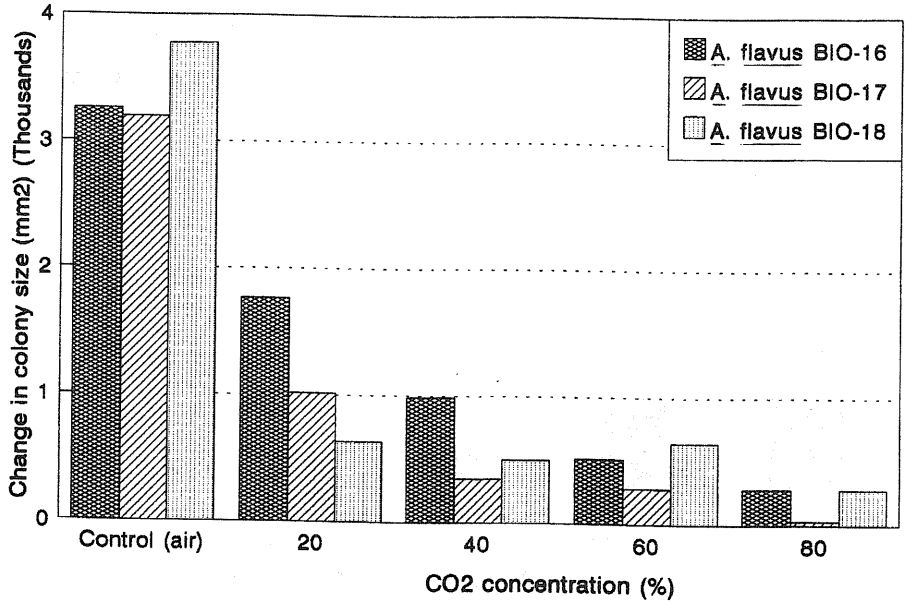


Fig. 1. Mycelial growth of *A. flavus* BIO-16, BIO-17 and BIO-18 after treatment with various concentrations of CO_2 for 7 d on potato dextrose agar (Dharmaputra *et al.*, 1990a).

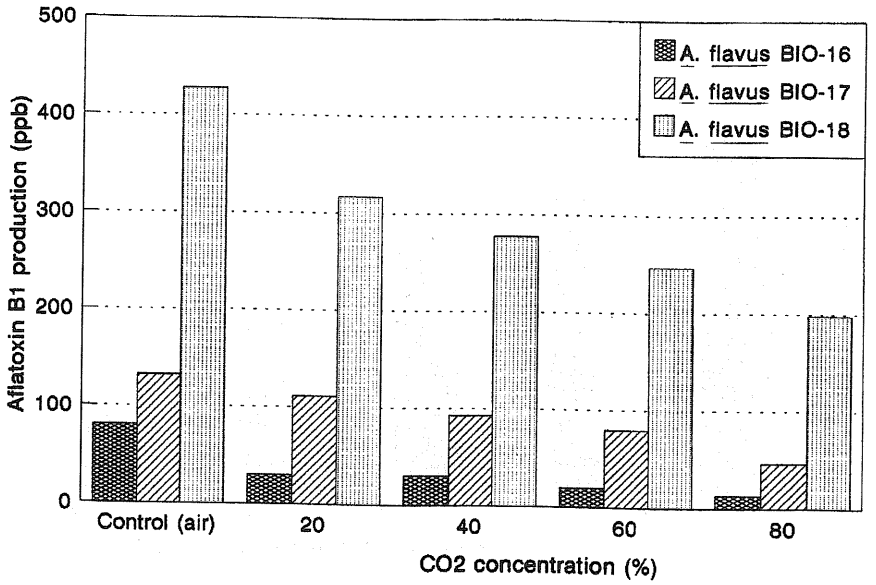


Fig. 2. Aflatoxin B_1 production of *A. flavus* BIO-16, BIO-17 and BIO-18 after treatment with various concentrations of CO_2 for 10 d on 10% coconut extract medium (Dharmaputra *et al.*, 1990a).

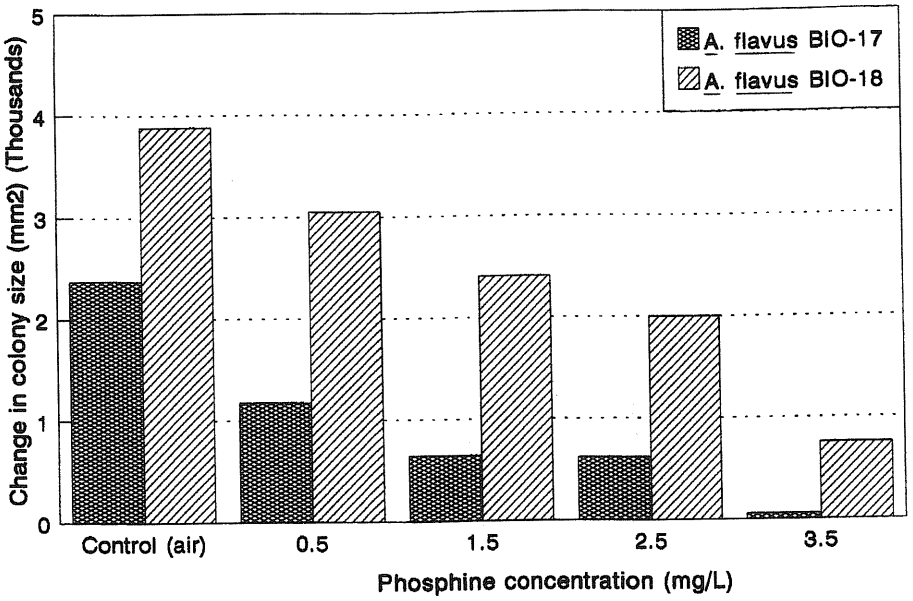


Fig. 3. Mycelial growth of *A. flavus* BIO-17 and BIO-18 after treatment with various concentrations of PH_3 for 5 d on potato dextrose agar (Dharmaputra *et al.*, 1991a).

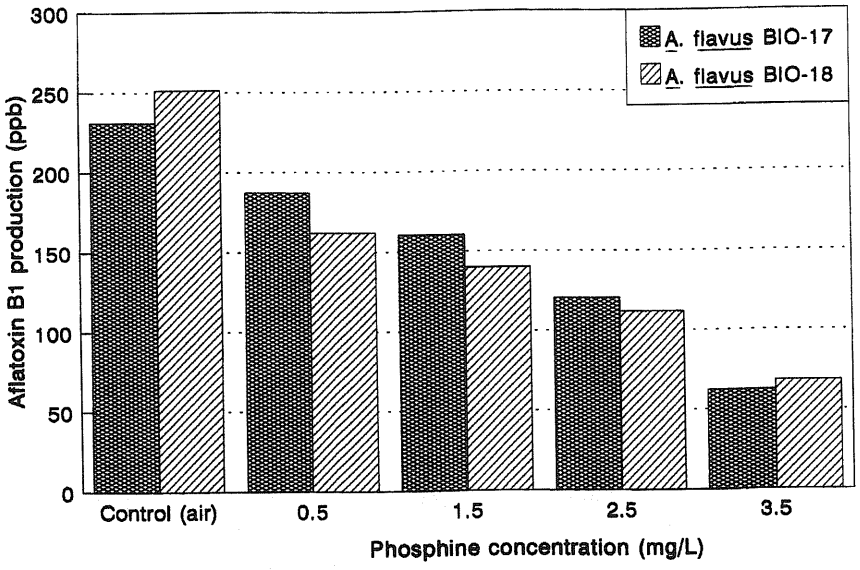


Fig. 4. Aflatoxin B_1 production of *A. flavus* BIO-17 and BIO-18 after treatment with various concentrations of PH_3 for 5 d on 10% coconut extract medium (Dharmaputra *et al.*, 1991a).

The effects of CO_2 on the development of fungi and aflatoxin production in stored maize

Dharmaputra *et al.* (1990b) studied the effects of CO_2 on the development of fungi and aflatoxin production in stored maize. Stacks of stored maize were sealed with PVC sheeting and treated with CO_2 for storage periods varying from 10 to 120 d. The concentration of CO_2 used was 2.4 kg/t. The control groups consisted of both stacks of maize sealed in plastic sheets, but not treated with CO_2, and stacks not sealed in plastic sheets. Twelve species of fungi were isolated from the stored maize using the dilution method. They were *A. candidus*, *A. flavus*, *A. niger*, *A. penicillioides*, *A. tamarii*, *A. versicolor*, *A. wentii*, *Cladosporium cladosporioides*, *Eurotium chevalieri*, *E. repens*, *Mucor hiemalis* and *Penicillium citrinum*.

The concentration of applied CO_2 had no significant effect on either the total population of fungi or the individual population of each fungal species, with the exception of *E. chevalieri*, whose population was reduced (Fig. 5). The total population of fungi increased significantly with the prolongation of storage duration.

The aflatoxin B_1 content of maize, whether in plastic sheets and treated with CO_2 or only in plastic sheets, was lower than that of the unsheeted and untreated stacks (Fig. 6). The control showed that the aflatoxin content increased with the increasing duration of storage.

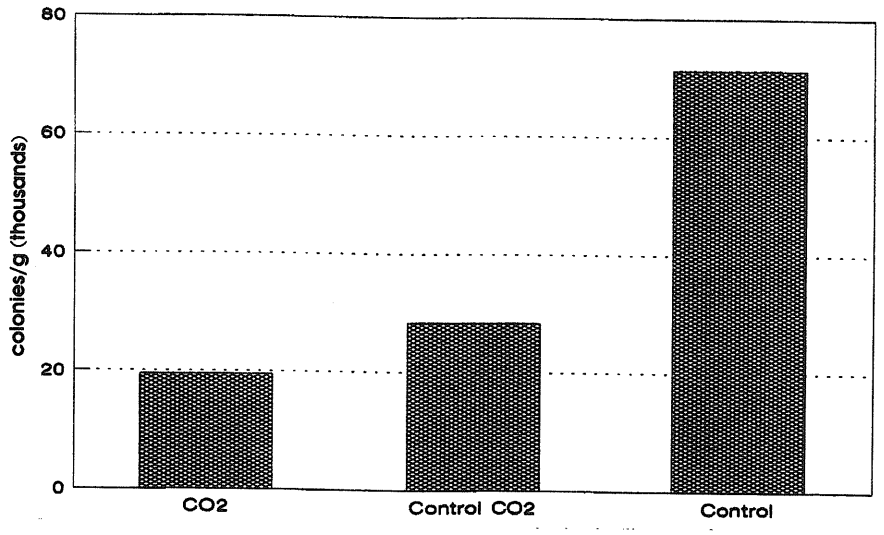


Fig. 5. Population of *Eurotium chevalieri* on maize treated with CO_2 and control (Dharmaputra *et al.*, 1990b). Control CO_2 = stacks of maize sealed in PVC sheeting but not treated with CO_2; control = stacks not sealed.

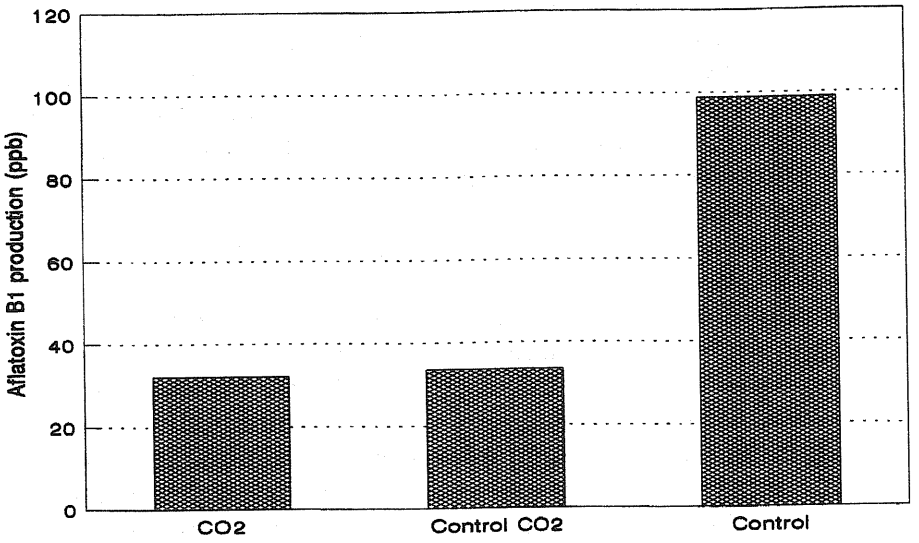


Fig. 6. Aflatoxin B_1 production on maize treated with CO_2 and control (Dharmaputra *et al.*, 1990b). Control CO_2 = stacks of maize sealed in PVC sheeting but not treated with CO_2; control = stacks not sealed.

The effects of PH_3 and bag type on the development of fungi in stored milled rice

An investigation was carried out by Dharmaputra *et al.* (1997) on the effects of PH_3 and bag type on the development of storage fungi in milled rice. The milled rice was stored in either jute or open-weave polypropylene bags for 49 weeks. The rice was fumigated with PH_3 at a dosage of 10 tablets/stack or 2 tablets/t for an exposure period of 5 d. Fumigation was repeated at 3-month intervals throughout the experiment.

The fungal population on rice was determined before each fumigation (at the beginning of storage and then at 11, 23, 35 and 47 weeks of storage) and immediately after fumigation (at 1, 13, 25, 37 and 49 weeks of storage).

During the 49 weeks of storage, 21 and 22 species of fungi were isolated from milled rice packed in jute and polypropylene bags, respectively. The predominant fungus in both bag types was *A. penicillioides*, which increased sharply in the first week; thereafter, the population remained relatively constant, both before and after fumigation (Fig. 7).

On milled rice packed in jute bags both the total fungal count and that of *A. penicillioides* were lower than on milled rice packed in polypropylene bags. The populations increased until the eleventh week of storage; thereafter, populations declined slowly with lengthening storage periods.

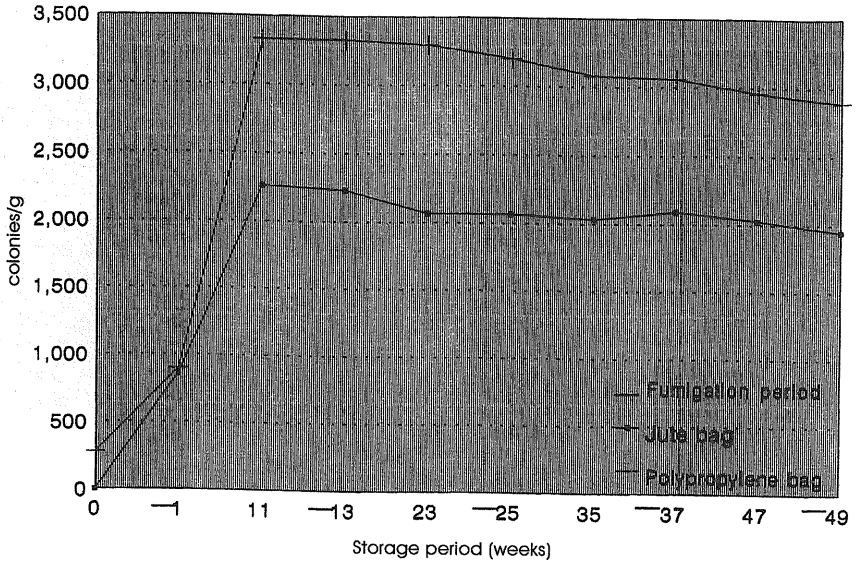


Fig. 7. Population of *A. penicilloides* on milled rice packed in jute or polypropylene bags both before and after fumigation with PH_3 (Dharmaputra *et al.*, 1996).

The effects of PH_3 on the development of fungi and aflatoxin production in stored maize

Dharmaputra *et al.* (1992a) studied the effects of PH_3 on both the development of fungi and aflatoxin production in stored maize. Stacks of stored maize were treated with 2 g/t PH_3 for 5 d. The control consisted of stacks of maize not treated with PH_3.

Using the dilution method, 11 species of fungi were isolated: *A. candidus*, *A. flavus*, *A. niger*, *A. tamarii*, *A. versicolor*, *A. wentii*, *C. cladosporioides*, *E. chevalieri*, *E. repens*, *M. hiemalis* and *P. citrinum*. PH_3 at the stated concentration reduced the population of *A. wentii* but increased that of *E. chevalieri* (Fig. 8). There was no significant difference between the stacks treated with PH_3 and the control in the aflatoxin B_1 content of maize (Table 1).

The effects of PH_3 on the development of fungi and aflatoxin production in stored soybean meal

Investigations were carried out by Dharmaputra *et al.* (1992b, 1993) on the effects of PH_3 on both the development of fungi and aflatoxin production in stored soybean meal. Soybean meal was stored in polypropylene bags for 190 d. Four stacks were treated with PH_3 (2.1 g/t) for 5 d, once at the beginning of storage and again after 95 d. Four untreated stacks served as control.

Using the dilution method, 17 species of fungi were isolated from the stored soybean

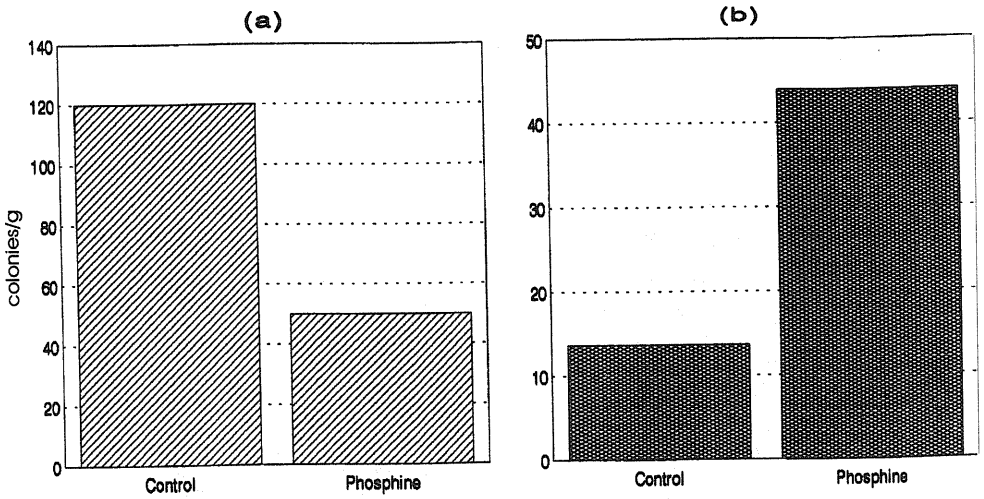


Fig. 8. Population of *A. wentii* (a) and *Eurotium chevalieri* (b) on maize treated with PH_3 and on control (Dharmaputra *et al.*, 1992a).

TABLE 1

The effects of treatment with PH_3 and fumigation period, and their interaction, on aflatoxin production on maize (Dharmaputra *et al.*, 1992a)

Effect	Aflatoxin B_1 production (ppb)
Treatment	
Control	38.87 a
Phosphine	37.78 a
Fumigation period	
0 d	24.43 a
5 d	26.22 a
Interaction between treatment and fumigation period	
Control, 0 d	36.76 a
Control, 5 d	39.98 a
Phosphine, 0 d	32.10 a
Phosphine, 5 d	43.46 a

Numbers followed by the same letter do not differ significantly according to Tukey's Multiple Comparison Test at 95% confidence level.

meal. The predominant species found in both treated and untreated stacks were *A. sydowii*, *E. chevalieri* and *Wallemia sebi*. There were fluctuations in the total populations of fungi during storage, both in the stacks treated with PH_3 and the untreated ones. The total populations decreased after treatment but then increased during storage (Fig. 9).

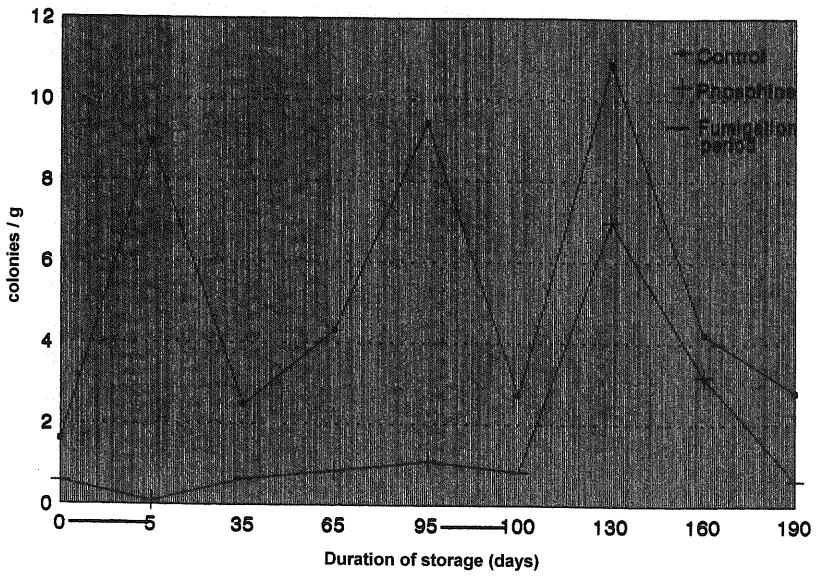


Fig. 9. Total population of fungi on soybean meal treated with PH_3 and on control (Dharmaputra *et al.*, 1992b, 1993).

There was a significant difference in aflatoxin B_1 production between the treated and the untreated samples (Table 2). Aflatoxin B_1 production increased during prolonged storage in both treated and untreated soybean meal (Fig. 10). These results indicate that PH_3 somehow inhibits aflatoxin B_1 production.

TABLE 2
Aflatoxin B_1 content on soybean meal treated with PH_3 and on control (Dharmaputra *et al.*, 1992b)

Duration of storage (d)	Control (ppb)	PH_3 (ppb)	F-value
0[a]	5.82	0.00	–
5	14.49	9.42	0.46
35	18.35	9.92	14.77*
65	18.42	11.21	23.50**
95[b]	22.42	12.67	10,255.91**
100	24.94	14.06	32,828.57**
130	27.85	14.84	497.96**
160	30.25	15.28	1,625.72**
190	31.12	23.55	10.33*

a = 1st PH_3 treatment; b = 2nd PH_3 treatment; * = significant difference at 95% confidence level; ** = significant difference at 99% confidence level.

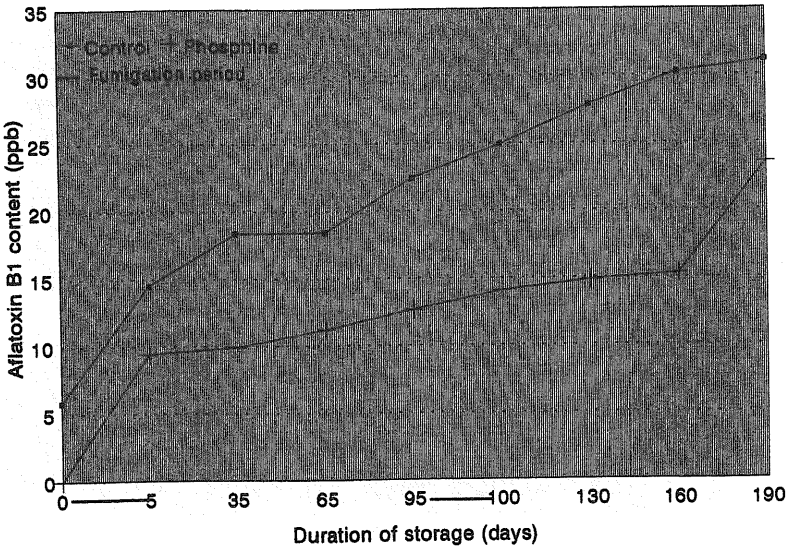


Fig. 10. Aflatoxin B_1 content on soybean meal treated with PH_3 and on control (Dharmaputra *et al.*, 1992b).

The effects of MB on fungi of stored milled rice and soybeans

Dharmaputra and Retnowati (1993) studied the effects of MB on fungal populations of stored milled rice and soybeans. Milled rice and soybeans were treated with 21 g/t MB for 24 h.

Both the total fungal population and the individual populations of *E. chevalieri* and *E. rubrum* in milled rice showed a decrease 2 d after fumigation but again increased 45 d after fumigation (Table 3). *A. niger* and *A. wentii* were not isolated either 2 d or 45 d after fumigation. The population of *A. flavus* decreased 2 d and again 45 d after fumigation, whereas populations of *A. versicolor* and *C. cladosporioides* increased. In soybeans, both the total fungal population and the individual populations of *A. sydowii* and *E. chevalieri* showed decreases 2 d after fumigation (Table 4).

CONCLUSIONS

In pure culture, CO_2 and PH_3 inhibited both mycelial growth and aflatoxin production of *A. flavus*. CO_2 at 80% had no significant effect on the total fungal population of maize but did reduce populations of certain individual species. The concentration of CO_2 which was applied reduced aflatoxin production.

PH_3 at 2 g/t reduced the population of certain fungal species and increased that of others; however, at the concentration applied, there was no significant difference in the treated and untreated samples in aflatoxin content of maize.

TABLE 3
Population of each fungal species on milled rice before and then 2 and 45 d after fumigation with methyl bromide (Dharmaputra and Retnowati, 1993)

Fungi	Fungal population (colonies/g)[1]		
	Before fumigation	2 d after fumigation	45 d after fumigation
A. flavus	19.2 a	1.4 b	0.7 b
A. niger	10.9 c	0.0 d	0.0 d
A. versicolor	0.0 e	2.4 e	9.3 f
A. wentii	6.5 g	0.0 h	0.0 h
C. cladosporioides	2.6 i	3.6 i	24.3 j
E. chevalieri	18.9 k	0.9 l	4.8 m
E. rubrum	6.2 n	0.0 o	0.3 o
Total[2]	100.1 p	42.4 q	195.1 r

[1]Numbers followed by the same letter do not differ significantly according to Tukey's Multiple Comparison Test at 95% confidence level.
[2]Total population of isolated fungi.

TABLE 4
Population of each fungal species on soybean before fumigation and 2 d after fumigation with methyl bromide (Dharmaputra and Retnowati, 1993)

Sampling time	Fungal population (colonies/g)[1]		
	Aspergillus sydowii	*Eurotium chevalieri*	Total[2]
Before fumigation	38.3 a	60.7 a	101.8 a
Two days after fumigation	10.7 b	1.0 b	24.0 b

[1]Numbers followed by the same letter do not differ significantly according to Tukey's Multiple Comparison Test at 95% confidence level.
[2]Total population of isolated fungi.

In soybean meal, PH_3 at 2.1 g/t reduced the total fungal population, but its effect was not persistent. It seems that PH_3 at this dosage level inhibits aflatoxin production.

On milled rice, 2 tablets/t of PH_3 retarded fungal growth. On milled rice and soybeans, 21 g/t of MB reduced populations of some fungal species.

ACKNOWLEDGEMENTS

The author gratefully acknowledges the research funds provided by the National Logistics Agency (BULOG), Jakarta, Indonesia. The author is also thankful to Dr M. Sidik and Dr H. Halid of the Ministry of Food Affairs/BULOG, Jakarta, to the scientists and technicians of the Laboratory of Stored-Product Pests, SEAMEO BIOTROP, to SEAMEO BIOTROP, P.T. Aneka Gas Industry, Jakarta, and to the Cyprus Organizing Committee for the financial assistance which enabled participation at the CAF Conference.

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EFFECT OF GASTIGHT STORAGE ON GROWTH OF FUNGI IN PADDY STORED OUTDOORS

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ABSTRACT

Due to high temperature and relative humidity in the Philippines, significant losses occur in stored paddy as a result of mold contamination, a loss exacerbated when grains are not properly or uniformly dried before storage. This led to the investigation of the effect of hermetic storage using gastight flexible liners on population changes of fungi in dry paddy (below 14% moisture content) and of the growth of mold in paddy with intermediate moisture content (18%) when the paddy is stored outdoors.

The concept of gastight storage was tested using especially designed enclosures and employing heavy duty flexible liners developed in Israel. Field evaluations showed that insect infestation was controlled in bagged paddy, with the added benefit that mold growth was controlled. Following one-and-a-half to six months in storage, most fungi generally did not develop. This may be attributed to the effect of the modified atmosphere generated inside the gastight liners. Higher fungal counts were noted at the periphery and top grain layers in the gastight enclosures. Further observations revealed that the proliferation and prevalence of mold species was affected by both storage time and the modified atmosphere present inside the gastight liners.

INTRODUCTION

The growth of fungi in paddy often leads to loss in grain quality. In the Philippines, the problem is exacerbated during the rainy season when immediate drying is not possible, leading to storage of non-uniformly dried grain or paddy of intermediate moisture contents (m.c.) of about 18%, that is frequently termed moist paddy.

A shortage of permanent storage facilities has promoted the development of temporary storage systems. Heavy duty flexible liners developed in Israel were examined for gastight storage of paddy in the open in order to provide alternative temporary or emergency

storage facilities which would both maintain grain quality and prevent the growth of insects and microflora. These storage facilities are intended for use by farmer organizations, cooperatives and grain processors. Field evaluations conducted in the Philippines have demonstrated that this flexible and transportable structure, using the principle of gastight storage, can preserve the quality of paddy, maintain its viability and keep insect infestation and damage below the economic threshold level. The effect of gastight storage on the growth of mold in dry (14% m.c. and below) and intermediate moisture (18% m.c.) paddy stored outdoors will be discussed in this paper.

MATERIALS AND METHODS

Site and duration of study

This study was conducted on nine 14-t capacity Volcani cubes, two 31-t capacity plastic Volcani silos and two 5-t capacity control stacks during a series of field trials conducted at the Naphire compound, Nueva Ecija, Philippines, from October 1991 to January 1994.

Grain supplies

Locally grown paddy packed in 50-kg polypropylene bags was used in the field trials. Paddy of mixed varieties was obtained from the National Food Authority, Cabanatuan City, and from the farmers of Muñoz, Nueva Ecija. The dry and moist paddy had 14% and 18.21% m.c., respectively. The duration of storage was 3–6 months for the dry paddy and 1.5 months for the moist paddy.

The storage structures

a. *Volcani storage cubes*. Flexible cube-shaped envelopes were designed for stack storage in which the stack itself forms the rigid structure of the system. The cube-shaped structures were planned for use on open ground under rigorous field conditions. The Volcani cubes consist of two sections: a lower floor-wall and an upper roof-wall. For this purpose, a UV-protected heavy-duty plastic tarpaulin of food-grade quality was manufactured in two sections. The lower section was laid on the ground and the bags of grain were placed directly on the floor area, pallets not being required. The dimensions of the floor section determined the size of the stack to be built. After the stack had been built up to the required height, the top tarp was placed over the stack. The wall-margin of the bottom tarp was raised to meet the overhang of the upper tarp half way up the side. Both the "undersection" and the "oversection" were provided with a gastight multiple tongue and groove zipper with which to zip the sections together, forming a continuous envelope. Made of polyurethane, the zipper has the advantage of being pliable over a wide range of temperatures and is resistant to deformation.

The design was intended to be user-friendly so its dimensions are such that neither mechanical loading nor high stacking are required. Special tension straps, designed to take up slack in the walls and pull the liner tight around the curve of the sacks at floor level, are situated around the cube. This prevents rodents from gaining a tooth-hold on the

slippery surface and thereby prevents damage to the hermetic seal. The cubes used in the trials were either all sections of PVC or top sections of CPE and bottom sections of PVC.

The "20-t" cubes measured 4.45 × 3.36 × 2.00 m (l × w × h) with a maximum storage volume of approximately 30 m^3. They weighed approximately 76 kg when empty. Each cube was provided with a light carrying bag for storage and protection from rodent attack when it was not in use.

b. *Volcani plastic silos.* The silos were constructed of two units: a welded-mesh circular wall, formed from sections bolted together to provide the structural enclosure, and an inside liner, made of heavy duty PVC sheeting welded to form a continuous wall-floor unit into which the grain was to be placed. The lining came in two sections: a lower wall-floor unit and an upper liner forming a roof cone. The roof cone was secured to the metal welded-mesh walls by ropes. The enclosures were also provided with hooks to which the wire mesh could be fixed. These liners, when zipped together with a gastight zipper, provided the gastight seal for the storage container. The silos were designed for either bulk or bag storage and mechanical loading or unloading. The intention was to provide a useful transition phase between bag and bulk handling. The silos used in these experiments had a diameter of 5.25 m and a wall height of 2.2 m with a storage volume of 52 m^3 and a capacity of 35–40 t, depending on commodity.

c. *Gas applicators.* Each plastic structure was provided with a gas applicator that consisted of a ball-and-socket gas tap, attached to the expansion chamber, that passed through the plastic sheeting into the cube and was screwed onto the tarp with a gasket seal. The tap may be connected directly to the gas cylinder by tubing provided with snap-on connectors. The cube is adaptable to modified atmosphere (MA) treatments; gas, preferably carbon dioxide (CO_2), can be applied in liquid form fairly rapidly for flushing through the stack and displacing the intergranular air which is expelled through an upper screw-capped opening situated in the opposite corner of the oversection.

d. *Control stacks.* An ordinary flat tarpaulin sheet was used to cover the 5-t control stacks set up on pallets in the open.

Preparation of storage site

A level plot of ground was selected and cleared of sharp objects. A 2-cm layer of rice hull ash followed by a 4-cm layer of rice hulls was laid down in an area corresponding to that upon which the cube was to be erected. The rice ash and hulls were intended to protect the plastic sheeting from punctures by sharp objects, rodents and (to a limited extent) termites. Rice hulls, an agricultural waste, are readily available in many parts of the Philippines.

Construction and insulation of stack

The bottom section was spread on the ground and the bulk grain or bags of grains were loaded directly on the liner. Pallets were not required. After the stack had been built up to

the required height, the top section was placed over the stack so the overhang met the raised margin of the lower section halfway up the side. The two sections were then zipped together to form the envelope. The stacks were pyramidal to allow rain water to run-off down the sides of the enclosure. With Volcani cubes, the top layer of the stack was insulated with 2–3 layers of sacks containing rice hulls. With plastic silos, the cone-shaped top surface was covered with a plastic sheet upon which 1–2 layers of bagged rice hulls were placed. The plastic sheet was designed to catch any condensed moisture that might seep through the insulator, thereby preventing the grain bulk from getting wet. The layers of rice hull in both cases were used to reduce temperature gradients within the grain mass.

Measurement of gas concentration and temperature

Changes in the levels of CO_2 and oxygen (O_2) inside the gastight dry-paddy Volcani cubes and plastic silos were measured every 2 weeks, using a Gow-Mac gas analyzer and Bacharrach Oxygen meter, respectively. The concentrations of the same gases in the gastight enclosures containing intermediate m.c. grain were recorded daily for the first 10 d and then every second day for 20 d. Subsequent monitoring was done at 5-d intervals.

Temperature measurements were made from thermocouple cables inserted at different points within the paddy stacks.

Sampling

Initial samples were collected during the building of stacks in both the Volcani cubes and the plastic silos, and final sampling was done when the stacks were opened. Using sampling spears, three composite samples of 1 kg each were collected from each bag in each stack. Samples from the Volcani cubes were also obtained from the periphery (4 sides), the top (uppermost layer) and the core (middle and bottom). Additional samples from the plastic silos were taken from the periphery (east and west), the core (top, middle and bottom) and the uppermost layer of the cone. Composite samples were used to determine the general condition of the stack; representative samples were used to assess grain quality at pre-determined points.

Microbial analysis

The enumeration of mold species and the extent of mold infection were assayed by plating ten seeds, taken at random from composite and representative samples, on each of the following media for the isolation of mold: Aspergillus Flavus and Parasiticus Agar or AFPA (Pitt *et al.*, 1983); Dichloran Chloramphenicol Peptone Agar or DCPA (Nash and Snyder, 1962); Dichloran Rose Bengal Chloramphenicol Agar or DRBC (King *et al.*, 1979) and Dichloran 18% Glycerol or DG18 (Hocking and Pitt, 1980). As the names suggest, AFPA is for isolating *Aspergillus flavus* and *A. parasiticus*, whereas DCPA is for isolating *Fusarium*, *Alternaria* and other "field" molds. *Aspergillus*, *Penicillium* and other "storage" molds can be isolated by DRBC, and xerophilic mold by DG18. Samples were surface sterilized with 0.5% sodium hypochlorite before plating. Paddy plated on AFPA

were incubated for 48 h at 25°C; those plated on other media were incubated for 7 d at the same temperature. Identification of *Aspergillus* species was based on Raper and Fennel (1965), *Penicillium* species on Pitt (1985), and *Fusarium* species on Burgess *et al.* (1988) and Singh *et al.* (1991). The following keys were consulted: Barnett and Hunter (1972), Ainsworth (1965), Sutton (1980), Commonwealth Mycological Institute (1978) and Klinch and Pitt (1988).

RESULTS AND DISCUSSION

Mold growth in dry paddy

The various mold species associated with dry paddy are designated in Table 1. The most prevalent molds, with a frequency of occurrence from 14% to 93%, were *A. flavus*, *A. niger*, *A. japonicus*, *A. fumigatus* and *Eurotium chevalieri*. The less prevalent mold species, with a frequency of occurrence ranging up to 13%, were *A. wentii*, *E. rubrum*, *P. funiculosum*, *P. variabile*, *P. citrinum*, *P. crustosum*, *F. moniliforme* and *F. semitectum*. A 30% to 100% reduction in mold growth was observed in paddy stored in Volcani cubes, with the exception of *E. chevalieri* which was reduced by only 9% (Table 2). Mold reduction in the gastight plastic silos (0–10%) was comparatively lower than in Volcani cubes. In general, mold infection in dry paddy stored in both Volcani cubes and plastic silos decreased, whereas mold growth in control stacks increased (Table 1).

TABLE 1
Percentage mold infection in dry paddy stored in plastic Volcani cubes, Volcani silos and ordinary tarpaulin sheets (control stacks)

	Volcani cubes (n = 6)		Volcani silos (n = 1)		Control stacks (n = 2)	
Mold species	Initial	Final	Initial	Final	Initial	Final
Aspergillus flavus	23	13	35	33	30	40
Aspergillus niger	17	7	25	23	30	33
Aspergillus japonicus	26	13	27	25	17	23
Aspergillus fumigatus	23	13	33	30	20	23
Aspergillus terreus	ND	ND	ND	ND	7	7
Aspergillus wentii	10	7	ND	ND	0	12
Aspergillus ochraceous	ND	ND	ND	ND	0	3
Eurotium amstelodami	ND	ND	ND	ND	7	13
Eurotium chevalieri	47	43	83	80	90	93
Eurotium rubrum	ND	ND	10	10	0	7
Penicillium funiculosum	10	0	10	10	0	3
Penicillium variabile	3	0	13	13	10	10
Penicillium citrinum	7	3	7	7	13	10
Penicillium crustosum	13	7	3	3	0	10
Fusarium moniliforme	10	7	ND	ND	10	13
Fusarium semitectum	13	7	ND	ND	10	13

ND = not detected; n = number of stacks observed.

TABLE 2
Percentage reduction of mold infection in dry paddy stored in plastic Volcani cubes and Volcani silos

Mold species	Percentage reduction	
	Volcani cubes (n = 6)	Volcani silos (n = 1)
Aspergillus flavus	43	6
Aspergillus niger	59	8
Aspergillus japonicus	50	7
Aspergillus fumigatus	43	9
Aspergillus wentii	30	ND
Eurotium chevalieri	9	4
Penicillium funiculosum	100	0
Penicillium variabile	100	0
Penicillium citrinum	57	0
Penicillium crustosum	46	0
Fusarium moniliforme	30	ND
Fusarium semitectum	46	ND

ND = not detected; n = number of stacks observed.

The suppression of mold growth in Volcani cubes could be attributed to the relatively higher concentration of CO_2 generated (Fig. 1), whereas the low reduction of mold growth in dry paddy stored in plastic silos could be due to leaks found upon examination of the

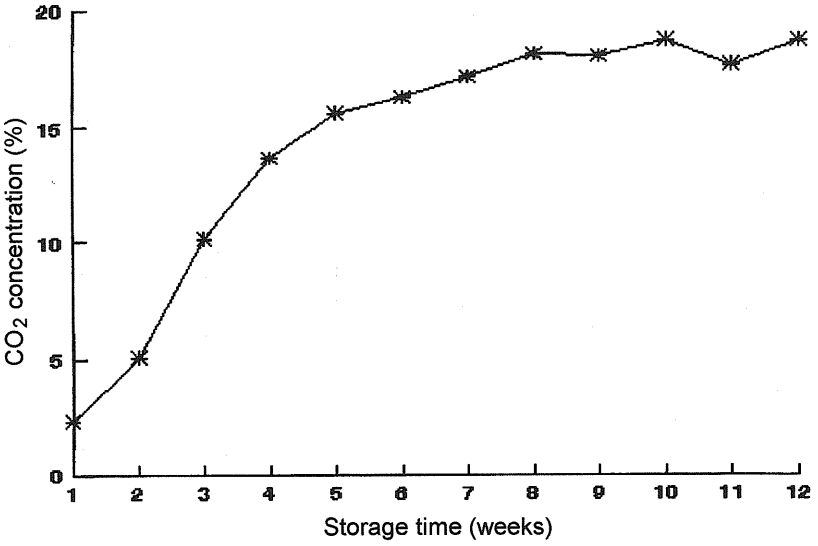


Fig. 1. Typical CO_2 concentration recorded during hermetic storage of dry paddy (up to 14% m.c.) in Volcani cubes.

plastic. The paddy grains used in the silo trial were heavily infested, and filling and sealing took a few days during which the lesser grain borer (*Rhizopertha dominica* F.) bored tiny holes in the plastic, thus causing a leaky structure.

Mold population in dry paddy stored both in gastight Volcani cubes and gastight plastic silos was affected by the location of the paddy in the stack. Higher mold counts were found in the uppermost layer and at the periphery, whereas a reduction was found at the core of the stack (Table 3). Mold increases in the uppermost layer and at the periphery were due to moisture migration. Rice hulls serving as insulation became saturated at some point and the excess water dripped onto the uppermost layer and sides of the stacks. This was very evident when the stacks stored for more than 4 months were opened and moldy bags were observed on the top layer, immediately below the insulator, and on the sides. Further examination of the moldy bags, however, indicated that moldy grain was limited to a depth of approximately 1–2 cm from the surface of the bag. This observation correlates with the low mold counts recorded in the core of the stack.

TABLE 3
Percentage reduction or increase of mold species at predetermined points in dry paddy stored under gastight Volcani cubes

Mold species	Percentage reduction or increase					
	Periphery (4 sides)		Top (uppermost layer)		Core (middle and bottom)	
Aspergillus flavus	35	(20–13)	6*	(33–35)	13	(23–20)
Aspergillus niger	57	(7–3)	11*	(18–20)	23	(30–23)
Aspergillus fumigatus	30	(33–23)	9	(33–30)	25	(20–15)
Eurotium chevalieri	9	(47–43)	4	(83–80)	7	(90–83)
Penicillium funiculosum	–	(13–0)	0	(7–7)	0	(3–3)
Penicillium variabile	–	(3–0)	0	(13–13)	0	(10–10)
Fusarium moniliforme	15*	(20–23)	–*	(0–13)	ND	(0–0)
Fusarium semitectum	30*	(13–17)	–*	(0–15)	30*	(10–13)

ND = not detected; numbers in parenthesis represent initial and final mold count; * = increase; – = percentage cannot be calculated.

Mold growth in moist paddy

A. flavus, *E. chevalieri*, *P. citrinum* and *F. moniliforme* were the prevalent mold species isolated from moist paddy. *A. niger*, *A. japonicus*, *A. fumigatus*, *A. terreus*, *A. ochraceous*, *P. variabile* and *P. funiculosum* were isolated at lower counts (Table 4). Infection by *A. flavus* increased after 43 d of gastight storage, whereas infection by other mold species decreased markedly. The decrease in the incidence of infection by *A. niger*, *A. japonicus*, *A. fumigatus*, *A. terreus*, *A. ochraceous*, *E. chevalieri*, *P. funiculosum*, *P. variabile* and *P. citrinum* was attributed to the modified atmosphere (19% CO_2, 0% O_2) caused by the respiration of high moisture grains and molds which contributed to the depletion of O_2 and the increased evolution of CO_2 (Fig. 2). On the other hand, there was a

TABLE 4
Initial and final percentage of various mold species in moist paddy stored in plastic Volcani silos for 43 d

Mold species	Percentage mold growth	
	Initial	Final
Aspergillus flavus	74	80
Aspergillus niger	10	8
Aspergillus japonicus	10	8
Aspergillus fumigatus	10	6
Aspergillus terreus	8	6
Aspergillus ochraceous	3	3
Eurotium chevalieri	18	15
Penicillium citrinum	57	1
Penicillium funiculosum	8	8
Penicillium variabile	10	8
Fusarium moniliforme	14	12

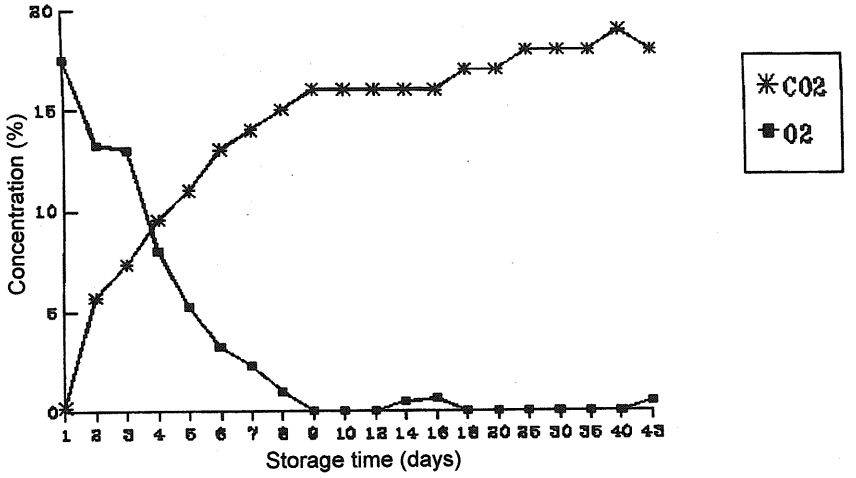


Fig. 2. CO_2 and O_2 concentrations recorded during hermetic storage of moist paddy (18% m.c.) in plastic Volcani silos.

decrease in the incidence of *F. moniliforme* which was reported to tolerate 14–15% CO_2 and 0.5–1% O_2 (Wilson and Jay, 1975). This decrease, however, may be due to the significant reduction in m.c. (from 18.21% to 14.87%).

It is evident that the population of *A. flavus* was not affected by the altered atmosphere. An atmosphere containing about 20% CO_2, needed to inhibit the growth of *A. flavus*, was not attained inside the silo. It is assumed, however, that if moist paddy had been stored as long as dry paddy, reduction of *A. flavus* could have been attained.

CONCLUSION AND RECOMMENDATION

Gastight storage outdoors using especially designed enclosures and the heavy duty flexible liners developed in Israel can preserve grain quality and has the added benefit of controlling mold growth. Mold growth in paddy stored in gastight Volcani cubes and gastight plastic silos is affected by grain m.c. and by the storage period. Moist grains (18% m.c.) have relatively higher respiration rates and mycofloral loads than dry grains (14%). In gastight storage this leads to faster evolution of CO_2 and accompanying depletion of O_2 which in turn suppresses mold development. Gastight storage of dry paddy for periods exceeding 4 months will enable mold development only at the top and periphery of the stack. The use of thin plastic film placed between the uppermost layer and an insulating layer of rice hulls, however, will help minimize grain molding at these locations.

Storage of moist paddy for approximately one-and-a-half months using gastight heavy duty flexible liners did not result in any serious quality deterioration. The potential of hermetic storage for grain above the critical moisture level has thus been established. This finding has great potential for addressing the problem of moist grain handling in the tropics.

ACKNOWLEDGEMENT

This study was part of USAID CDR project number C7-053. The authors would like to thank Mr Don David T. Julian, Mr Joel V. Dator, Mr Elijah Z. Davalos, Ms Marilyn T. dela Cruz and Mr Nelson C. Santiago for their technical support during the conduct of this study. The support extended by the National Food Authority of Regions II and III in the provision of the experimental stocks used in this study is also greatly appreciated.

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EFFECT OF PHOSPHINE AND BAG TYPE ON STORAGE FUNGI OF MILLED RICE

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ABSTRACT

The effects of phosphine (PH_3) and bag type on storage fungi of milled rice, together with changes in moisture content (m.c.), were investigated during storage. Milled rice was stored in either jute or polypropylene bags under warehouse conditions for 49 weeks. Five stacks of each bag type were constructed, and their positions were selected at random. Each stack consisted of 50 jute bags of 100 kg or 100 polypropylene bags of 50 kg milled rice. The rice was fumigated with PH_3 at a dosage rate of 10 tablets/stack or 2 tablets/t with an exposure period of 5 d. Fumigations were repeated at 3-month intervals throughout the experiment. Fungal populations on the rice were determined using the dilution method on Dichloran 18% Glycerol Agar (DG18) before each fumigation (at the beginning of storage, and subsequently at 11, 23, 35 and 47 weeks of storage) and again immediately after fumigation (at 1, 13, 25, 37 and 49 weeks of storage).

During the 49 weeks of storage, 21 species of fungi were isolated from milled rice packed in jute and 22 species from that in and polypropylene bags. In both bag types the predominant fungus was *Aspergillus penicilloides*, which increased sharply from the first week and was thereafter almost constant both before and after fumigation. The total count of fungal and *A. penicilloides* populations on milled rice packed in jute bags was lower than the count on milled rice in polypropylene bags. Both populations increased until the eleventh week of storage and thereafter declined slowly as the storage period continued.

The m.c. of milled rice packed in jute bags was lower than that on milled rice in polypropylene bags. The m.c. varied with increase in storage duration both in jute and polypropylene bags.

INTRODUCTION

During storage, milled rice may be infested by insects, mites, microorganisms and rodents. Among microorganisms, fungi are the most important cause of deterioration of stored products.

In the tropics, *Eurotium* species and other *Aspergilli* are dominant, with *Penicillium* species playing only a minor role (Pitt and Hocking, 1991). They can cause weight loss, seed discoloration, heating with accompanying mustiness and also the production of mycotoxins.

Fumigation is primarily used for insect control and little is known about its effect on the development of storage fungi in milled rice. Among fumigants, phosphine (PH_3) is effective for controlling insects during storage of rice.

The type of bag used to store milled rice may affect fungal development. The National Logistics Agency (BULOG) uses jute and polypropylene bags for the storage of milled rice.

The objective of this study was to obtain information on the effects of PH_3 and bag type on storage fungi of milled rice. The m.c. of the rice was also observed. This study was part of a research project on "pest management of psocids (*Liposcelis* spp.) in milled rice stores".

MATERIALS AND METHODS

The study, initiated in November 1993 in a warehouse belonging to BULOG (located in Darmaga, West Java), lasted for 49 weeks. One end of the warehouse was allocated to the study while the other contained normal rice stocks.

Stacks of milled rice

The experimental units were 5-t bag stacks constructed with either 100 open-weave polypropylene bags (50 kg) or 50 jute bags (100 kg). The jute bags were arranged in nine layers. In each layer two bags were accessible for sampling at the front/back of each stack, three bags at the left/right side and two bags at the top. The polypropylene bags were arranged in ten layers. In each layer five bags were accessible at the front/back of each stack, two bags on the left/right side and ten bags at the top. There were five stacks of each bag type.

The rice used in this trial had already been in store for 1 year prior to the test. It was harvested in Karawang in 1992. According to BULOG standards, it was 1B quality, with a milling degree about 90% and 35% broken grains. It was composed of a mixture of varieties, mostly IR64 and Cisadane (local variety). The initial m.c. of rice in jute and polypropylene bags was 13.6 and 13.8%, respectively.

Pest control

Pest control measures were routinely carried out during this study. Store surfaces were sprayed with insecticide S-fenvalerate (Sumistore 25EC) at 4-week intervals. The insecticide was applied at the rate of 0.125 g a.i./m^2 to floor, stack and wall surfaces using a semi-automatic knapsack sprayer. The stacks were fumigated with PH_3 (phostoxin at 10 tablets/stack or 2 tablets/t) under gastight sheets for 5 d at 12-week intervals. The insecticidal treatment described above was always applied just before fumigation. During fumigation, PH_3 concentrations were monitored daily at 13:00 h with a Bedfont EC80 PH_3 meter. The gas concentrations obtained are presented in Table 1.

These pest control activities were also applied to other stacks in the warehouse, although not necessarily at the same time.

TABLE 1
Daily changes in PH_3 concentration (ppm) in rice stacks built of jute or polypropylene bags, during five fumigations

Rice stacks	Day	Fumigation				
		1st	2nd	3rd	4th	5th
Jute bags	1	1135	1130	1227	1097	1189
	2	971	846	1074	808	1107
	3	748	629	814	563	825
	4	531	427	650	389	640
	5	411	307	242	226	422
Polypropylene bags	1	1299	1189	1260	1140	1156
	2	1271	939	1048	1020	1080
	3	1075	640	797	776	786
	4	857	455	591	591	585
	5	738	330	221	302	346

Sampling methods, moisture content and fungal analysis

Samples of rice were taken from 80 positions around each stack (20 positions/side). A sampling spear 77 cm long and 1.6 cm in diameter was used. Samples were taken at the beginning of storage and thereafter at intervals, as shown in Fig. 1. The depth of insertion

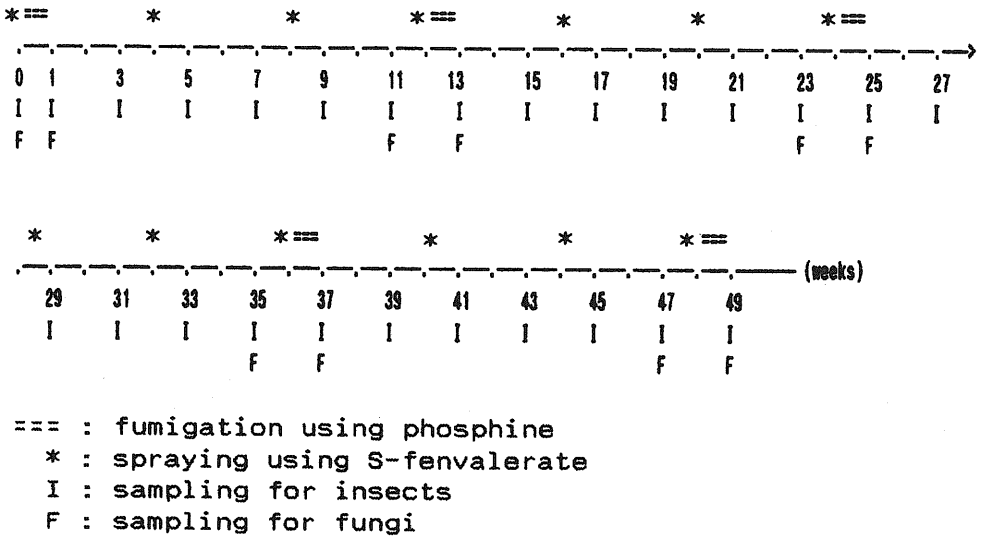


Fig. 1. Plan of activity carried out at BULOG warehouse in Darmaga.

of the sample probe was 70.5 cm, and these samples were grouped to create a final sample of about 3 kg/stack. In the laboratory, psocids were separated from the rice using graded sieves of 2 mm, 1 mm and 250 μ, respectively. Each sample was then twice divided, using a sample divider, to obtain working samples of 0.75 kg for m.c. determination and fungal analysis.

Using the oven method, m.c. was determined at 130°C for 2 h (BSI, 1980). Fungal populations on the rice were determined using the dilution method on Dichloran 18% Glycerol Agar (DG18). Observations were made 6 d after incubation at 28°C and fungal species identified mainly using the method of Pitt and Hocking (1985).

Ambient temperatures and relative humidities were recorded continuously using a Wilh Lambrecht thermohygrograph type 252.

RESULTS AND DISCUSSION

Environmental conditions

Daily average temperatures during the trial ranged from 26.7 to 34.5°C (Fig. 2), whereas relative humidity varied from 55.5 to 88.1%. From May, as the drier season of the year began, there was a trend toward slightly lower humidities.

There was very little difference between the mean m.c. of rice packed in the two bag types. In jute bags, the mean value was 13.8% m.c. (±0.5 SD), while that in the

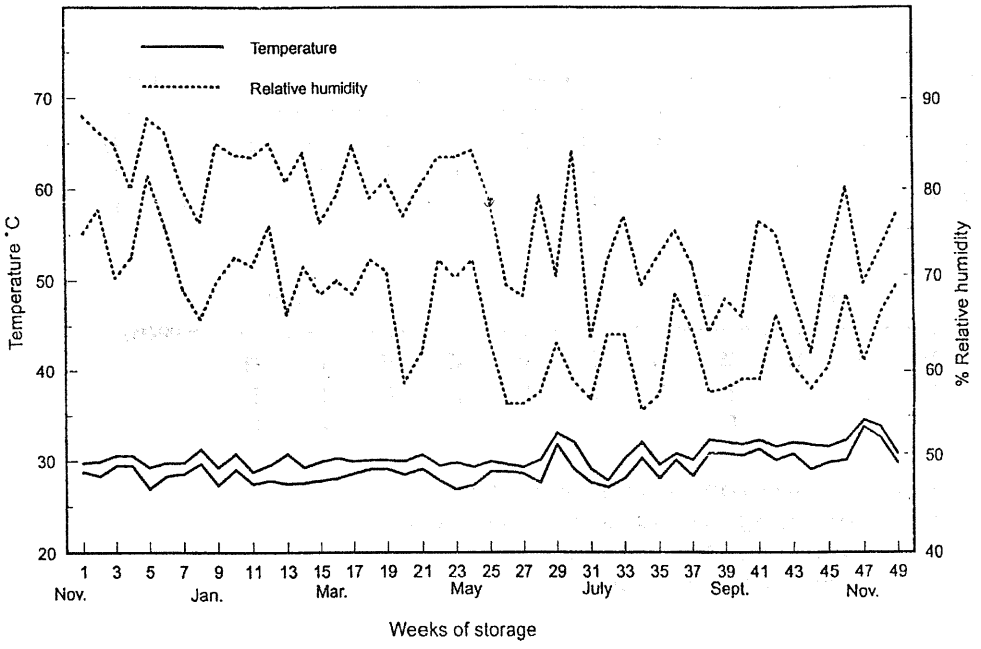


Fig. 2. Maximum and minimum daily mean temperatures and r.h. for each week of the field trial.

polypropylene bags was a little higher at 13.9% (±0.4 SD). The initial mean m.c. in the jute and polypropylene bags was 13.6 and 13.8%, respectively; they rose slightly for some months and then fall again to 13.3 and 13.2%, respectively, at the end of the trial (Table 2).

Analysis of m.c. variance showed that although the difference between bag types was small, it was nonetheless significant. The values were very significantly affected by storage period, and there was a significant interaction between bag type and length of storage (Table 3).

TABLE 2
The effects of bag type, duration of storage and their interaction on moisture content

	Moisture content (%)		
Duration of storage (weeks)	Jute	Polypropylene	Overall
0	13.62	13.82	13.72
1	13.24	13.94	13.59
3	13.36	14.54	13.95
5	13.86	14.42	14.14
7	13.80	14.12	13.96
9	15.30	14.46	14.88
11	14.24	14.34	14.29
13	13.80	13.96	13.88
15	14.22	14.22	14.22
17	14.96	14.06	14.51
19	13.98	14.32	14.15
21	14.08	14.00	14.04
23	14.38	13.80	14.09
25	13.70	13.94	13.82
27	13.64	13.80	13.72
29	13.82	13.96	13.89
31	13.84	13.80	13.82
33	13.54	13.68	13.61
35	13.40	13.48	13.44
37	13.76	13.74	13.75
39	13.62	13.62	13.62
41	13.38	13.48	13.43
43	13.16	13.12	13.14
45	13.28	13.22	13.25
47	13.32	13.38	13.35
49	13.34	13.22	13.28
Standard error			
Mean	13.794	13.863	13.828
SE	0.1004	0.0772	0.0796

TABLE 3
Analysis of variance on the effect of bag type, duration of storage and their interaction on moisture content

Source of variance	Df	SS	MS	F-value
A	1	0.246	0.246	4.043*
B	25	41.105	1.644	27.014**
A × B	25	11.023	0.441	7.244*
Error	208	12.660	0.610	

A = bag type; B = duration of storage; A × B = interaction between variables. * = significant difference with 95% confidence; ** = significant difference with 99% confidence.

The effects of PH_3, bag type and duration of storage on fungal population

During 49 weeks of storage, 21 species of fungi were isolated from milled rice packed in jute bags, and 22 from that in polypropylene bags (Table 4). In both bag types the predominant fungus was *A. penicilloides*, the population of which increased sharply from the first week until the eleventh week of storage, after which the population remained about constant. It was assumed that the constant population was a result of several fumigations using PH_3. Dharmaputra *et al.* (1991) reported that PH_3 at a concentration of 3.5 mg/L (equivalent to 2 g/t grain) almost totally inhibited the mycelial growth of *A. flavus*. It is not clear why the fungal population should increase at the beginning of the trial, considering that at that time the rice had already been stored for 1 year.

According to both Hocking (1981) and Pitt and Hocking (1985), *A. penicilloides* was quite rare primarily because it does not grow on the media commonly used for fungal isolation and enumeration. If suitable media were used, the fungus could be recovered in large numbers from a variety of such dried foods as spices and cereals. Ryckaert *et al.* (1981) reported that the abundance of the fungus was greatly influenced by the outdoor climate.

As the fungal population was so dominated by *A. penicilloides* that other fungal counts were more or less insignificant, statistical analysis would be confined to this species. The population of this species in relation to treatment variables is presented in Table 5 and Fig. 3. The *A. penicilloides* population on milled rice packed in jute bags was lower than that on milled rice in polypropylene bags. It was assumed that the difference was due to the difference in airflow rates through jute and polypropylene bags, and/or to the m.c. differential. Christensen and Kaufmann (1975) reported that m.c. was the most important factor in determining the development of storage fungi in grain. The population of *A. penicilloides* was very significantly affected by bag type and storage duration, although the interaction between these two factors was not significant. An analysis of *A. penicilloides* populations, transformed to logs to stabilise variance, is presented in Table 6.

TABLE 4

Population of each fungal species (colonies/g) on milled rice packed in jute (J) or polypropylene (P) bags, at the beginning of storage and after 1, 11, 13, 23, 25, 35, 37, 47 and 49 weeks of storage (* = anomalous result)

		Storage period (weeks)									
Fungal species	Bag	0	1	11	13	23	25	35	37	47	49
Arthrinium phaeospermum	J	0	0	0.9	0.3	0.6	0.3	0	0	0	0
	P	0	0	0.6	0.6	0	0	0	0	0	0
Arthrobotrys sp.	J	0	0.6	0	0	0	0	0	0	0	0
	P	0	0	0	0	0	0	0	0	0	0
Aspergillus candidus	J	1.2	0	0	0.3	2.3	0	0	2.3	0	0
	P	0	0	0	0	0.6	0	0.3	0	0	0
Aspergillus clavatus	J	0	0	0	0	0	0	0	3.3	3.7	2.7
	P	0	0	0	0	0	0	0	0	6.3	2.7
Aspergillus flavus	J	8.2	1.6	4.7	1.9	6.3	3.4	20.8	0	6.7	2.6
	P	4.9	0.9	5.2	0.3	4.7	5.3	2.5	0	2.5	3.9
Aspergillus fumigatus	J	0	0	0.7	8.3	0.6	0	7.2	0.7	0	0
	P	0	0	0.7	0	1.6	0	6.7	0.6	0	0
Aspergillus niger	J	4.9	0	0	0	0	0	0	0	0	0
	P	1.2	3.6	0	0	0	1.7	0	0	0	0
Aspergillus oryzae	J	0	0	0	0	0	0	0	0	0	0
	P	0	0	0	0	0	0	0.9	0	0	0
Aspergillus penicilloides	J	0*	861.7	2264.0	2233.2	2066.7	2067.9	2032.2	2092.7	2028.0	1945.1
	P	277.3	900.7	3329.9	3326.6	3293.3	3201.6	3079.5	3059.7	2958.1	2888.0
Aspergillus restrictus	J	0	0	0	0	0	0	0	0	0	0
	P	0	0	0	0	0	0	2.7	0	0.7	0
Aspergillus versicolor	J	0.6	0	0	0	0	0	0	0	0	0.9
	P	0	0	0	0	0	0	0.9	0	0	0
Aspergillus wentii	J	0	0	0	0	0	0	3.9	0.6	0	0
	P	0	0	0	0	1.3	0	0.9	0	0	0
Cladosporium cladosporioides	J	4.0	0.6	0.3	0	1.9	1.0	18.0	2.9	4.5	2.0
	P	2.6	1.7	1.2	0.6	2.2	3.7	5.9	3.3	5.4	2.6

TABLE 4 (continued)

Fungal species	Bag	Storage period (weeks) 0	1	11	13	23	25	35	37	47	49
Cladosporium sphaerospermum	J	0	0	0	0	0	0	1.6	0	0	0
	P	0	0	0	0	0	0	1.8	0	0	0
Endomyces fibuliger	J	0	0	0	0	0.3	0	0	0	0	0
	P	0	0	0	0	0	0	0	0	0	0
Eurotium chevalieri	J	6.4	7.7	8.0	6.1	2.6	2.6	4.0	4.1	4.2	4.3
	P	4.6	6.9	5.5	3.3	2.2	0	5.4	5.6	6.0	1.7
Fusarium semitectum	J	0	0	0	0	0	0	0	0	0	0
	P	0.9	0	0	0	0	0	0	0	0	0
Mucor circinelloides	J	0	0	0	0	0	0	0	0	0	0
	P	0	0	0	0	0.3	0	0	0	0	0
Mucor hiemalis	J	0	0	0	0	0	0	0	0	0	0
	P	0	0	0	0	0	0.3	0	0.3	0	0
Penicillium citrinum	J	2.7	2.6	5.6	9.1	15.8	10.1	28.1	3.0	2.3	0.6
	P	3.6	0	6.3	3.2	15.1	9.6	4.4	3.8	1.0	1.0
Penicillium herquei	J	0	0	0	0	0	0	2.4	0	0	0
	P	0	0	0	0	0	0	0	0	0	0
Penicillium islandicum	J	0	0	0	1.3	0	0	0	0	0	0
	P	0	0	0	0.6	0	0	0	0	0.6	0
Penicillium sclerotiorum	J	0	0	0	0	0	0	0	0.3	0.6	0.7
	P	0	0	0	0	0	0	2.0	0	0	0.9
Rhizopus microsporus	J	0	0	0	0	0	0	0	0	0.3	0.7
	P	0	0	0	0	0	0	0	0	1.7	0
Syncephalastrum racemosum	J	0	0	6.0	0	0	0	0	0	1.0	0.3
	P	0	0	0	0	0	0	0	0	0	0
Wallemia sebi	J	0	0	0	0	0	0	0	0	0	0
	P	0	0	0.7	0.3	0	0	0	0	0	0
Total	J	28.0	874.8	2290.2	2265.3	2097.1	2085.3	2118.2	2109.9	2054.0	1960.2
	P	295.1	913.8	3350.1	3336.5	3321.3	3222.2	3115.9	3073.3	2982.3	2901.5
Difference (%)		953	5	46	47	58	55	47	46	45	48

TABLE 5
The effects of bag type and duration of storage on the population of *Aspergillus penicilloides*

Variable	Mean fungal population (colonies/g)		
	Not transformed	Transformed log (x + 1)	SE
Bag type			
Jute	1954	3.30	0.026
Polypropylene	2893	3.40	0.055
Duration of storage (weeks)			
1	881	2.76	
11	2797	3.43	
13	2780	3.43	
23	2680	3.40	
25	2635	3.41	
35	2556	3.39	
37	2576	3.40	
47	2493	3.39	
49	2417	3.38	

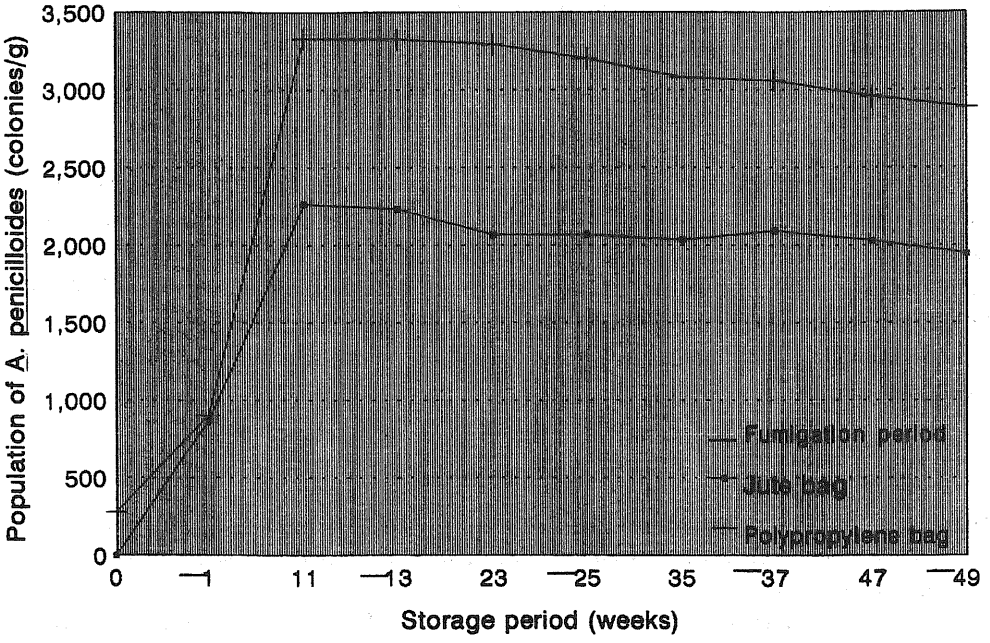


Fig. 3. *Aspergillus penicilloides* population on milled rice, packed in jute or propylene bags, before and after fumigation with PH_3 (Dharmaputra *et al.*, 1991).

TABLE 6
Analysis of variance on the effect of bag type, duration of storage and their interaction on the population of *Aspergillus penicilloides* (transformed into log (x + 1))

Souce of variance	Df	SS	MS	F-value
A	1	0.502	0.502	16.88**
B	8	3.716	0.465	15.61**
A × B	8	0.157	0.020	0.66*
Error	72	2.142	0.030	

A = bag type; B = duration of storage; A × B = interaction between variables.
** = significant difference with 99% confidence.

CONCLUSIONS

The predominant fungus which infected milled rice packed in jute and polypropylene bags was *A. penicilloides*, which increased sharply from the first week and thereafter remained almost constant both before and after fumigation. PH_3 at 2 tablets/t of milled rice appears to contribute to the slowing down of fungal growth. The total fungal population in milled rice packed in jute bags was lower than that in milled rice packed in polypropylene bags. The m.c. of milled rice packed in jute bags was also lower than that of milled rice packed in polypropylene bags.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the Natural Resources Institute (NRI) of the UK for providing research funds. The authors are also grateful to Dr Rick Hedges of the NRI; to the Head of BULOG, Jakarta; to Dr H. Halid of the Ministry of Food Affairs/BULOG; to Mr Sunjaya and the technicians of the Laboratory of Stored-Product Pests, SEAMEO BIOTROP; to SEAMEO BIOTROP; to P.T. Aneka Gas Industry, Jakarta; and to the Cyprus Organizing Committee for financial assistance enabling their participation in the CAF Conference.

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SESSION 3

INFLUENCE OF CONTROLLED ATMOSPHERES AND/OR FUMIGATION ON QUALITY PRESERVATION OF STORED PRODUCTS

Chairpersons:
Darka Hamel, *Croatia*
M. Sidik, *Indonesia*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 159–167

QUALITY CONSERVATION OF PADDY STORED UNDER GASTIGHT SEAL OUTDOORS IN THE PHILIPPINES

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ABSTRACT

Paddy stacks of capacities ranging from 13.4 to 31.9 t were stored outdoors in flexible enclosures for 78 to 183 d. The quality of the paddy was compared with that of three control stacks (5.3–5.6 t capacity) held under tarpaulins in the open for 78–117 d. The trials were conducted at the NAPHIRE compound, Nueva Ecija, the Philippines. Two varieties of enclosures were tested, heavy-duty PVC-based sheeting sufficiently gastight to control insect infestations (called "Volcani cubes" and designed in Israel for stacked bag storage) and weld-mesh supported silos, in both of which the paddy was stored in bulk. In both systems, the upper layers of paddy were covered with a layer of rice hulls aimed at reducing temperature gradients, and plastic sheeting was placed between this insulating layer and the grain, serving to prevent the transfer of moisture to the top grain layer. Moisture content, grain temperature and gas concentrations were measured throughout the trials. Initial and final samples were taken to determine changes in paddy quality; insect infestation, fungal infection, milling recovery, head rice, yellow kernels, brokens, germination and weight loss were analyzed. The percentage of milling recovery and the levels of yellowing in the gastight stacks showed no significant change. The levels of head rice and brokens were preserved in seven out of nine stacks. A decrease in the percentage of head rice in two stacks was attributed to the biological aging phenomenon. The two control stacks showed a decrease in head rice and an increase in brokens. Rice yellowing was very pronounced in one of the control stacks.

INTRODUCTION

This report forms part of a more comprehensive study designed to provide alternative temporary or emergency outdoor storage facilities for use by farmers' organizations, cooperatives, village grain merchants and other intermediary parties in the Philippines (and other countries) where countryside storage is an important component of the national grain reserve.

The storage facilities used in this project were designed as gastight structures that can provide affordable and user-friendly grain-conservation systems without the need for chemical pesticides. This report describes storage trials with paddy undertaken in the Philippines. It details the storage conditions recorded during the trials and the findings relating to quality conservation, including insect infestation and damage. Other sections of the study have been reported elsewhere (Alvindia *et al.*, 1994; Caliboso *et al.*, 1997).

MATERIALS AND METHODS

The paddy storage trials were conducted at the National Post Harvest Institute for Research and Extension (NAPHIRE) compound, Muñoz, Nueva Ecija, Philippines.

Storage methodology

Descriptions of the storage structures, preparation of the storage site, construction of the stacks, insulation of the stacks, sealing, periodic monitoring of gas compositions within the storage structures and grain sampling methodology are provided in a paper by Caliboso *et al.* (1997).

Quality evaluation

Moisture content (m.c.) was determined by drying grain samples for 1 h in an oven (Anon., 1982). Live insects, sieved from the composite and representative samples, were sorted according to group and species.

Quality parameters were calculated by hand counting the number of insect-damaged, discolored, moldy and germinated kernels in 1,000 kernel samples taken from composite samples. Viability tests were done by the rag-doll method.

Data were statistically analyzed using the Multi-Factor Analysis of Variance (AVMF) and Least Square Difference (LSD) tests.

RESULTS AND DISCUSSION

A total of nine trials were carried out on storage of paddy in plastic Volcani cubes, two in plastic silos and two in control stacks under tarpaulins. Table 1 lists the details and durations of the trials.

Moisture content

The average m.c. of gastight sealed paddy in stacks P2 and P11 increased slightly but significantly from the beginning to end of storage, whereas no significant increase was noted in the rest (Table 2). There was a real trend towards an increase in m.c. in the two control stacks stored during the wet season (P3 and P6) and towards a decrease in m.c. in the control stack stored during the dry season (P9). These differences indicate the importance of the gastight sheet in preventing moisture diffusion. The field trials show that

TABLE 1
List of paddy trials carried out in the Philippines

Trial no.	Stack code	Structure		Treatment		Handling		Capacity (t)	Duration of storage (d)
		Cube	Silo	Gas-tight	Control	Sacks	Bulk		
I-1	P1	×		×		×		14.00	94
I-2	P2	×		×		×		13.43	94
I-3	P3	×			×	×		5.30	94
II-1	P4	×		×		×		13.63	117
II-2	P5	×		×		×		13.84	117
II-3	P6	×			×	×		5.30	117
III-1	P7	×		×		×		14.77	78
III-2	P8	×		×		×		15.06	78
III-3	P9	×			×	×		5.56	78
IV-1	P10	×		×		×		14.78	97
IV-2	P11	×		×		×		14.73	97
V-1	P12		×	×			×	31.86	183
VI-1	P13		×	×		×		30.38	43

TABLE 2
Moisture content of composite samples taken from paddy stacks during the trials

Trial no.	Stack code	Treatment	Moisture content	
			Initial	Final
I-1	P1	Gastight	10.30 a	10.56 a
I-2	P2	Gastight	9.87 a	10.83 b
I-3	P3	Control	9.67 a	11.60 b
II-1	P4	Gastight	11.68 a	12.37 b
II-2	P5	Gastight	12.07 a	12.28 a
II-3	P6	Control	12.15 a	12.80 b
III-1	P7	Gastight	11.16 a	11.27 a
III-2	P8	Gastight	11.61 a	11.07 b
III-3	P9	Control	12.34 a	10.67 b
IV-1	P10	Gastight	12.23 a	13.08 a
IV-2	P11	Gastight	13.46 a	14.24 b
V-1	P12	Gastight silo	10.75 a	10.63 b
VI-1	P13	Gastight silo	18.21 a	14.87 b

In a row, means followed by the same letter are not significantly different at 5% level using LSD test.

there was no critical moisture build-up or localization in any of the treatments and controls with the exception of P11, which exceeded the critical m.c. of 14% by 0.24%. The overall m.c. of paddy stored in bulk under gastight conditions in the silo did not change significantly during the trial (P12). This result suggests the feasibility of bulk storage in the silo without an adverse effect on the m.c. of paddy.

A significant reduction from 18.21 to 14.87% in m.c. of the wet in-silo paddy (P13) was recorded after storage for 43 d. This reduction may be explained by the thermal gradients that caused the mass transfer of moisture. The air movement induced by diurnal temperature differences and the diffusion of moisture as a result of moisture equilibration between the grain and the air both resulted in the air's taking up moisture from the wet paddy, resulting in the formation of dew under the liner during the cool hours of the evening. Water that condensed below the liner was initially absorbed by the rice hulls. This was evidenced by the fact that the rice hulls, which initially averaged 7.97% m.c., more than doubled their m.c. to 18.58% by the end of the 43-d trial. A total of 92 bags of rice hull, each weighing about 5 kg, were used. Most of the rest of the condensed water was apparently absorbed by the upper layer of the grain mass, with some water also accumulating on the thin plastic sheet placed between the rice hulls and the paddy bags. The plastic sheet was provided to catch any condensed water that might have dripped between the sacks of rice hulls, and also to catch any excess moisture that was not absorbed by the rice hulls. This change in m.c. was not reflected in all the grain samples since moisture translocation appears to have been confined to the upper layer. That the weight of the grain changed only very slightly indicates that excess water remained within the stacks and silos.

Gas concentration

Maximum carbon dioxide (CO_2) concentrations recorded in the gastight silos and cubes of paddy are shown in Table 3. The maximum CO_2 concentration, found in the

TABLE 3
Maximum CO_2 concentrations generated in gastight storage cubes containing stored paddy and initial moisture contents

Trial no.	Stack code	Percent CO_2	Initial moisture content
I-1	P1	6	10.30
I-2	P2	7	9.87
II-1	P4	13	11.68
II-2	P5	10	12.07
IV-1	P10	15	12.23
IV-2	P11	18	13.46
V-1	P12	5	10.75
VI-1	P13	19	18.21

silo containing high m.c. paddy, reached 19%. Lower CO_2 concentrations, reported in stacks P1, P2 and P12, were due to leaks in the plastic sheeting caused by insects or mechanical damage. In stack P1, it was discovered only after 6 weeks of storage that the zipper was unlocked. In the first silo trial, P12, there was a heavy infestation by the lesser grain borer. The silo remained unsealed for several days, enabling insects to attack the liner. After the damage to the liner was detected, it was decided to fumigate the bulk with phosphine at a dosage of 6 g/t.

The high CO_2 concentrations recorded in P11 and P13 indicate that the initial m.c. of the commodity caused intensive biogeneration of CO_2 by microorganisms.

Insect infestation

Initial and final counts of live insects revealed no significant population increase in the gastight sealed paddy stacks (Table 4), whereas in the control stacks (P3, P6 and P9) there were marked increases in insect density and many of the insects were still alive at the end of the storage period. These results show that in all the gastight sealed stacks complete disinfestation of paddy was not achieved. In spite of the presence of a few live insects at the end of storage, however, all the treatments in the trials were successful. This is matched by a much lower percentage of weight loss in the treated stacks in comparison with the control stacks.

TABLE 4
Average density of insects per kg of sample in paddy trials

Trial no.	Stack code	Treatment	Insect density		Significance level
			Initial	Final	
I-1	P1	Gastight	5.33 (–)	8.00 (–)	ns (–)
I-2	P2	Gastight	9.67 (–)	1.67 (–)	* (–)
I-3	P3	Control	13.67 (–)	35.33 (–)	ns (–)
II-1	P4	Gastight	8.67 (0)	0.33 (36.33)	** (**)
II-2	P5	Gastight	17.00 (0)	2.33 (63)	* (**)
II-3	P6	Control	16.67 (0)	51.00 (91.00)	ns (**)
III-1	P7	Gastight	0 (18.00)	4.33 (122.67)	** (**)
III-2	P8	Gastight	0 (11.33)	6.67 (26.67)	* (ns)
III-3	P9	Control	0 (12.33)	47.33 (89.33)	** (ns)
IV-1	P10	Gastight	3.33 (0.33)	6.33 (2.67)	ns (ns)
IV-2	P11	Gastight	3.00 (0.33)	0 (4.67)	ns (*)
V-1	P12	Gastight silo	24.67 (18.67)	15.00 (261.67)	ns (**)
VI-1	P13	Gastight silo	10.33 (0)	1.33 (1.00)	** (ns)

ns = not significant; * = significant at 5% level; ** = significant at 1% level.
Numbers represent live insects per kg of sample; numbers in brackets, dead insects per kg of sample.
– = not recorded.

The insect species found in the silo trial of bulk paddy (P12) before storage were a few dead *Tribolium castaneum* and live *Rhyzopertha dominica*, *Sitophilus oryzae*, *Lophocateres pusillus*, *Oryzaephilus surinamensis* and *Carpophilus dimidiatus*. During unloading large numbers of dead insects of various species and live *R. dominica*, *L. pusillus* and *O. surinamensis* were retrieved. The density of live insects at the end of the trial was reduced, whereas an increase was noted in the density of dead insects (Table 4). These results indicate that gastight conditions inside the silo did inhibit insect development but could leave a residual insect population below the economic threshold. Initial flushing with CO_2, however, could be used to control initial infestations.

Paddy qualities

Changes in the percentage of milling recovery of head rice, broken grains and yellow grains in dry paddy are presented in Table 5. Neither the milling yield nor the level of the yellowing grain in the gastight paddy stacks were significantly altered.

In general, gastight storage preserved grain quality by maintaining the level of head rice. The levels of head rice and brokens were preserved in 8 out of 13 stacks while in P1 and P4 a rise in the percentage of head rice was recorded. This may be explained by the biological aging phenomenon which occurs in paddy during the first 3 to 4 months of storage. It is theorized that the aging phenomenon is the result of a sol-gel transformation of colloidal starch and protein deposited during ripening and its transformation into a more stable, water-insoluble physical form during storage. Tensile strength of the grain increases, and this increased hardness is translated into higher total and head rice yields and a greater resistance to milling.

As expected, the two control stacks (P3 and P6) showed a decrease in head rice and an increase in brokens. Because the trial was conducted during the dry season, and due to the short duration of storage, the control stacks for trial P9 did not exhibit severe quality deterioration. Rice yellowing, however, was very pronounced in untreated control stack P3. Although yellowing increased in the wet paddy stack (P13), the final level remained low.

The above data suggest that gastight storage of dried bagged and bulk paddy has no adverse effect on grain yellowing, milling yield and other quality parameters.

Viability

The germination of paddy stored under gastight sealed conditions did not change significantly during the trials (Table 6).

Weight loss

The weight losses observed during the field trials are shown in Table 7. The magnitude of loss recorded from the gastight sealed paddy stacks was about 18 times lower than that in the control stacks and resulted in 3.75–4.85% weight loss.

TABLE 5
Comparison of mean quality parameters in paddy at the beginning and end of storage

Stack code	Treatment	Milling recovery (%)		Head rice (%)		Yellow kernels (%)		Broken kernels (%)	
		Initial	Final	Initial	Final	Initial	Final	Initial	Final
P1	Gastight	68.71	69.67**	75.47	77.27*	0.32	0.26^{ns}	25.43	22.73*
P2	Gastight	69.66	70.16^{ns}	83.20	82.53^{ns}	0.16	0.23^{ns}	16.80	17.46^{ns}
P3	Control	70.33	70.31^{ns}	82.80	78.33*	0.16	4.67**	17.20	21.67*
P4	Gastight	66.12	67.61*	73.60	77.33**	0.27	0.23^{ns}	26.07	22.67^{ns}
P5	Gastight	66.47	65.35^{ns}	72.47	72.47^{ns}	0.33	0.34^{ns}	27.53	26.30^{ns}
P6	Control	66.89	67.00^{ns}	77.73	70.90**	0.33	0.22^{ns}	22.07	20.10^{ns}
P7	Gastight	64.62	63.90^{ns}	76.55	77.71^{ns}	1.00	0.69^{ns}	23.45	22.56^{ns}
P8	Gastight	64.37	64.68^{ns}	81.32	81.81^{ns}	0.08	0.05^{ns}	18.68	18.19^{ns}
P9	Control	64.26	65.57^{ns}	82.61	82.85^{ns}	0.23	0.13^{ns}	17.39	17.19^{ns}
P10	Gastight	64.09	63.15^{ns}	82.24	74.64*	0.07	0.16^{ns}	17.76	24.70*
P11	Gastight	62.96	61.85^{ns}	81.74	77.24^{ns}	0.02	0.06^{ns}	18.24	22.76^{ns}
P12	Gastight silo	65.27	65.06^{ns}	87.32	79.47*	0.13	0.12^{ns}	12.68	19.88**
P13	Gastight silo	65.60	65.67^{ns}	84.66	79.88^{ns}	1.83	2.52*	10.88	15.11**

^{ns} = not significant; * = significant at 5% level; ** = significant at 1% level.

TABLE 6
Percent germination of paddy during field trials

Trial no.	Stack code	Treatment	Initial	Final
II	P4	Gastight	98.67 a	89.33 b
II	P5	Gastight	97.67 a	100.00 a
II	P6	Control	97.67 a	98.33 a
III	P7	Gastight	95.67 a	93.33 b
III	P8	Gastight	88.33 a	86.00 a
III	P9	Control	95.00 a	91.67 a
IV	P10	Gastight	86.00 a	89.33 a
IV	P11	Gastight	83.67 a	83.00 a
V	P12	Gastight silo	93.67 a	93.00 a
VI	P13	Gastight silo	95.33 a	94.77 a

In a row, means followed by the same letter are not significantly different at 5% level using LSD test.

TABLE 7
Percentage weight loss in paddy stacks during the trials

Trial number	Stack code	Treatment	Percent weight loss
II	P4	Gastight	0.32
II	P5	Gastight	0.21
II	**P6**	**Control**	**4.85**
III	P7	Gastight	0.29
III	P8	Gastight	0.27
III	**P9**	**Control**	**3.75**
IV	P10	Gastight	0.19
IV	P11	Gastight	0.10
V	P12	Gastight silo	0.26
VI	P13	Gastight silo	0.13

ACKNOWLEDGEMENT

This study was undertaken within the framework of USAID CDR project number C7-053. We wish to thank Mr Don David T. Julian, Mr Joel V. Dator, Mr Elijah Z. Davalos, Ms Marilyn T. dela Cruz and Mr Nelson C. Santiago for their technical support during the conduct of this study. We also wish to express our appreciation to the National Food Authority of Regions II and III for providing the experimental paddy stocks used in this study.

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CONTROL OF BEESWAX MOTHS USING CARBON DIOXIDE IN FLEXIBLE PLASTIC AND METAL STRUCTURES

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ABSTRACT

The building structure of bee hives is based on the wax cells produced by the bees themselves. The most important of the species that attack beeswax is *Galleria mellonella* (L.), the greater wax moth, and protection of stored combs from this insect is of high priority, especially in hot climates.

A new approach to control of this pest has been to replace the traditionally used chemical insecticides, such as EDB, by environmentally sound methods. We propose the use of controlled atmosphere methodology with CO_2 for insect control and subsequent protection of honey supers during storage. The results of field tests using mixtures of CO_2 in air at initial concentrations ranging from 96 to 63%, for prolonged exposure periods, are presented. The tests were undertaken using 12-m^3 plastic enclosures and specially sealed 34-m^3 20-foot freight containers. Both methods enabled complete control of the wax moths and subsequent protection from reinfestation. The rigid structure of the containers had the inherent advantage of giving better protection from rodents and pilfering. However, each container requires specific sealing procedures followed by sealing tests. The plastic enclosures, found to be suitable for smaller enterprises, had the advantage of ease of portability and installation at the storage site. Their structural seals were checked during manufacture. So far they have been under continual use for 5 years.

INTRODUCTION

A number of moth species, the most important of which are the greater wax moth, *Galleria mellonella* (L.), and to a lesser extent the lesser wax moth, *Achrois grisella* (F.), attack beeswax. They cause major losses to commercial beekeepers every year by damaging wax combs in storage (Eckert, 1951; Smith, 1990; Singh, 1962). Wax moths can cause rapid and

complete destruction of unprotected combs. Since the combs can only be made by the honeybees themselves, beekeeing ventures can become losing propositions without a ready supply of empty combs during the brood rearing and honey flow season.

In the past, the accepted method for controlling these pests was fumigation with ethylene dibromide or methyl bromide (MB) (Burges, 1978). Since 1983 the use of ethylene dibromide has been banned because of its carcinogenic properties, while MB, as a result of the unprecedented international cooperation for ridding the world of ozone-destroying chemicals, was listed at the Montreal Protocol (UNEP, 1992) among the chemicals that contribute to the depletion of the ozone layer of the atmosphere. For developed countries, complete phase-out of MB is programmed for the year 2010 (UNEP, 1995).

The use of modified atmospheres (MA's) as a fumigation alternative that retains the special capacity for *in-situ* treatment of agricultural commodities has been developed to control storage pests (Navarro, 1987; Navarro and Donahaye, 1990). MA technology has been considered for wax moth control; however, only scanty information has so far been published on the effect of exposure period and MA concentrations on wax moth mortality (Cantwell *et al.*, 1972; Greatti and D'Agaro, 1992).

On-farm storage of honey combs is problematic since storage structures are generally not sufficiently sealable for an effective gas composition of the treated storage space to be maintained in order to both obtain control of the pests and prevent subsequent reinfestation. Therefore, to implement the MA technology, appropriate storage structures need to be developed (Yakobson *et al.*, 1990). Such structures should be sufficiently gastight to maintain the desired MA concentrations and, at the same time, should be durable and resistant to harsh environments. Such structures have been developed for the preservation of grain and are in use (Donahaye and Navarro, 1989). However, similar structures are lacking in the beekeeping profession. This study reports on both the development and on-site testing of a flexible structure and the adaptation of old transport containers, taken out of circulation, as MA treatment and storage facilities for beehive supers and combs.

MATERIALS AND METHODS

The commercial-scale field trials to control wax moths using carbon dioxide (CO_2) were based on laboratory studies undertaken to examine the sensitivity of all stages of *G. mellonella* to 60, 70 and 80% CO_2 in air at 25 and 30°C. These studies are now being summarized and will be published elsewhere.

Plastic treatment enclosures

The structure. Of the several prototype fumigation enclosures investigated, the one reported on here consists of a flexible 0.83-mm liner, made of reinforced chlorinated polyethylene (CPE), welded to the shape shown in Fig. 1. The enclosure was provided with a walk-in flap-door, zip-closed with a tongue-and-groove gastight zipper, and an inner protective curtain of netting to prevent ingress of bees when the flap was open. The

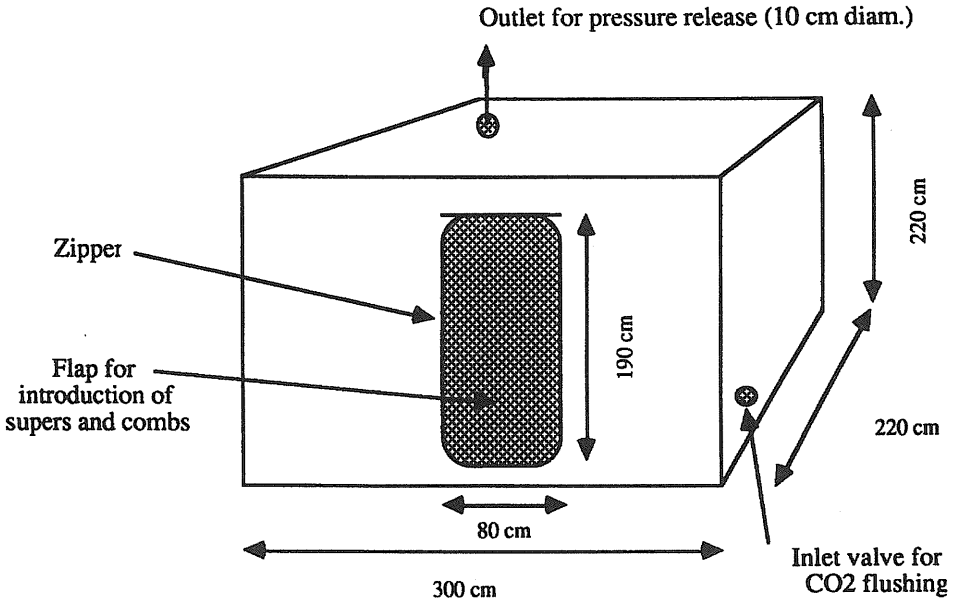


Fig. 1. Plastic enclosure for MA treatments of beehive supers (to control wax moths) and subsequent protection from infestation during storage.

liner was characterized by its ability to withstand prolonged periods of intense solar radiation. The enclosure was structurally supported by sets of vertically and horizontally positioned 19-mm fibre-glass rods held in place by connecting sockets in the corners of the liner. In this trial the enclosure was designed to contain 120 Langstroth-type supers holding wax combs. The supers were removed from bee hives in late spring, and after the honey had been extracted they were placed directly in the enclosure.

The treatment. CO_2 in the liquid phase was applied as a single shot at a rate of up to 6 kg/min from a pressurized cylinder through a release valve and pressure hose attached by snap-on connectors to the inlet valve of the enclosure. CO_2 concentrations were monitored during release (using a Gow-Mac thermal conductivity gas analyzer) until a concentration of approximately 65% CO_2 was obtained within the enclosure. This was equivalent to about 18 kg liquid CO_2 removed from the cylinder. Concentrations were measured daily for the first month. Then the gastight seal of the door was broken to permit the first inspection for infestation, and from then on the enclosure served as a physical barrier to prevent reinfestation.

Monitoring for infestation. Among the supers in the enclosure, one was heavily infested initially with all developmental stages of the wax moths. The wax combs in this super were examined monthly for 5 months for the presence of live adults, eggs, larvae and pupae.

Modified containers

This trial was carried out on one of several 20-foot freight containers that had been taken out of service and purchased by commercial beekeepers for storage of supers. The containers were then modified to form treatment chambers. Modifications consisted of installation of an inner PVC liner over the floor; sealing of cracks and crevices, particularly around the doors, with acryllic and silicon sealer; installation of a 4-mm diameter gas inlet duct, equipped with ball-valve tap and snap-on connecter, and an upper 10-cm diameter outlet duct sealed with a screw cap; installation of a pressure release valve; and installation through the container wall of two 4-mm diameter gas sampling lines, one leading to the top and the other to the floor. In the trial container reported on here, because the door gaskets were in poor condition, an additional inner door, consisting of PVC sheeting attached to the walls and held in place between sections of aluminium profile bolted around the opening of the container, was made. The beehive supers were progressively loaded into the container as they were removed from the hives in late spring for the extraction of honey. An initial examination, before the container was closed and sealed, revealed considerable wax moth activity on many of the combs.

Pressure tests. Following the above modifications, after the doors had been closed and sealed the container was subjected to a pressure test by creating a positive pressure of 50 mm water, using a portable vacuum cleaner, and recording the time taken for pressure to decay to 25 mm water.

Treatment. CO_2 in liquid form was flushed into the container from inverted cylinders. The outlet pipe was then capped and the container was kept sealed for 21 d. Then the doors were opened and a thorough inspection was made of supers and combs. Combs were removed from the container for additional laboratory examination.

RESULTS

Plastic treatment enclosure

Measurements of CO_2 concentration in the enclosure are given in Fig. 2. It can be seen that the CO_2 concentrations dropped from an initial level of 63% to 43% after 9 d. The first inspection of combs in the enclosure, made 30 d after fumigation, revealed 100% mortality of all wax moth stages in the infested supers. No further wax moth activity or damage was observed in the supers for 5 months following the treatment with CO_2.

Modified containers

Pressure test. The pressure decay test from 50 to 25 mm water revealed a halving time of 48 sec. Additional attempts to improve the seal around the door, followed by a second pressure decay test, resulted in an increase in halving time to 1.2 min.

CO_2 treatment. Three cylinders, each containing 26–28 kg CO_2, were emptied at flow rates ranging from 0.7 to 1.2 kg/min. After the third cylinder had been emptied, CO_2 readings were 91% and 96.5% at the top and bottom of the container, respectively. The

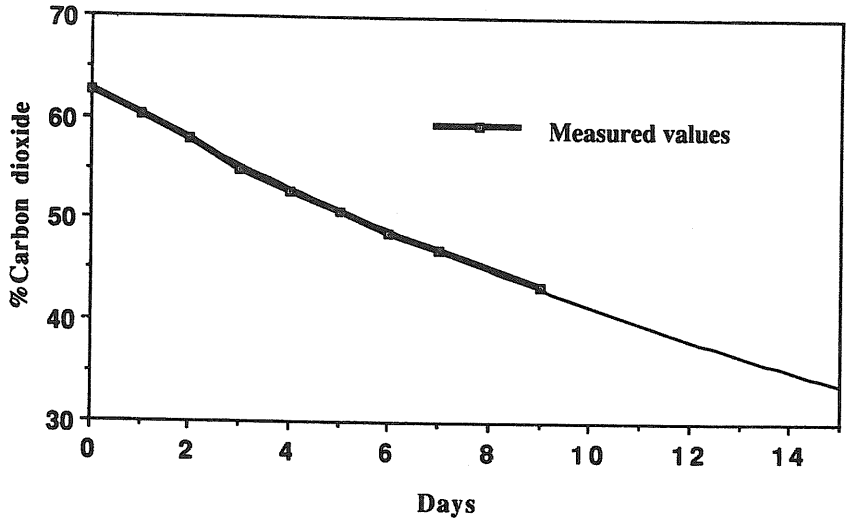


Fig. 2. Carbon dioxide concentrations recorded in plastic enclosure during the first 9 d of treatment.

CO_2 concentrations recorded over the first 10 d of the post-treatment period are shown in Fig. 3. This reveals that CO_2 concentrations decreased at about 5.4% per day.

At the inspection made upon opening the container after 21 d, no live insects were observed. Six combs that were taken from different regions of the container and held in the laboratory inside sealed plastic bags for a further 30 d revealed no live infestation upon examination.

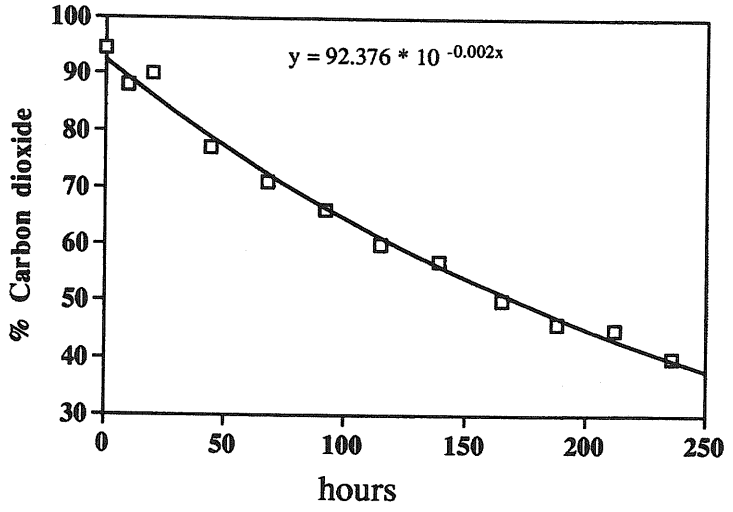


Fig. 3. Carbon dioxide concentrations recorded in modified freight container during the first 10 d of treatment.

CONCLUSION

One CO_2 treatment per season of the wax combs stored in a portable CPE fumigation chamber effectively controlled the wax moths. The modified freight container was also effective in controlling existing infestation and preventing wax-moth reinfestation and damage. Although the sensitivities of the developmental stages of the wax moths to different CO_2 concentrations under various climatic conditions have still to be investigated, it is clear from these field trials that very prolonged exposure to a range of high CO_2 concentrations was sufficient to obtain complete kill. This "single shot" CO_2 flushing treatment represents a user-safe and environmentally friendly alternative to fumigation with MB or phosphine.

ACKNOWLEDGEMENT

We would like to thank Yishai Ophir, apiculturalist of Moshav Hogla, for the cooperation and help extended to us during the course of this work. We are also indebted to Ben Ami Zilberstein of the Institute of Agricultural Engineering, ARO, for assistance in modification of the container.

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TRADITIONAL HERMETIC METHODS OF GRAIN STORAGE USED IN CYPRUS

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ABSTRACT

Four structures, traditionally used since medieval times for storing grain in Cyprus, are described. Storage in those structures was primarily based on the concept of hermetic storage. The first structure, *goufa* (plural: *goufes*) or *voufa* (cavity, hole, well), was an underground space built in the open. Walls were covered with mud, and a fire was lit inside before storing grain in it. When temperature had been reduced and the floor cleaned, grain was poured in. The opening was then sealed with a lid and mud. The second structure, *fournos* (oven, kiln), was built with stones and mud in a hemispherical shape and located outside in the courtyard. Its capacity was about 500–1,000 L. Before storing grain, a fire was lit in it. Grain was placed inside the *fournos* after its temperature had fallen sufficiently. The third structure, *pilini* (*pilos* in Greek, mud or clay), was a 50–500-L clay pot. After the pot was filled with grain, its opening was closed with a wooden or stone lid. In some cases larger pots, with an opening on top for pouring in grain and another one at bottom for emptying, were built inside the houses. Finally, a fourth method was the storage of grain in thickly woven woollen or cotton bags placed in the middle of a heap of straw in a store-room.

In the first two structures, *goufa* and *fournos*, fire disinfested the space and reduced air humidity. The thick walls of these structures and the mud sealant provided the systems with a certain degree of airtightness. In them, oxygen concentration was reduced and carbon dioxide increased gradually as a result of grain and insect respiration.

INTRODUCTION

Throughout the ages, people have tried to protect their agricultural products from attack by pests and deterioration due to pests and diseases. In this paper, four methods of storage which were used in rural regions of Cyprus until at least the first part of this century are described. One of these structures was underground, the other three above-ground. In all cases, storage in these structures was based primarily on the concept of hermetic storage.

DESCRIPTION

Goufa

The first grain storage structure is called a *goufa* (plural *goufes*) (Yennadios, 1972). The name is derived from the medieval Italic "gueffa", meaning "underground prison". In Cyprus it has the connotation "hole" or "well" (and at least until recently this word was used to describe a room with a pit). This structure was an underground store-pit, shaped like a flask, with an opening narrower than the periphery. The villagers co-operatively constructed *goufes* near the fields or threshing places, preferably on a slope. They chose soil which was dry, hard, heavy and free of sand. In some cases *goufes* were hollowed into limestone rocks (Figs. 1 and 2).

Before storing grain in one of these structures, the walls were covered with mud and a fire lit inside. When the temperature returned to normal, grain was poured into the *goufa* and the opening was well closed with a flat stone lid and mud. These structures had a capacity of 50–150 kg, and grain was stored in this way for up to one year.

This type of grain storage was in use until at least 1930 in the isolated region of Karpassia and in Akanthou village. In some cases, groups of up to fifty pits were built in one place.

Similar storage structures hollowed out of solid limestone rock can be seen in Malta (Hyde and Daubney, 1960).



Fig. 1. A group of underground storage structures (*goufes*) outside the village of Akanthou, Karpassia (front right), 1971.



Fig. 2. A group of underground *goufes* outside the village of Akanthou, 1971.

Fournos

The second structure is called *fournos*, meaning "oven" or "kiln". This is an above-ground store constructed outdoors in courtyards or gardens or even close to the field. It was constructed (on a raised base) of stones, brick and mud with straw. It was conical, with walls 25–30 cm thick (Figs. 3 and 4).

At the top there was an opening for pouring the grain into the *fournos* and at the bottom a second opening for emptying.

The *fournos*, like the *goufa*, was fired with burning tree branches before being used for storing grain. Both openings were sealed by mud after the grain was introduced. Grain was kept in these structures for up to one year.

Similar storage structures can be seen on an ancient Egypt papyrus (Attia, 1948).

Pilini

The third structure used for grain storage (Fig. 5) is called *pilini*, from Greek *pilos*, meaning mud (Kythreotes, 1972). This was a large cylindrical pot, 1–1.5 m high, usually standing on a base with three small feet. It was made of mud mixed with fine pieces of straw. First the base was made, and after it dried, the sides were built up. The walls were about 10 cm thick.



Fig. 3. *Fournos*, an aboveground store, Moni Sina, Eleousa, Karpassia, 1910 (Richter, 1913).



Fig. 4. *Fournos*, Karpassia, 1972.



Fig. 5: *Pilini*, a mud-pot for storing grain, Messaoria, 1970.

After pouring the grain or flour inside, the pot opening was closed with a wooden or stone lid. An opening in the side close to the base was used for emptying the contents. Before being stored, the grain was washed and dried.

Pilini were in use for storage up to 1974 in the Karpassia and Messaoria regions (Papademetriou, 1992; Xistouris, 1972).

Bags

The last method of storage is placing the grain in thickly-woven woollen or cotton bags placed at the center of a heap of straw in a store-room. The bags were woven from strips of woolly or hairy material. As the store-room was being filled, the straw was compressed by children. Farmers used to store seed-grain in this way for up to one year. This method of storage was used primarily in Messaoria (in Lisi and other villages) and Pafos (Panaretos, 1974).

DISCUSSION

The concept of hermetic storage (Vayssiere, 1948), on which these storage methods were based, is described in Tables 1, 2, 3 and 4.

TABLE 1
Hermetic concept of storage in *goufes*

Parameter	Influence on the airtightness of the structure
Location	Underground Limited supply and penetration of oxygen into the structure
Walls	Covering the walls with a mud layer reduced gas exchange between the interior and the surrounding soil
Sealing of openings	There was only one opening on the top, well sealed by mud, preventing or reducing gas exchange between the interior and the atmosphere
Duration of storage and frequency of opening during storage	Storage lasted up to one year (enough time for the production of carbon dioxide and the reduction of oxygen). The structure was opened only at the end of storage, so the airtightness was not destroyed during the storage
Preparation of structure and grain before starting storage	Lighting a fire inside the structure before filling it with grain reduced both the relative humidity of the air and the amount of oxygen inside the structure as well as disinfestating the space
Degree of airtightness	Medium

TABLE 2
Hermetic concept of storage in *fournos*

Parameter	Influence on the airtightness of the structure
Location	Aboveground. Surrounding air rich in oxygen
Walls	20–30 cm thick walls (made of stones, brick and mud) and an outer covering of mud reduced gas exchange between the interior and the surrounding atmosphere
Sealing of openings	There were two openings, well sealed by mud which prevented or reduced gas exchange between the interior and the surrounding atmosphere
Duration of storage and frequency of opening during storage	Storage lasted up to one year (ample time for the production of carbon dioxide and the reduction of oxygen). The structure was opened only at the end of storage, so the airtightness was not destroyed during the storage period
Preparation of structure and grain before starting storage	Lighting a fire inside the structure before filling it with grain reduced both the relative humidity of the air and the amount of oxygen inside the structure
Degree of airtightness	Medium

TABLE 3
Hermetic concept of storage in *pilini*

Parameter	Influence on the airtightness of the structure
Location	Aboveground. Surrounding air rich in oxygen
Walls	10 cm thick walls made of mud are not expected to seriously affect gas exchange between the interior and the surrounding atmosphere
Sealing of openings	There were two openings which were not sealed hermetically
Duration of storage and frequency of opening during storage	Storage lasted up to one year, but the structure was frequently opened during storage
Preparation of structure and grain before starting storage	There was no preparation of the structure The grain was washed and dried before storage
Degree of airtightness	None

TABLE 4
Hermetic concept of storage in bags hidden inside a straw heap

Parameter	Influence on the airtightness of the structure
Location	The bags of grain were placed in the middle of a heap of straw in a store-room The surrounding air was poor in air and oxygen and enriched in carbon dioxide because of both the compression of the straw layers during filling and the respiration of insects on the top of the straw heap
Sealing of the flat store	The opening on the roof was well sealed by stone and mud The second opening (door) provided very poor airtightness
Duration of storage and frequency of opening during storage	Storage lasted up to one year, but the structure was frequently opened to obtain straw
Preparation of the structure and grain before starting storage	None
Access by insects and rats to the grain in bags	Difficult
Degree of airtightness	Poor Modification of air composition due to insect respiration

Some additional factors affected the success of grain storage in such structures as the *goufes* and *fournos*. These are the very low moisture content of grain (7–12%) during harvest and Cyprus's dry climatic conditions.

There are modern modifications of the traditional hermetic methods of grain storage in Cyprus described above. There are eight Cyprus "Ctesiphon" semi-underground pits, 1,200 t wheat capacity each, which were built in 1955 (Hall *et al.*, 1956). In Nicosia, two

platforms were constructed on a concrete base. With polyethylene covering the floor and a PVC liner covering the top of the grain, a good airtight seal is provided during storage. Their capacity is 2,500 and 4,000 t, respectively. In use since 1987, these platforms provide 1–3 years of storage for grain (Navarro *et al.*, 1992; Varnava *et al.*, 1995). Lastly, three airtight concrete bins of 2,500 t capacity each were erected in 1996 in the new silo at Limassol port.

ACKNOWLEDGEMENTS

The authors wish to thank Mr Ioannis Leandrou of the Cyprus Grain Commission for his assistance in presenting visually the old storage structures.

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7-YEAR RESULTS OF HERMETIC STORAGE OF BARLEY UNDER PVC LINERS: LOSSES AND JUSTIFICATION FOR FURTHER IMPLEMENTATION OF THIS METHOD FOR GRAIN STORAGE

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ABSTRACT

From 1988 to 1995 barley of 8–11% moisture content (m.c.) was stored at 32–38°C for 1–3 years on two concrete platforms (2,500 and 4,000 t, respectively) under UV-protected PVC liners 0.8 mm thick. In some cases platform floors were covered with a polyethylene liner 0.2 mm thick. Grain moisture, temperature, insect infestation, germination and some other quality parameters were measured during storage. Concentrations of oxygen (O_2) and carbon dioxide (CO_2) were also recorded. At the end of each storage period mold and total losses were determined.

The effect on intergranular O_2 and CO_2 concentrations of covering the floor with a polyethylene liner was significant. With a polyethylene underliner, O_2 and CO_2 were 2–6% and 8–12%, respectively. Without it, concentrations were 6–10% and 2–4%, respectively. However, this did not have a significant influence on losses. Zearalenone (760 ppb) and ochratoxin A (1,990 ppb) were detected in samples of mold-damaged grain, and the total fungal count was 5.7×10^9/g. In sound grain, concentrations of zearalenone and ochratoxin A were zero, and the total fungal count was 1.6×10^6/g. The longer the storage, the higher both mold losses and total losses. After 1 year of storage, they were 0.16 ± 0.1% and 0.32 ± 0.06%, respectively. After 2 years of storage, losses were 0.22 and 0.52%, respectively, and after 3 years of storage mold losses and total losses reached 0.66 and 0.96%, respectively. After 1 year of storage, germination remained above 95%, and after 3 years it was above 88%. Hermetic storage under the PVC liner successfully protected the grain against insects, birds, rodents and rain.

The total expenditure for both fixed and running expenses (building a concrete platform 25 m wide and 75 m long and hermetically storing 4,000 t of barley under the PVC liner) was US$4/t/1 year or US$2.50/t/3 years.

INTRODUCTION

It is quite frequent for many countries to face serious problems in undertaking the safe and timely collection and storage of newly harvested crops. In Cyprus, due to limited

storage capacity, almost every year thousands of tons of harvested barley and wheat remain stored in the open for 4–5 months. In some years considerable amounts of grain are destroyed by rain. Deterioration and loss of grain stored in the open is also caused by attacks by birds, rodents and insects. In Australia this problem was addressed by the development of bunker storage (Banks and Sticka, 1981; McCabe and Champ, 1981), while in Israel emphasis was placed on the development of hermetically sealed bunkers (Navarro *et al.*, 1984).

The need to provide a rapid and effective solution to the problem of storing newly harvested grain in Cyprus prompted the Cyprus Grain Commission, in cooperation with the Volcani Center of Israel, to develop a modified platform hermetic-storage system. Aspects of the Cyprus platform storage have been published (Navarro *et al.*, 1993; Varnava *et al.*, 1994), while this paper reports on the quality of hermetically stored grain, with particular reference to the problem of mycoflora and mycotoxins (Bullerman, 1979), and on a cost–benefit analysis of the platform method.

MATERIALS AND METHODS

Two platforms have been used for hermetic storage since 1987. Each consists of a re-inforced concrete floor with a 1 m high peripheral retaining wall. Grain is loaded either directly on the concrete floor or onto a polyethylene underliner 0.25 mm thick. A UV-protected PVC liner 0.8 mm thick is used to cover the grain. Where the floor is covered by a polyethylene liner, the overliner and underliner are brought together over the walls, folded over at the outer base of the walls, and weighted with sandbags, thus forming a hermetic seal.

The first platform is 75 m by 25 m and has a capacity of about 4,000 t of barley. The second platform is 50 m by 25 m and has a capacity of about 2,500 t of barley. The height of grain at the peak is 6.5 m.

Both platforms are used for storing local barley with a maximum m.c. of 11%. Normally, incoming grain is already infested by insects when it arrives at the platforms. However, no chemicals are used during loading or during storage of the grain on the platforms.

Grain has been stored on these platforms for 8–10 months, and in some cases 2 or 3 years. During storage grain samples have been taken from different depths and locations to monitor moisture content (m.c.), insect infestation, germination and mycotoxin deterioration. Grain temperature and intergranular oxygen (O_2) and carbon dioxide (CO_2) concentrations have also been measured. In addition, both mould losses and total loss have been evaluated.

Estimates were also made, for comparison, of losses caused in 1992 and 1995 by heavy rain during storage of grain in the open.

Finally the cost of on-platform storage per ton for 1, 2 and 3 years was calculated.

RESULTS AND DISCUSSIONS

Covering the floor with polyethylene liner

The influence on intergranular gas composition, insect infestation and grain losses of covering the platform floor with a polyethylene liner is given in Table 1.

TABLE 1
Influence of covering the platform floor with a polyethylene liner (duration of storage 8–10 months)

Liner	Gas concentration (%) 3–4 months after covering		Infestation[1]	Losses due to mould (%)	Total grain losses (%)
	O_2	CO_2			
Yes*	2–6	8–12	Rare	0.16 ± 0.1	0.32 ± 0.06
No**	6–9	2–4	Yes	0.13 ± 0.06	0.28 ± 0.06

* = data for 4 years; ** = data for 5 years.
[1]Presence of adult insects in samples from the upper grain layers after covering with the PVC liner.

The data obtained over several years indicate that covering the floor with polyethylene significantly influences change in intergranular gas composition and also provides better control of insect infestations. It would not appear to have any serious influence on the amount of grain on the top of the platform damaged by mould or on total grain losses by the end of storage.

Duration of storage

The influence of the duration of storage on grain loss, infestation and grain germination is given in Table 2. This table shows that satisfactory insect control can be achieved for 1, 2 and 3 years of storage on a platform with a polyethylene covering over the floor. Grain germination remained very high (94–97%) even after 2 years of storage, and it was reduced to 88% only after 3 years of storage.

TABLE 2
Influence of duration of storage on grain losses and other parameters
(platform floor covered with a polyethylene liner)

Duration of grain storage (months)	Platform capacity (t)	Infestation[1]	Germination (%)	Losses (%)	
				Mould	Total
8–10*	2,500–4,000	Rare	95–97	0.16 ± 0.1	0.32 ± 0.06
23	2,500	Rare	94–98**	0.22	0.52
34	4,000	Rare	88 ± 1.6	0.66	0.96

* = data for 4 years; ** = viability.
[1]Presence of adult insects in samples from the upper layers after opening the PVC liner.

TABLE 3

Microflora and mycotoxins in barley samples of different degrees of deterioration at the end of a 10-month platform storage under a PVC liner

Parameter	Threshold value for the occurrence of symptoms of mycotoxicosis to animals	Sample A Taken from center of mould spot Visible signs of mould deterioration Very spoilt grain	Sample B Taken 10–20 cm below mould spot Visible signs of mould deterioration Less spoilt grain	Sample C Taken from grain surface free from any mould spot High moisture grain (17% m.c.) Sound grain	Sample D Taken 0.5 m below grain surface Low moisture grain (12% m.c.) Sound grain
Mycotoxines (ppb)					
T-2	200	0	0	0	0
Zearalenone	200	760	260	0	0
Deoxynivalenol	400	0	0	60	0
Fusarenon X	400	0	0	0	0
Nivalenol	200	0	0	350	0
Diacetoxyscirpenol	400	0	0	0	0
Ochratoxin A	100	1990	1570	0	0
Aflatoxin B1	20	0	0	0	0
Microflora (fungi number per g)					
Total fungi		2.7×10^{10}	5.7×10^{9}	2.2×10^{6}	1.6×10^{6}
Fusarium		5.5×10^{4}	1.5×10^{4}	4.5×10^{5}	5.5×10^{5}
Alternaria		9.2×10^{7}	4.2×10^{7}	1.2×10^{4}	0.7×10^{4}
Penicillium		2.4×10^{10}	5.5×10^{9}	1.7×10^{6}	1.1×10^{6}
Aspergillus		3.2×10^{8}	9.7×10^{7}	9.7×10^{4}	4.7×10^{4}
F. graminearum		Few	Few	3.7×10^{5}	3.8×10^{5}
F. colmorum		Few	Few	0.7×10^{5}	1.7×10^{5}
F. moniliforme		Few	Few	9.5×10^{4}	2.5×10^{4}
P. ochraceus		2.4×10^{10}	4.4×10^{9}	1.4×10^{6}	0.8×10^{6}
P. urticea		3.1×10^{9}	7.2×10^{8}	0.2×10^{6}	0.2×10^{6}
Mucor		Many	Many	Few	Few

On the other hand, the duration of storage had a significant influence on both mould losses and total loss. At the end of 3 years of storage, mould losses were four times greater than average mould losses after 1 year of storage. Similarly, total loss after 3 years of storage was three times greater than average loss after 1 year of storage.

Microflora and mycotoxins

The initial temperature of grain stored on platforms was between 30–37°C, whereas the lowest ambient air temperature during the winter season was 2–6°C. This resulted in the establishment of strong temperature gradients within the bulk which in turn caused convection currents, leading to transfer of moisture to the peak area of the bulk and therefore to mould deterioration of the grain in some spots at the apex of the bulk. These spots were 5–50 cm deep.

Two samples of very spoilt barley and spoilt barley taken from these mould spots at a depth of 0–20 cm and two samples of sound barley at 17% m.c. and 12% m.c., respectively, were analysed for mycotoxins and microflora. The results are given in Table 3. These results demonstrate that spoilt grain from the mould spots could be seriously contaminated by zearalenone (260–760 ppb) and ochratoxin (1,600–2,000 ppb). Spoilt grain was contaminated primarily by *Alternaria, Penicillium, Aspergillus* and *Mucor* fungi. The quantity of spoilt grain at these spots was very small, but the importance of carefully removing all spoilt grain from the bulk surface must be emphasised.

Analyses of samples "C" and "D" show that the grain stored on platforms actually remains free from mycotoxins and is suitable for animal consumption.

Total costs of platform erection and hermetic storage of 4,000 t grain

The fixed cost of building a concrete platform (75 m by 25 m), together with the costs of PVC and polyethylene and the current cost for storing 4,000 t of barley for 1, 2 and 3 years are all shown in Table 4. The total per-year cost for storing 1 t of barley for a period of 3 years is C£1.23.

Table 5 shows the losses that occurred in 1992 and 1995 during storage of local barley in the open. Grain was affected by rain, and a part of it was completely destroyed. In the years 1992 and 1995, 65,500 t of barley stored in the open were affected by rain and, based on the weighted average loss of 5.23%, some 3,425 t were completely destroyed. The economic loss was about C£387,100.

Table 6 shows the estimated monetary saving in losses to the 65,500 t of barley, which in 1992 and 1995 remained in the open unprotected against the rain, had they been placed in hermetic storage. The total fixed and running costs for 1 year's hermetic storage of 65,500 t of local barley on a concrete platform under PVC is estimated to be about C£152,000. The economic loss due to the lack of appropriate protection was about C£387,100. In addition, hermetic storage of grain on platforms under PVC successfully protects the grain against insect, bird and rodent attacks and also prevents its contamination by mycotoxins.

TABLE 4
Costs of building a concrete platform and of hermetic storage of 4,000 t of barley under PVC liner

Costs	Total cost	Depreciation (years)	Costs per 1 year storage (C£)	Costs per 2 years storage (C£)	Costs per 3 years storage (C£)
Fixed costs					
Building platform	56,250	30	1,875	3,750	5,625
PVC sheet for covering grain	10,990	7	1,570	3,140	4,710
Polyethylene liner for covering floor	522	1	522	522	522
Total fixed costs			2,967	7,412	10,857
Current costs					
Electricity to inload 3000 t	75		75	75	75
Labour to inload 3000 t and level grain surface	1,711		1,711	1,711	1,711
Labour to cover grain and weld PVC sheet	1,100		1,100	1,100	1,100
Labour to cover floor with polyethylene liner	40		40	40	40
Labour to remove PVC sheet	155		155	155	155
Labour to inload grain into trucks using a tractor	800		800	800	800
Total current costs			3,881	3,881	3,881
Total of fixed + current costs					
Total costs per 4,000 t			7,848	11,293	14,738
Total costs per 4,000 t per year			7,848	5,647	4,913
Total costs per t per year			1.96	1.41	1.23

Platform 25 m wide, 75 m long, 7 m high; C£1 = US$2.1.

TABLE 5
Barley loss due to rain during open storage after harvest (1992 and 1995)

Year	Total quantity harvested (thousand t)	Quantity affected by rain (thousand t)	(%)	Quantity completely spoilt by rain (t)	Losses (completely spoilt grain), % of total quantity harvested	of quantity affected by rain	Losses (completely spoilt grain, C£113/t) (C£ × 1,000)
1992	161.4	48.3	30.0	2,463	1.5	5.1	278
1995	122.6	17.2	14.0	1,011	0.8	5.9	114
Average of 1992 and 1995	142	32.8	25.8*	1,737	1.2	5.23*	196

* = weighted average.

TABLE 6
Economic effectiveness of grain storage using the hermetic method on platforms under PVC liners

1-year hermetic storage of 65,500 t of barley under PVC	C£ × 1,000 (C£1 = US$2.1)
Total cost (fixed + current): 65,500 t × C£1.96/t/year	128
Total losses after 1-year hermetic storage: 65,500 t × 0.32% losses × C£113/t	24
Total (cost + losses)	152
Total losses due to deterioration by rain occurring in 1992 and 1995 during storage of 65,500 t of barley in the open 65,500 t × 5.23% losses × C£113/t	387

CONCLUSIONS

The results of trials over a period of 7 years clearly showed that using the hermetic method of storage on concrete platforms under PVC was suitable for prolonged storage of dry barley under the climatic conditions of Cyprus.

The hermetic method of storage on platforms under PVC successfully protected stored grain against rain and against insect, rodent and bird attack. Ecologically it is an advanced method for grain storage which also has a low structural cost.

The serious problems faced by Cyprus because of limited grain storage facilities, particularly during harvest time, could be addressed by widely implementing the hermetic method of storage on platforms.

ACKNOWLEDGEMENTS

The authors wish to thank the Chairman and Members of the Board of Directors of the Cyprus Grain Commission, the Director of the CGC, and the engineers, storekeepers, technicians, workers and other colleagues of the CGC for their support and assistance. Special thanks are extended to Mrs A. Eliadou and Mr A. Antoniou for the many thousands of analyses they carried out. Warm thanks are also extended to Dr Popi Kanari, Dr Eleni Kakouri and Dr R. Argyrides, of the General State Laboratory, Mr S. Xenofontos, of the Seed Production Centre, and Dr P. Hadjigeorgiou, of the Chemical Laboratory of the Dept. of Agriculture, and to their staffs.

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SESSION 4

PHYSICAL AND CHEMICAL PROCESSES IN THE APPLICATION OF CONTROLLED ATMOSPHERES AND/OR FUMIGATION

Chairpersons:
D.S. Jayas, *Canada*
Linda Mason, *USA*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 193–202

SORPTION OF CARBON DIOXIDE BY WHEAT

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ABSTRACT

Sorption of carbon dioxide (CO_2) by soft red winter wheat was tested in 1-L jars, each containing 700 g of grain with 12.9% moisture content and each filled to 93% of jar capacity. In one series of experiments, the jars of wheat were purged with CO_2 to obtain an initial concentration of 99.8%. The jars were sealed and pressure changes were recorded periodically. All tests were carried out at temperatures of 15, 20, 25 and 30°C. In a second series of experiments, different CO_2 partial pressures ranging between 30.6 and 98.6% were maintained at 25°C in the filled jars.

At the initial phase of sorption, the rate was proportional to the temperature of the wheat; the highest sorption was recorded at 30°C. As sorption progressed, the higher the temperature, the shorter the time required for the sorption to reach steady-state equilibrium. The times required to reach equilibrium varied inversely with temperature (65, 80, 95 and 120 h at temperatures of 30, 25, 20 and 15°C, respectively). The lowest pressure recorded was 520 mm Hg at 15°C. Maximum sorption also varied inversely with temperature (260, 297, 331 and 393 mg CO_2/kg of wheat at 30, 25, 20 and 15°C, respectively). A linear relationship was found between sorption of CO_2 and partial pressure at a constant temperature. This was in accordance with the classic adsorbtion isotherm of Freundlich. Using this equation, the sorption of wheat at 25°C for any given CO_2 concentration in the range included in the tests can be calculated.

INTRODUCTION

The effects of modified atmospheres (MA's) on stored-product insects involve altering the concentrations of the atmospheric gases normally present in a storage structure (Bailey and Banks, 1980; Navarro and Jay, 1987). One technique utilized to obtain a MA is the application of carbon dioxide (CO_2) to reach a concentration of 60 ± 10% (Jay, 1971). Using this technique, Jay (1980) found that sorption of CO_2 by grain makes the gas effective against those insect species whose immature stages feed inside the kernel.

The concentration of a gas in equilibrium with a solid is always greatest in the immediate vicinity of the solid. This phenomenon is termed adsorption, to be distinguished from absorption, which is the bulk penetration of gas by diffusion into the structure of a solid or liquid. In many cases where the two processes occur simultaneously, the term "sorption" is used (Monro, 1961; Young and Crowell, 1962). This term will be used in this paper.

Two processes contribute to the adsorption of a gas on a solid. The first is often called "physical adsorption" and is the result of molecular interaction forces called Van der Waal forces. In the case of physical adsorption, the gas forms a physically adsorbed layer on the solid, similar to the condensation of vapor to form a liquid film. The second process which contributes to adsorption is termed chemisorption and involves the transfer of electrons between the molecules of the solid and the gas. One way to distinguish between physical adsorption and chemisorption is to attempt to remove the adsorbed gas using reduced pressure. If the process is primarily physical adsorption, then desorption of the gas is easily accomplished; however, if the process is chemisorption, much more drastic methods are usually required to recover the gas. Both surface area and porosity (or pore volume) have been recognized as playing complementary parts in adsorption phenomena in a vast range of solids (Gregg and Sing, 1967). The adsorption mechanism of CO_2 into the grain has been found to be very similar to that observed in sorption of gases by charcoal and silica gel (Mitsuda *et al.*, 1973). Sorption causes the removal of some molecules of the gas from the free space present in the treated enclosure; this in turn causes a progressive lowering of the concentration (partial pressure) of the gas in the free space.

In the application of CO_2 into large bins containing wheat, an initial rapid decay of concentration shortly after purging was observed by Banks *et al.* (1980). This initial rapid decay seems to be associated with sorption of the CO_2 by the grain. Despite much interest in CO_2 treatment, little information has been published on the sorption mechanism of wheat (Donahaye and Navarro, 1983). The most detailed work on the mechanism of CO_2 sorption by different commodities has been reported by Mitsuda *et al.* (1973) in relation to skin-packaging. They found that when grain was confined in a bag made of flexible laminated plastic film containing a CO_2 atmosphere, both the bag and its contents became as rigid after sealing as if packed *in vacuo*. The adsorption responsible for this rigidity has led to the development of skin-packaging, the "carbon dioxide exchange method" (CEM), for the preservation of rice in storage (Mitsuda and Yamamoto, 1980). Although Mitsuda *et al.* (1973) were able to demonstrate that wheat at 20°C adsorbs 75 ml CO_2/kg in 3 h, the sorption mechanism of the CO_2 on wheat was not fully investigated. Cofie-Agblor *et al.* (1993, 1995) have examined the characteristics of CO_2 sorption by wheat at four temperatures and four moisture contents (m.c.). The maximum mass of CO_2 sorbed in 60 h was 0.510 g/kg wheat at 18% m.c. and 0°C. The present work was initiated to obtain additional and comparative information on both the sorption mechanism of CO_2 in relation to temperature and the maximum reduced pressures that can be obtained when wheat is maintained in gastight containers.

MATERIALS AND METHODS

Wheat

Soft red winter wheat harvested in 1983 and having an average m.c. of 12.97% (SE ± 0.0609) was used in the tests. This wheat contained foreign material of different particle sizes, as would be present in actual storage. The foreign material was composed of 0.15% (w/w) large particles retained by a 5-mesh sieve, 0.27% particles retained by a 30-mesh sieve and 0.04% smaller particles (dust). The remaining 99.54% of the tested material was wheat.

Manometry

The sorption of CO_2 was measured using a transducer type manometer (Hastings Vacuum Gauge, Model DNNV-800) connected to a 960-ml container. Prior to each experiment, the transducer was calibrated against a Fortin type barometer (US Signal Corps model 02-380) and against the very low absolute pressure of <2 mm Hg obtained from a high vacuum pump. The amount of CO_2 adsorbed by wheat created a negative pressure in the container which was recorded for periods of up to 8 d.

CO_2 concentration

The gas concentration in the headspace of the container was measured by a gas chromatograph (Fisher Model 1200) equipped with a thermal conductivity detector and two columns, one packed with molecular sieve 13X (60/80 mesh) and the other with Columpak PQ (80/100 mesh). An integrator (Hewlett-Packard Model 3390A) was used to measure the areas under the peaks.

Experimental procedure

The volume occupied by a predetermined weight (686 g) of wheat grain was measured using the manometric technique described by Day (1964). The grain m.c. was determined using a capacitance moisture meter (Motomco Model 919). The grain was then poured into containers having a predetermined volume (960 ml), and the metal screw-on lid was tightly closed. The 68-mm diameter lid was equipped with two 1.15-mm i.d. copper tubes. One tube extended 160 mm and the other 35 mm into the container. They extended 45 mm above the outside edge of the lid and were used for the gas inlet and outlet.

The containers were submerged in constant-temperature water baths equipped during the experiments with refrigeration and heating systems so that the temperature could be adjusted to both below and above room temperature. The grain temperature was measured daily by a thermistor placed in the center of a container of the same volume which also held 686 g of wheat. This method of temperature measurement in a separate container was adopted in order to avoid leaks from the container lids. The containers were purged with CO_2. Prior to the CO_2 purge, the wheat was maintained for 24 h in the baths to ensure temperature stabilization.

Each experiment began with an initial purge of CO_2 supplied from pressurized cylinders at a flow rate of approximately 200 ml/min for 9 min. During this purge phase, gas

samples were taken for CO_2 analysis. At the end of the purge, the transducer was immediately connected to the gas inlet tube, the container was sealed and both the initial container pressure and the barometric pressure were recorded.

Two sets of experiments were conducted. In the first set, an initial average CO_2 concentration of 99.8% was attained immediately after purge and the pressure changes due to adsorption were periodically measured. Since the containers were maintained without additional CO_2 supply, in this set of experiments the pressure drop due to adsorption decreased the partial pressure of CO_2. These experiments were replicated three times at temperatures of 15, 20, 25 and 30°C. In the second set of experiments, a constant CO_2 concentration was periodically supplied from CO_2 cylinders so that a constant partial pressure was maintained in the containers. The pressure in the containers was subsequently brought to the original pressure, determined by daily observations, by supplying CO_2 at the tested concentration; 30-ml or 1-ml gastight syringes equipped with a three-way Luer-lock attachment were used for this. In these experiments five different CO_2 concentrations ranging from 30.64% to 99.81% were tested at 25°C. Sorption of CO_2 in mg CO_2 sorbed/kg of grain was calculated after corrections had been made for STP conditions.

RESULTS AND DISCUSSION

Effect of sorption on changes in pressure

Typical reduced pressures created by sorption in the gastight containers are shown in Fig. 1. Since the amount of CO_2 sorbed is proportional to the amount of grain in the container at a given partial pressure of CO_2 and temperature, the resulting reduced pressure will also be proportional to the void space of the system. Therefore, the drop in pressure shown in Fig. 1 can only represent the experimental conditions when the grain bulk volume occupied 93% of the total container capacity. Under these conditions, the lowest pressure recorded was 520 mm Hg at 15°C. The sorption rate changed inversely with the temperature of the wheat, and the highest absolute pressure of 606 mm Hg was obtained at 30°C. Mitsuda and Yamamota (1980) reported that a 0.8-L container filled with grain (apparently rice) developed a negative pressure of 0.27 kg/cm^2 after 7 d. This negative pressure in terms of absolute pressure at STP conditions was calculated to be approximately 555 mm Hg. This pressure falls in the range obtained in our experiments (close to the 25°C line in Fig. 1). However, since the commodity and the temperature were not mentioned in this paper, the results can not be compared quantitatively.

Sorption rate of CO_2

The initial sorption rate of CO_2 by wheat was found to follow a linear relationship during the first 3 h of the process when results were plotted on a log-log scale (Fig. 2). In these experiments, the containers in which the sorption process took place were maintained without addition of CO_2 to compensate for the changing partial pressure conditions. Therefore, the sorption rate in these experiments does not follow the conventional kinetic laws (Daniels and Alberty, 1963) and the rates shown in Fig. 2 have the restriction of

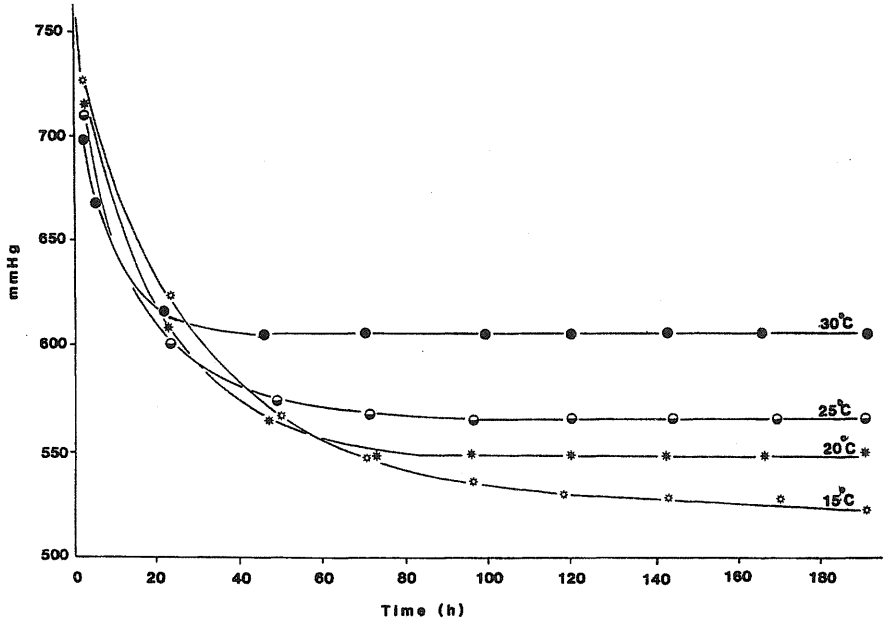


Fig. 1. Pressure decay due to sorption of CO_2 in gastight containers filled to 93% capacity with wheat (bulk volume to container) at four different temperatures, with an initial CO_2 concentration of 99.8% and an initial pressure of 768 mm Hg.

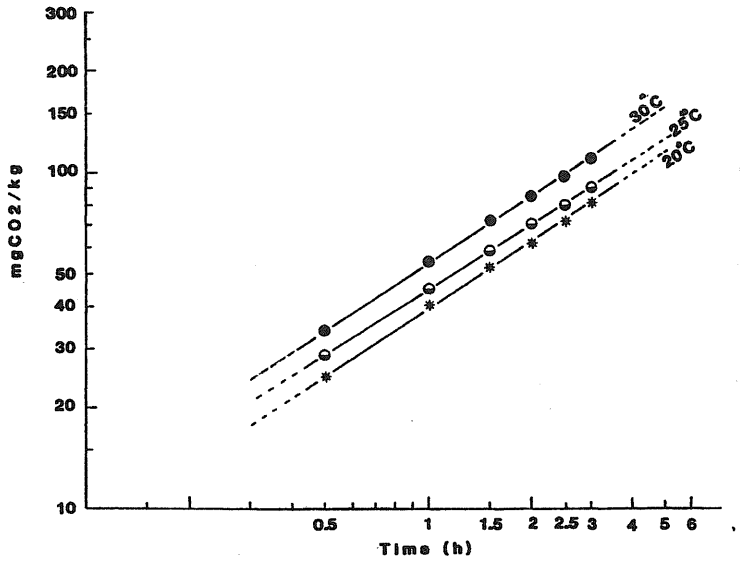


Fig. 2. Sorption rate of CO_2 by wheat during the first 3 h of the process at three different temperatures with an initial CO_2 concentration of 99.8% in containers filled to 93% capacity with wheat.

being dependent on the intensity of the changing CO_2 partial pressures throughout the sorption process. Since the grain comprised 93% of the container volume, the sorption rates shown in Fig. 2 could be considered similar to pressure changes that may occur in practice in grain bulks.

The slopes for the time–sorption curves were almost parallel with values of 0.664, 0.635 and 0.659 for 20, 25 and 30°C, respectively. A similar analysis was performed at 15°C, but the initial CO_2 purge probably caused a temperature change in the container; therefore during the first hour a sorption rate near the value of that found for 20°C was obtained. The calculated slope at 15°C was 0.512, which was markedly different from the slope obtained at the higher temperatures. The correlation factors calculated for the relationship with the sorption rate were $r^2 = 0.9994$, 0.9994, 0.9995 and 0.9982 for 15, 20, 25 and 30°C, respectively. Results obtained by Yamamoto and Mitsuda (1980) indicate that at 20°C, wheat adsorbed 75 ml CO_2/kg in 3 h. This amount of CO_2 is equivalent to 147.2 mg CO_2/kg wheat. Cofie-Agblor *et al.* (1995) also showed that sorption of CO_2 was about 135 mg/kg at 20°C after 3 h for wheat at 14% m.c. In contrast our results indicate that at 20°C only 81.6 mg CO_2/kg was sorbed after 3 h.

The sorption rate in our experiments was calculated under decreasing partial pressure. In practice, when a commodity is kept in a container a decrease in the partial pressure due to the sorption process would be expected, and in a completely sealed container the drop in pressure would cause a decrease in the partial pressure of CO_2. Similarly, in a container with leaks sorption would cause air to enter the system as the pressure decreases, although the total pressure would be maintained close to that of the surrounding atmosphere. Therefore, the partial pressure of CO_2 would decrease, resulting in further lowering the sorption rate. Our results clearly show that at the initial phase of sorption the higher the temperature the higher is the sorption rate (Fig. 2).

Sorption at equilibrium

The length of time needed for the sorption process to reach steady-state equilibrium at varying pressures, due to adsorption at different temperatures, is shown in Fig. 3. These data demonstrate that the higher the temperature, the shorter the time required to reach equilibrium. Figure 3 shows that the time required to reach equilibrium was 65, 80, 95 and 120 h at 30, 25, 20 and 15°C, respectively. This is in contrast to the initial sorption process, which showed a higher initial rate at higher temperatures (Fig. 2). In the study by Cofie-Agblor *et al.* (1995) the longest duration was 60 h which was not sufficient to reach equilibrium, as they also indicated. The amount of CO_2 sorbed at equilibrium could only be calculated from their equations, whereas in our work the values were the times recorded until equilibrium was reached, and for the experiments at 15°C this lasted up to 120 h.

Calculations of pressure drop or air entry into a CO_2-treated wheat container could be made from the results shown in Figs. 2 and 3, provided the initial CO_2 concentration is 99.8% and the filling ratio is 93%. Since a higher sorption was found at 15°C than at 30°C, it would be advantageous to apply MA's at high temperatures with subsequent reduction of a substantial amount of the CO_2 concentration.

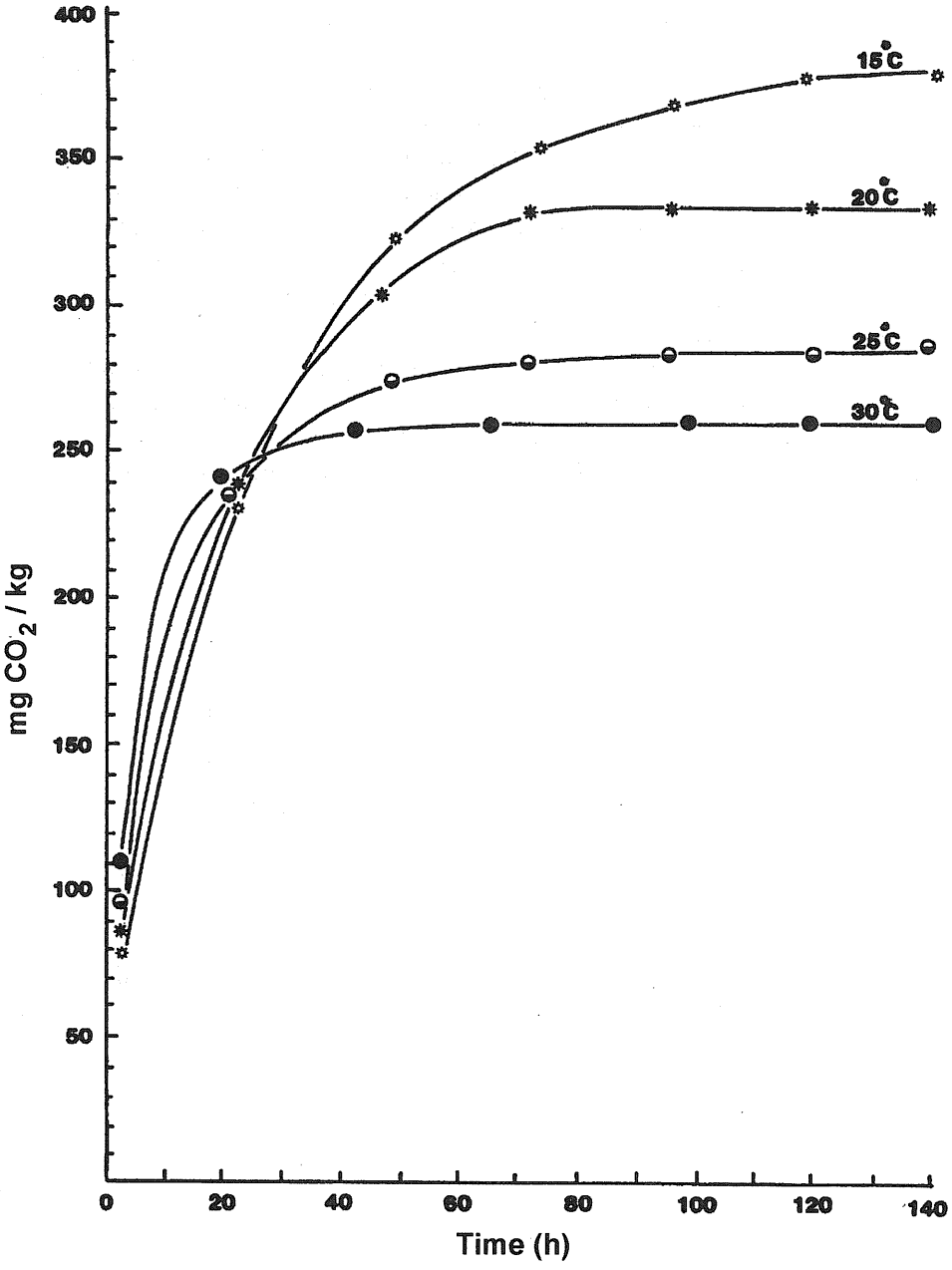


Fig. 3. Time to reach sorption equilibrium between CO_2 and wheat at four different temperatures, with an initial CO_2 concentration of 99.8% in containers filled to 93% capacity with wheat.

Sorption isotherm

An attempt was made in these experiments to simulate field conditions where, after an initial CO_2 purge, sorption would create a partial pressure differential. The amount of CO_2 sorbed to reach equilibrium was measured at 25°C in order to demonstrate the relationship between sorption and constant partial pressures. This relationship was tested for linearity using the classic adsorption isotherm of Freundlich (Daniels and Alberty, 1963). The calculated correlation factor was $r^2 = 0.9890$ for the tested CO_2 concentrations varying between 30.64% and 99.81%. This relationship was described by the equation: log mg CO_2/kg = 2.643 + 1.719 log P CO_2. A plot of our data and the calculated line are shown in Fig. 4. Using this equation, the adsorption of wheat at 25°C for any given CO_2 concentration in the range of our tests can be calculated. A similar relationship for rice has been described by Mitsuda *et al.* (1973).

The expected amount of CO_2 sorbed at a constant CO_2 partial pressure may well describe a specific situation in which the supply of CO_2 to the commodity is almost constant. However, since in a field situation (or even in a small container) there is always a changing CO_2 partial pressure, the rate at which sorption takes place needs further investigation.

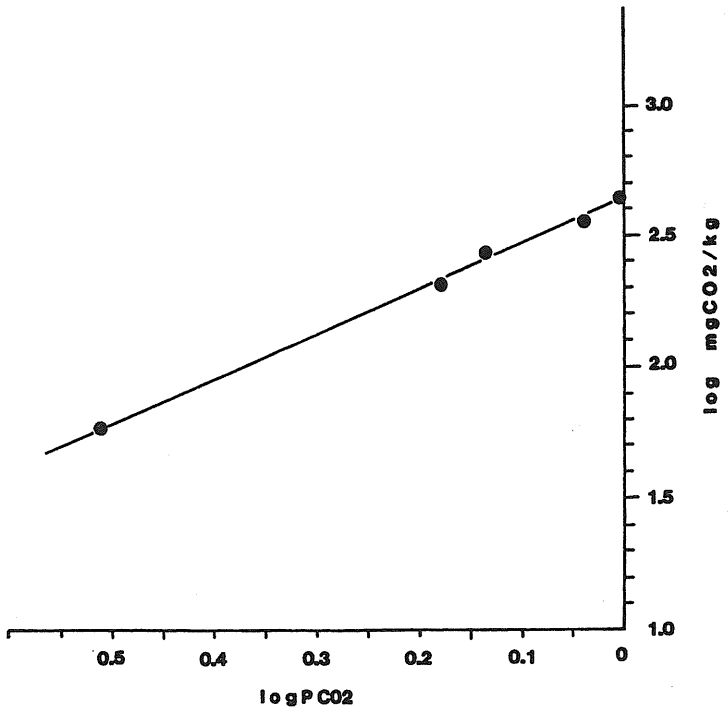


Fig. 4. Relation between the amount of CO_2 sorbed and the partial pressure of CO_2 at 25°C until equilibrium is reached between the CO_2 and the wheat.

Once the sorption rates at different temperatures and varying partial pressures are known, it may be possible to formulate an equation to describe the CO_2 concentration for any given ratio of the volume occupied by commodity to the total volume of the container.

ACKNOWLEDGEMENT

The author wishes to thank Drs E.G. Jay and J. Leesch, USDA, ARS, Stored-Product Insects Research and Development Laboratory, Savannah, GA, for their support, and Mr G.C. Pearman Jr. of the same laboratory, for his technical assistance during these experiments. This research was supported in part by Grant No. I-303-80 from BARD, the United States–Israel Binational Agricultural Research and Development Fund.

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SORPTION OF FUMIGANTS BY CUT FLOWERS

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ABSTRACT

Sorption of fumigants (carbonyl sulphide, methyl bromide and phosphine) by two flower species (*Dianthus* sp. and *Anigozanthos* sp.) was studied. Exposures were carried out in stainless steel chambers at concentrations suggested for insect disinfestation of cut flowers or similar commodities; carbonyl sulphide (COS), 15 mg L^{-1}; phosphine (PH_3), 0.25 mg L^{-1}; and methyl bromide (MB), 32 mg L^{-1}.

Sorption profiles differed for each of the fumigants but were relatively constant across the flower species. The greatest fumigant loss was observed with COS; after 7 h, no measurable levels of fumigant were detected, indicating that the sorption process is accompanied by a metabolic process in the flowers. Over the 12-h exposure period, PH_3 and MB displayed a 33% and 5% sorption of fumigant, respectively. Such levels of sorption may be easily accounted for in the development of a fumigation procedure. PH_3 may have potential as a replacement for MB as a fumigant for cut flowers.

INTRODUCTION

A need to identify new fumigants and fumigation regimes for a range of commodities became apparent following the identification of methyl bromide (MB) as a powerful ozone depletor (UNEP, 1994). MB has been routinely used to disinfest cut flowers, one of the commodities for which alternative fumigants are being sought. Cut flowers, which by their very nature attract insects, are not acceptable for either domestic or international markets if infested.

A recent study of the deleterious effects of five potential fumigants on flower quality identified phosphine (PH_3), carbonyl sulphide (COS) and hydrogen cyanide (HCN) as potential replacements for MB as a quarantine fumigant for cut flowers (Weller *et al.*, 1995).

Any successful fumigation requires that the concentration of fumigant be maintained at, or above, a given level long enough to kill all target organisms. Fumigant concentrations do not remain static during fumigation; they decline through leakage from the system and physical and chemical binding to the fumigation chamber and the commodity,

or they may be broken down through chemical reaction (Banks, 1986, 1990a). In developing fumigation regimes for any commodity, it is necessary to understand these interactions and compensate, where possible (Banks, 1990b). Sorption, the physical and chemical bonding of the fumigant to the fumigation chamber and to the commodity, is a predictable loss which can be overcome by using either a higher initial dose or by boosting the concentration during the fumigation (Stout, 1983).

In comparing the sorption response of durable commodities with that for cut flowers, very different responses might be expected because of the different properties of the commodities. The high moisture content of flowers and the presence of free water, whether associated with storage of cut flowers or condensation, will undoubtedly produce an effect due to the varying solubility of fumigants. Likewise, the more active metabolic state of cut flowers, compared with that of durable commodities, may effect the perceived sorption rates.

This paper reports on work undertaken to study sorption of three fumigants by cut flowers. Two of them have been identified as possible MB replacements: PH_3 and COS.

MATERIALS AND METHODS

The flowers used in this study were field carnations (*Dianthus* sp.) and kangaroo paw (*Anigozanthos* sp. hybrid).

Fumigations were carried out in stainless steel chambers (approximately 2.37 L) fitted with Swagelock-fittings to facilitate circulation of fumigants. A measured amount of concentrated source gas was introduced into the fumigation chamber through a septum (M-type, Alltech) insert to achieve the desired fumigation dose. Gases in the chamber were circulated using a diaphragm pump (5 L min^{-1}) for 5 min to ensure an even distribution of gas. Gas samples were taken from the chamber at regular intervals to measure any change in fumigant concentration during the exposure period.

Fresh flowers were purchased through a local wholesaler. Individual bunches of each flower type were mixed and sub-samples taken, weighed and placed in the fumigation chambers. The weight of flowers used in each experiment was 110–120 g. Following fumigation the dry weight of the flowers was determined by drying in a forced draught stainless steel lined oven at 70°C for 72 h.

Sorption profiles of COS, MB and PH_3 were determined for both carnations and kangaroo paw over a 12 h period. The initial dose applied for each fumigant was that suggested for cut flowers or similar commodities: MB 32 g m^{-3} (Stout, 1983), COS 15 g m^{-3} (Desmarchelier, 1994) and PH_3 0.25 g m^{-3} (Winks, 1986). All exposures were carried out at 21°C.

Controls consisting of sealed, empty fumigation chambers were also dosed as above to determine the "chamber effect", if any, on fumigant concentrations.

Concentrated sources of COS and PH_3 were generated in the laboratory. COS was produced by the addition of potassium thiocyanate to sulphuric acid, and the resultant gas

was passed through a lead acetate solution to remove associated hydrogen sulphide (Ferm, 1957). PH_3 was generated by the addition of aluminium phosphide tablets (Phostoxin, Degesch, Germany) to 5% (v/v) aqueous sulphuric acid (Anon., 1975).

The concentrations of the generated gases (COS and PH_3) were determined using a Gow Mac model 11-625 gas density detector on a Tracor MT150 gas chromatograph (GC) fitted with a Porapak Q 80/100 column. Fumigant loss was determined over the 12-h exposure period by analysis of gas concentrations in the head space of the chambers. Fumigant concentrations were measured in each chamber, during exposure, using gas chromatography techniques. MB was measured using a Shimadzu 6AM GC fitted with an FID and a 20% OV101 on Gas Chrom Q column run at 50°C isothermal. COS and PH_3 were both detected using a Tracor MT-220 GC fitted with a FPD (sulphur mode for COS and phosphorus mode for PH_3) and a Haye Sep Q 80/100 mesh column run at 110°C isothermal.

The effect on the rate and amount of sorption of the weight of flowers fumigated was studied for both PH_3 and COS using kangaroo paw. These experiments were carried out using the same methodology as above. The weight of flowers fumigated was varied by steps between 0 and 100 g. Tests were carried out using initial doses of 0.25 mg L^{-1} for PH_3 and 15 mg L^{-1} for COS. In calculating the amount of concentrated source to deliver to the chamber, the volume of the fumigation chamber was assumed to be that of the empty chamber (the reduction of headspace due to the mass of flowers was not taken into consideration).

RESULTS

Sorption of methyl bromide

Loss of MB observed over six replicates was averaged and is presented in Fig. 1. All plots are expressed as a fraction of the original concentration in the head space (C/C_0) against time (h). The lowest rate of loss occurred in the empty fumigation chamber

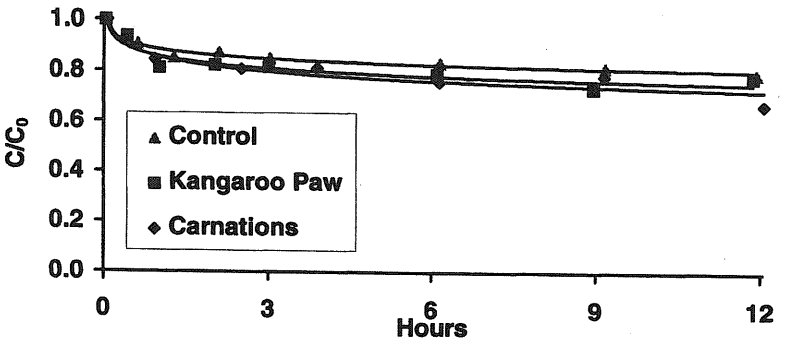


Fig. 1. Sorption of MB by cut flowers. Fraction of original head space concentration (C/C_0) vs time (h).

(control). The difference between the sorption profile of the control and the sorption profiles for the fumigation of kangaroo paw and carnations can be attributed to sorption by the flowers. It is apparent that the loss of a large proportion of the MB can be attributed to sorption by the fumigation chamber and a smaller proportion to sorption by the flowers.

All three tests followed a logarithmic curve which can be described thus:

— empty fumigation chamber: $y = -0.033 \ln(x) + 0.89$ (R^2 value 0.97);
— kangaroo paw: $y = -0.042 \ln(x) + 0.86$ (R^2 value 0.86);
— carnations: $y = -0.052 \ln(x) + 0.86$ (R^2 value 0.90).

Sorption of phosphine

Losses of PH_3 observed over five replicates were averaged and are presented in Fig. 2. The empty fumigation chamber displayed an initial loss of gas after which the concentration remained constant. The fraction of the original concentration in the head space (C/C_0) extrapolated to time zero (the intercept value) of 0.98 is indicative of a low level of adsorption to the chamber surface.

In the chambers containing flowers, the plots follow a logarithmic loss and are described thus:

— kangaroo paw: $y = -0.06 \ln(x) + 0.70$ (R^2 value 0.92);
— carnations: $y = -0.10 \ln(x) + 0.75$ (R^2 value 0.99).

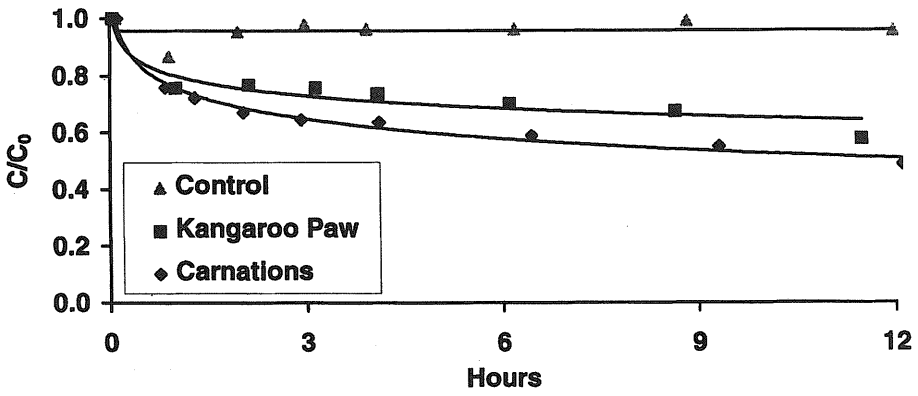


Fig. 2. Sorption of PH_3 by cut flowers. Fraction of original head space concentration (C/C_0) vs time (h).

Sorption of carbonyl sulphide

Losses of COS for six replicates were averaged and are presented in Fig. 3. The gas loss observed in the empty fumigation chamber was minimal. It can best be described as a straight line with a C/C_0 intercept value of 0.99.

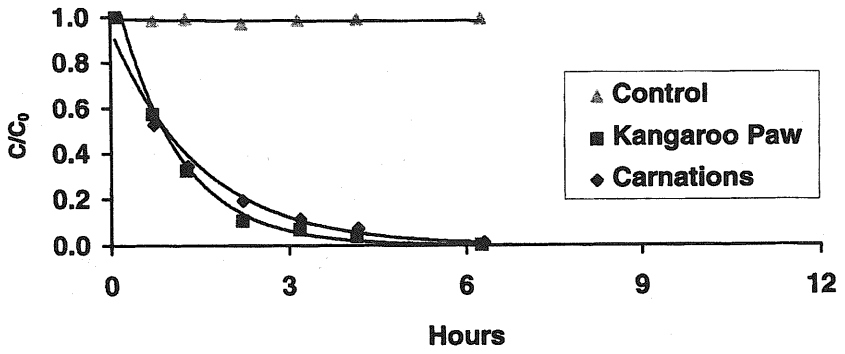


Fig. 3. Sorption of COS by cut flowers. Fraction of original head space concentration (C/C_0) vs time (h).

The loss of COS during the fumigation of flowers was so rapid that within 6 h of commencing fumigation there was no detectable COS. Hydrogen sulphide was detected in the chamber after 1 h and continued to increase in concentration over the duration of the fumigation. Fumigations were terminated when the concentration of COS fell below detectable levels.

In the chambers containing flowers the fumigant losses are described by logarithmic relationships thus:

— kangaroo paw: $y = -0.23 \ln(x) + 0.41$ (R^2 value of 0.99)

— carnations: $y = -0.25 \ln(x) + 0.40$ (R^2 value of 0.97).

Effect of fill ratio on sorption

Phosphine. Results where the weight of flowers being fumigated was varied are given in Fig. 4. The loss of PH_3 observed over the first hour increased with the increasing weight of flowers. Thereafter, the sorption profiles described by different masses of flowers were

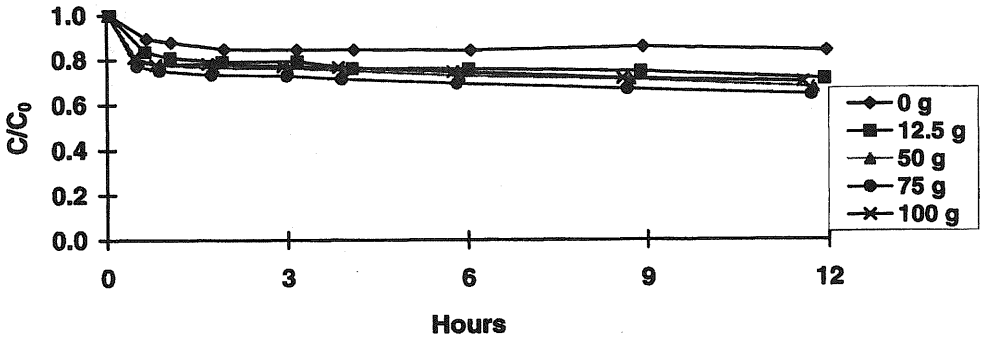


Fig. 4. Effect of weight of flowers (kangaroo paw) on sorption of PH_3 by cut flowers. Fraction of original head space concentration (C/C_0) vs time (h).

very similar. This could be explained by increased physical sorption due to the increasing surface area of the flowers.

Carbonyl sulphide. Results for the variation in weight of flowers for COS are given in Fig. 5. Uptake of COS was affected by the weight of flowers fumigated and can be plotted as a semi-logarithmic relationship (Fig. 6). Uptake of COS increased with increasing flower weight. Plotting the slopes from Fig. 6 (rate constant) against the weight of flowers fumigated gave a linear relationship (Fig. 7). This relationship enabled us to make predictions of the loss of COS that might be expected in a commercial scale fumigation facility at various fill levels. It may be noted that all of the fill ratios (even the lowest) used during this trial were significantly higher than those encountered in most commercial situations.

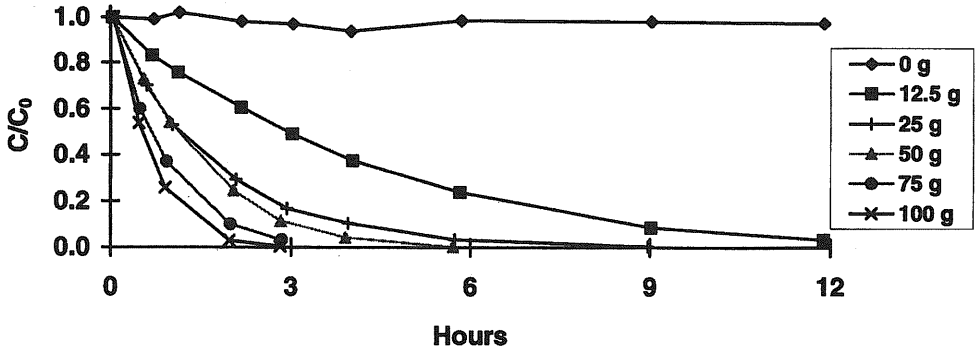


Fig. 5. Effect of weight of flowers (kangaroo paw) on sorption of COS by cut flowers. Fraction of original head space concentration (C/C_0) vs time (h).

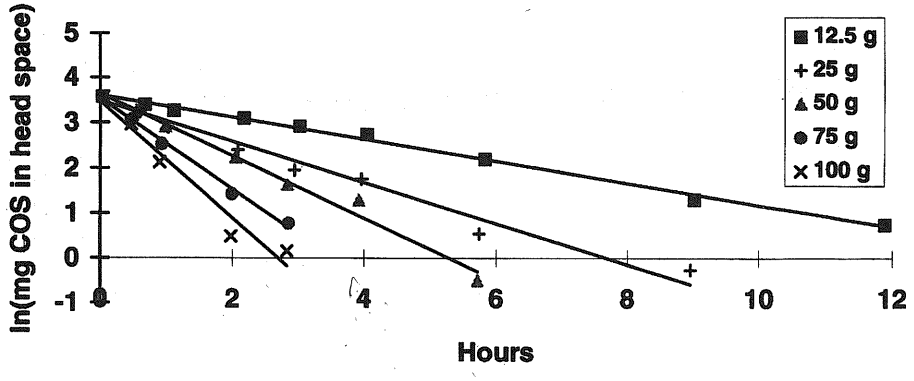


Fig. 6. Effect of weight of flowers (kangaroo paw) on sorption of COS by cut flowers (ln mg COS in the head space) vs time (h).

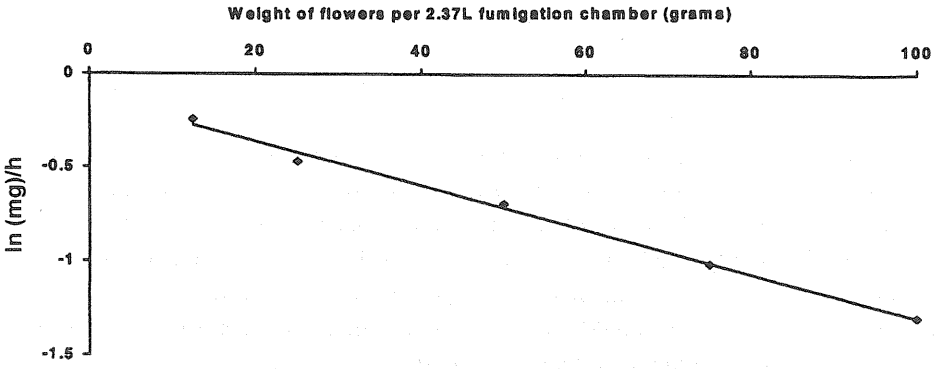


Fig. 7. Rate of COS sorption by cut flowers (kangaroo paw).

DISCUSSION

It is generally accepted that the mechanism of sorption incorporates two processes. The first is a rapid uptake of the fumigant, described as physical adsorption of the fumigant, whereby the gas molecules are loosely bound to the surfaces of the commodity and the fumigation enclosure. The second is a slower uptake of the fumigant, thought to be related to diffusion of the fumigant into the commodity, whereby the gas molecules are more tightly bound to them. In a gas tight system an equilibrium is reached between the "free gas" in the chamber and the sorbed or "bound gas". Gasses which have been sorbed by commodities can be recovered from the system.

Banks (1986) describes the diffusion/sorption process mathematically as a semi-logarithmic relationship, between concentration and time, which is dependent upon the commodity, the fumigant and the fill ratio.

Although there was no significant difference between the sorption of PH_3 and of MB by the two flower genera tested, carnations consistently sorbed more of both fumigants than kangaroo paw. This may be related to the different surface area and physical properties of the two flowers, and it may also correspond to their relative moisture content. The percentage weight lost through drying carnations at 70°C for 72 h was 83%, compared with an 81% loss in kangaroo paw.

Solubility of MB and PH_3 in the water component of flowers may, in part, account for the sorption observed. The solubility of both MB and PH_3 in water at 25°C is fairly low. MB has a solubility of 1.3 g/100 ml (Anon., 1979) and PH_3 0.03 g/100 ml (Weston, 1954).

COS fumigation of cut flowers followed a semi-logarithmic relationship. The fumigant concentration fell rapidly to levels which we were unable to detect. The production of hydrogen sulphide within the chamber indicated that, rather than being sorbed, the fumigant was reacting with the commodity or being metabolised. COS is only slightly soluble in water, 0.12 g/100 ml at 25°C (NIOSH, 1979) and it hydrolyses slowly to produce carbon dioxide and hydrogen sulphide. The reaction is catalysed by many substances and

ions, especially the hydroxyl ion (Thompson *et al.*, 1935). In higher plants metabolic pathways for the consumption of COS have been described, most significantly where carbonic anhydrase catalyses the hydrolysis of COS to produce H_2S and CO_2 (Protoschill-Krebbs and Kesselmeier, 1992).

In developing new fumigation procedures for cut flowers, PH_3 offers potential. The sorption properties of cut flowers with respect to PH_3 are measurable and may be easily accounted for in the development of a fumigation procedure. Unfortunately, the short exposure periods usually used for cut flowers do not kill the eggs and pupae of many insects and are also ineffective against many species of mites (Hole *et al.*, 1977). As a consequence PH_3 fumigation could form part of an integrated control program but is unlikely to offer a stand-alone treatment for all pests.

Despite the rapid consumption of COS by cut flowers, reducing the fill ratio to levels more akin to those experienced in commercial fumigation facilities would reduce the relative speed of decay. If the fill ratio is small enough, the concentration could be maintained at fumigation levels for a period long enough to achieve mortality; therefore, COS could be an effective fumigant for cut flowers.

ACKNOWLEDGEMENTS

The research reported in this paper was funded by the Australian Environment Protection Agency, the Flower Exporters Council of Australia, and the Rural Industries Research and Development Corporation.

We are grateful to Chris Whittle and Jonathan Banks for their comments and advice during the preparation of this paper.

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MATHEMATICAL MODELS FOR CARBON DIOXIDE DISTRIBUTION AND LOSS IN BULKS OF GRAIN

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ABSTRACT

A mathematical model was developed for predicting the distribution of carbon dioxide (CO_2) through grain bulks. The model was solved using a finite element method and can be used for any shape or size grain bulk, provided that the bulk can be discretized into 8-node brick elements and input data can be prepared for the model. The model was developed in stages. Initially, the model was based on diffusion of CO_2 through bulk grain. In the current form, the model includes diffusion and movement of CO_2 within the grain bulk and sorption or desorption of CO_2 by the grain. The predictions of the model, in its various stages of development, were compared with experimental data on the distributions of CO_2 in three pilot-scale bins (about 1.4 m in diameter and 1.5 m high). We compared our experimental data with predictions based on two previously-published models of the loss of CO_2 from storage structures. Areas for further research in the development of mathematical models are also identified in this paper.

INTRODUCTION

A stored-grain bulk is a man-made ecological system in which deterioration of the stored-product results from interactions among physical, chemical and biological factors. Heat, moisture and carbon dioxide (CO_2) are produced during the deterioration of grain; therefore, increases in grain temperature, moisture content and CO_2 concentration in the intergranular air can all be used as indicators of incipient grain spoilage. An increase in the CO_2 concentration is a better indicator of incipient spoilage than the increase in temperature or moisture of grain because the normal concentration of CO_2 in the atmospheric air is low (0.03%) and therefore any increase can be easily detected. In addition, the movement of CO_2 through bulk grain is faster than is the movement of heat or moisture.

Modified atmospheres (MA's) provide an environment which is lethal to stored-grain pests. This is achieved by altering the proportions of CO_2, nitrogen (N_2) and oxygen (O_2) in the intergranular atmosphere. MA's containing elevated CO_2 (>60%), depleted O_2

(<10%) and low relative humidities (<50%) at high temperatures (>27°C) are, within a few days of exposure, lethal to most of the insect and mite species that are responsible for losses in stored grain (White *et al.*, 1990; Jayas *et al.*, 1991; White and Jayas, 1991; Rameshbabu *et al.*, 1991). Under a given set of environmental conditions, exposure times and gas concentrations are the critical factors, and the required minimum of these factors to cause death varies with pest species. To create MA's, CO_2 or N_2 is usually introduced into the grain mass at single or multiple points. The uniform distribution of introduced gases and the maintenance of the lethal concentration for the required duration are critical for effective control of pest populations.

The movement of CO_2 through bulk grain is dependent on the characteristics of the grain, the storage structure and the weather. Mathematical models can be developed to predict CO_2 concentrations at various locations in the stored grain when CO_2 is produced during grain spoilage or when CO_2 is introduced for disinfestation. The predictions of such models can be used either to determine the resolution required and the location of CO_2 sensors for the detection of incipient spoilage in stored grain, or to design systems that will provide a uniform distribution of CO_2 in the bulk grain. Mathematical models of the loss of CO_2 from non-airtight structures can be used to determine the amount, frequency and location of the gas injections needed to maintain lethal atmospheres.

Mathematical models, if based on the principles of physical and biological sciences and properly validated, can be used to study the effects of various parameters, such as weather, grain condition, and the size, shape and material of the storage structure, on the distribution and loss of CO_2. The major advantages of validated models are their ability to answer "what if?" questions and their transferability to different climatic regions of the world, thus making them globally applicable rather than only locally applicable (as is expected of experimental studies). Only a few studies on the movement of CO_2 within the grain bulk and the loss of CO_2 from the stored grain have been reported in the literature (Singh *et al.*, 1983; Banks and Annis, 1984; Navarro *et al.*, 1985; Jayas *et al.*, 1988; Alagusundaram *et al.*, 1991; Navarro *et al.*, 1991). Our research group has been working on the development and validation of a mathematical model of the distribution of CO_2 and on the evaluation of reported models for their ability to predict the loss of CO_2 from the storage structures. Progress on our model and the results of our evaluation of two CO_2-loss models are summarized in this paper.

MATHEMATICAL MODEL FOR CO_2 DISTRIBUTION

Earlier models

Singh *et al.* (1983) solved an axisymmetric diffusion equation using the finite element method and then predicted CO_2 concentrations in grain masses containing localized spoiling grain. The model predictions were not compared with experimental data. Based on simulations, we determined that the best location for installation of a CO_2 sensor for detection of incipient spoilage was at the centre of the grain mass. This was later confirmed experimentally by Muir *et al.* (1985). Later Jayas *et al.* (1988) used the model of

Singh *et al.* (1983) for predicting the CO_2 concentrations in a 5.8-m-diameter bin containing wheat 4.9 m deep. The predicted concentrations at various locations were compared with values measured 23 h after injecting CO_2 both at the centre and 1 m below the grain surface (all CO_2 concentrations were measured by gas chromatography). In the bottom two-thirds of the bin, the predicted CO_2 concentrations (assuming the bin wall to be impermeable to CO_2) were higher than the measured concentrations. We concluded that the model prediction could be improved by allowing some leakage through the bin wall.

In a stored-grain bulk, CO_2 may be introduced at points near the wall and then sorbed (absorbed and adsorbed) by grain, or it can be produced at various locations by pockets of spoilage containing respiring grain, microflora, mites and insects, thus making the problem of CO_2 movement three-dimensional. Alagusundaram *et al.* (1991) solved a three-dimensional diffusion equation using the finite element method for predicting CO_2 distribution in bulk-stored wheat. The predicted CO_2 concentrations were much lower than the measured concentrations in three wheat-filled, 1.42-m-diameter bins (refer to the section on experimental data for model validation). In this model, the bulk movement of CO_2 caused by the expansion of the CO_2 during sublimation was not considered. Later, Alagusundaram *et al.* (1996a) used an experimentally-determined apparent flow coefficient (D_{app}) during the application period to model the bulk movement of CO_2. Although the model predictions were improved using this approach, the errors continued to be high during the initial 3-h period after the introduction of dry ice. To use the approach of Alagusundaram *et al.* (1996a), in addition to the diffusion coefficient the values of D_{app} must be determined for every grain. Such an empirical approach is not an efficient method for use in a mathematical model.

Present model

The transient transport of miscible fluids in an anisotropic porous medium is governed by the following partial differential equation (Bachmat and Bear, 1964; Huyakorn *et al.*, 1986; Bundus *et al.*, 1996):

$$\frac{\partial C}{\partial t} = \frac{\partial}{\partial x_i}\left(D_i \frac{\partial C}{\partial x_i} - V_i C\right) + q \tag{1}$$

subject to the boundary conditions:

$$C = C_{S1} \qquad \text{on boundary S1} \tag{2}$$

$$D_i \frac{\partial C}{\partial x_i} l_i - V_i C l_i = Q \qquad \text{on boundary S2} \tag{3}$$

and the initial condition:

$$C\,(i, t = 0) = C_0 \qquad \text{in the domain } \Omega \tag{4}$$

where C = concentration of CO_2 at time $t > 0$ (kg/m^3), C_0 = initial concentration of CO_2 in

the domain Ω (kg/m^3), D_i = diffusion coefficient of CO_2 through bulk grain in the ith direction (m^2/s), l_i = direction cosines of outward drawn normal to the boundary, q = amount of CO_2 sorbed or produced by the grain (kg m^{-3} s^{-1}), Q = total surface flux across the boundary S2 (kg m^{-2} s^{-1}), S1 and S2 = boundary segments (defined in the following paragraph), t = time (s), V_i = velocity of CO_2 flow in the ith direction (m/s), and Ω = domain consisting of the stored-grain bulk; subscript $i = x$, y and z in a Cartesian coordinate system.

The boundary S1 represents the segment of the boundary where concentration of CO_2 can be specified. It may consist of more than one part of the boundary. For example, part of S1 may represent the surface of the grain where concentration may be specified as constant at the atmospheric level in a ventilated head space; another part of S1 may represent the portion of a grain boundary where CO_2 is injected and thus maintained at a constant concentration. Similarly, S2 may be made up of more than one part of the boundary. For example, the bin floor may be assumed to be impermeable to the flow of CO_2, whereas bin walls may have a specified flux of CO_2 to the surroundings depending on the rate of loss through the bin wall. Segments S1 and S2 together make the total boundary of the domain Ω. The specified CO_2 concentration along boundary S1 is known as the Dirichlet boundary condition, and the specified flux across boundary S2 is known as the Neumann boundary condition. When the surface flux, Q, is zero, Eq. (3) represents a homogenous Neumann boundary condition.

To handle the period of CO_2 sublimation from dry ice, we calculated and used effective diffusivities that take into account the effects of both bulk movement and diffusion. Replacing diffusion coefficients with effective diffusivities during the CO_2-sublimation period eliminates the velocity terms in Eq. (1), thus eliminating the computational difficulties engendered by the presence of the velocity term. The effective diffusivities were calculated using the equations of Saffman (1960) and Bear (1972):

$$D_L = \frac{1}{2} V_a L \left(\frac{\ln 3 V_a \tau_0}{L} - \frac{1}{12} \right) \tag{5}$$

$$D_T = \frac{3}{16} V_a L \tag{6}$$

where D_L = longitudinal diffusivity (diffusivity in the major direction of flow) (m^2/s), D_T = lateral diffusivity (diffusivity perpendicular to the major direction of flow) (m^2/s), L = grain size (m), V_a = average Darcy velocity (m/s) and $\tau_0 = L^2/2D_i$ (1/s).

The diffusion coefficient of 6.46 mm^2/s (Shunmugam *et al.*, 1994) was used after the sublimation of CO_2. It was assumed that the diffusion coefficient was independent of the direction of diffusion (Singh *et al.*, 1984) and of CO_2 concentration (Cunningham and Williams, 1980).

Experimental data for model validation

The measured CO_2 concentration data of Alagusundaram *et al.* (1996b) were used to validate the model. The CO_2 concentrations were measured at various locations in wheat

bulks contained in three bins 1.42-m diameter by 1.47-m height (Fig. 1). Each of the three bins was equipped with a different partially-perforated floor opening (circular at the centre, rectangular and circular near the wall). The CO_2 data were collected from five levels in each bin (near the floor and then spaced 0.33 m apart vertically). At each level there were 11, 13 and 12 sampling points for Bins 1, 2 and 3, respectively. Metal boxes ($0.5 \times 0.5 \times 0.37$ m for Bins 1 and 3 and $1.22 \times 0.46 \times 0.36$ m for Bin 2) were mounted centrally under the floor openings. Known quantities of dry ice were introduced in these boxes to create high CO_2 concentrations in the wheat bulk. The CO_2 concentrations were recorded at 1, 3, 6, 9, 12 and 21 h after the introduction of dry ice. The tops of the grain bulks were either left open or covered with a polyvinylidene chloride (PVDC) sheet impermeable to CO_2.

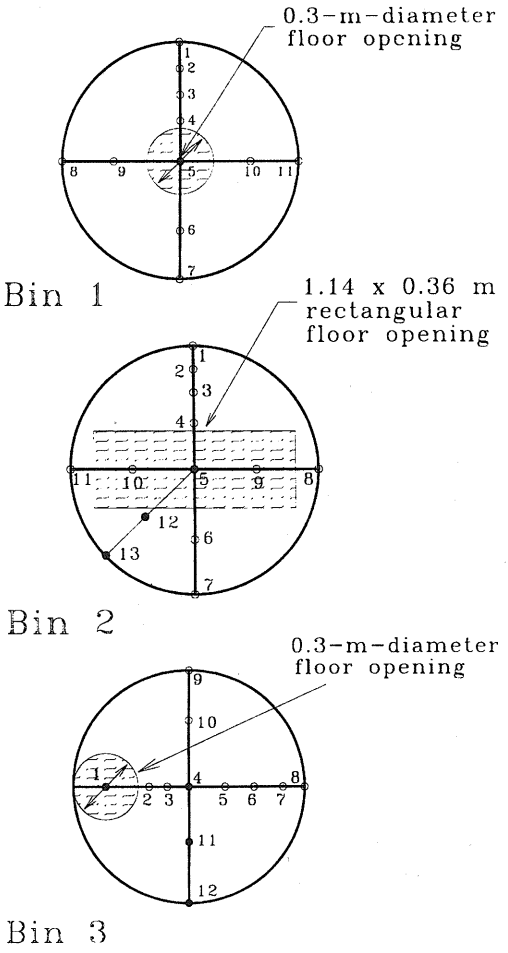


Fig. 1 Schematic diagram of the temperature and gas sampling locations in 1.42-m-diameter by 1.47-m-tall bins. ● = temperature and gas samples; ○ = only gas samples.

Validation of the present model

The CO_2 concentrations predicted by the convective-diffusive model were compared with the measured CO_2 concentrations by calculating the mean relative-percent-error (hereafter referred to as error):

$$e = \frac{1}{N}\sum_{i=1}^{N}\frac{|M_i - P_i|}{M_i} \times 100 \tag{7}$$

where e = mean relative percent error (%), M_i = measured CO_2 concentration at sampling location i (%), N = number of data points and P_i = predicted CO_2 concentration at sampling location i (%).

The errors in all three bins (Fig. 1) at different sampling times are given in Table 1. These data can be used to judge the accuracy of the model prediction. The accuracy of model predictions improved considerably in comparison with both the predictions of a pure diffusion model (Alagusundaram *et al.*, 1991) and the predictions using experimentally determined apparent flow coefficients during the dry ice sublimation period (Alagusundaram *et al.*, 1996a). However, the errors were still high in the initial time periods. There are two probable reasons for such high errors in the initial time periods:

1. The horizontal velocity, V_x, was estimated using the airflow-pressure drop relationship of wheat. The flow characteristics of CO_2 through wheat could be different from that of the flow characteristics of air. Data on the low-velocity flow characteristics of CO_2 through agricultural grains are essential for accurately predicting the CO_2 distribution in MA storage.

2. The gravity effect was not included in the model. CO_2 is about 1.5 times heavier than air (CO_2 density at a temperature of 20°C is 1.82 kg/m^3 compared with air density of 1.19 kg/m^3 at the same temperature). The gravitational forces acting on the heavier CO_2 molecules will reduce their rate of vertical movement. This hypothesis was supported by the fact that the differences between the measured and the predicted CO_2 concentrations at 0.33 and 0.66 m above the floor were larger than the differences near the floor. For example, in Bin 1 with 180 g dry ice and a covered grain surface, the average errors at 3 h were 11.7% near the floor and 18.7 and 37.7% at 0.33 and 0.66 m, respectively, above the floor. Thus, it is essential to include the gravity effect in the model.

The predictions agreed well with the CO_2 concentrations at 12 h. In Bin 2 with 370 g dry ice and a covered grain surface, for example, the error at 12 h was only 6%. The errors, however, were higher at 21 and 24 h, when the predicted concentrations were higher than the measured concentrations. This could be due to sorption of CO_2 by the wheat. To determine the effect of sorption, we compared predicted concentrations, with and without sorption, for the 740-g dry-ice experiment in Bin 2 with a covered grain surface. The sorption of CO_2 by wheat was estimated using the data of Cofie-Agblor *et al.* (1993). The error at 21 h was 22.4% with sorption, compared with 34.9% without sorption. Therefore, including sorption appeared to improve the accuracy of the model.

We did not simulate concentrations with sorption for other experiments because

TABLE 1

Mean relative errors[1] (%) between the measured CO_2 concentrations in 1.42-m-diameter bins filled with wheat and the concentrations predicted by the convective-diffusive model. Effective diffusivities were used in the first hour and the diffusion coefficient was used thereafter

Bin	Grain surface	Mass of dry ice (g)	Replicate	Time since introduction of dry ice											
				1 h	3 h	6 h		9 h		12 h		21 h		24 h	
						5 L*	4 L**	5 L	4 L	5 L	4 L	5 L	4 L	5 L	4 L
1	Open	180	1	34.8	21.4	16.0		10.9	6.4	6.0	4.5	21.9	10.6		
			2	31.7	24.9	19.8		11.2	7.8	5.9	4.7	16.0	13.8		
			3	33.9	24.7	17.6		7.1	6.0	6.0	3.5	26.0	17.2		
			4[2]	33.7	23.6	18.0		9.5	6.4	4.5	3.5	20.0	16.3		
	Covered		1	17.9	22.2	18.1				12.1	10.3	15.6	15.9		
			2	29.6	24.0	14.9				9.5	7.4	11.1	12.4		
			3	28.7	22.1	17.8				11.5	8.5	10.5	10.5		
			4[2]	25.4	22.7	17.0				10.8	8.5	12.3	12.9		
		540	1	60.2	50.5	36.5	34.5	20.0	18.8	27.3	13.8	21.5	4.2		
			2	61.5	52.5	36.0	37.0	31.8	31.0	33.2	25.3	30.2	4.4		
			3	59.7	55.9	41.3	38.2	26.9	29.2	16.1	16.3	9.0	6.6		
			4[2]	60.5	53.0	38.4	36.7	24.4	26.8	20.6	18.6	14.3	4.5		
2	Open	370	1	47.2	32.2	20.6				11.7	7.6	21.5	20.2		
			2	46.1	28.2	17.8				10.9	10.0	26.0	21.4		
			3	42.8	31.9	22.4				9.5	4.9	13.6	15.3		
			4[2]	45.4	30.3	20.2				8.0	5.6	17.9	18.1		

TABLE 1 (continued)

Bin	Grain surface	Mass of dry ice (g)	Replicate	Time since introduction of dry ice											
				1 h	3 h	6 h		9 h		12 h		21 h		24 h	
						5 L*	4 L**	5 L	4 L	5 L	4 L	5 L	4 L	5 L	4 L
	Covered		1	47.0	31.7	22.1				5.9	5.2	15.2	13.2		
			2	45.5	31.7	21.0				8.3	7.6	15.3	13.4		
			3	46.0	31.4	19.4				7.5	7.9	18.1	15.5		
			4 [2]	46.2	31.6	20.8				6.3	6.2	15.8	13.6		
		740	1	47.8	39.3	28.6				7.11	6.3	31.7	23.5	35.2	25.7
			2	46.2	36.1	22.2				8.8	7.5	37.6	28.8	43.4	31.2
			3	46.4	40.3	25.9				9.2	7.4	39.0	29.6	44.1	31.9
			4 [2]	46.5	38.0	26.2				6.8	5.6	34.9	26.4	39.8	29.1
3	Open	180	1	42.0	50.7	44.5		39.1	35.5	30.8	28.9	15.9	13.3		
			2	46.0	52.5	45.6		37.8	34.3	29.7	27.7	15.3	12.3		
			3	46.5	57.1	48.5		38.8	40.1	32.1	33.9	17.6	18.7		
			4 [2]	43.9	53.7	46.4		39.1	36.8	30.9	30.4	16.0	14.8		
	Covered		1	48.1	53.6	49.5		43.3	40.6	34.7	33.1	17.5	17.2		
			2	49.1	55.3	49.4		42.6	39.0	34.9	33.8	20.1	20.0		
			3	43.2	55.5	46.1		43.5	39.8	34.3	31.9	19.1	18.8		
			4 [2]	43.9	54.1	48.5		42.9	39.4	35.6	32.8	18.3	18.4		

[1]Mean relative error (%) = $\frac{1}{N}\sum\frac{|\text{Measured}-\text{Predicted}|}{\text{Measured}} \times 100$. The CO_2 concentrations in the bins ranged from 5 to 60%.

[2]Measured data for the three replicates were averaged and compared with the simulation results.

N = number of data points (25 in Bin 1, 50 in Bin 2 and 45 in Bin 3).

*Mean relative percent errors were calculated for all 5 levels (5 L).

**Mean relative percent errors were calculated for the bottom 4 levels (4 L).

the available sorption data are only available for a 100% initial CO_2 concentration (Cofie-Agblor *et al.*, 1993). It is possible that at lower initial concentrations the sorption rate could be equal to the sorption rate at 100% initial concentration. However, there is no experimental evidence to prove this hypothesis, and Alagusundaram *et al.* (1996a) demonstrated, by predicting CO_2 concentrations with various rates of sorption, that the model predictions are sensitive to the sorption rate. Therefore, to obtain more accurate model predictions, we are currently determining the CO_2 sorption behaviour of wheat at various initial concentrations.

EVALUATION OF CO_2-LOSS MODELS

Mathematical models of CO_2 loss from bins (caused by wind, temperature and chimney effects) can be used to predict the amount of CO_2 needed to maintain the required concentration and thus improve the efficiency and effectiveness of MA fumigation of stored grain. Predictions of two mathematical models from the literature, those of Lawrence Berkeley Laboratory (LBL) (Sherman and Grimsrud, 1980) and of Banks and Annis (BA) (1984), were compared with experimental data on gas loss from a pilot bin (diameter 1.42 m, height 1.47 m) and a full-size bin (diameter 5.56 m, height 6.60 m), both filled with wheat (Peck *et al.*, 1994).

The effective leakage areas (ELA) of the bins were determined using fan pressurization tests. An ELA of 4.6 cm^2 was measured for the pilot bin. In the full-size bin, a CO_2 impermeable plastic sheet was attached to the inside wall at 2.5 m above the floor. This excluded the upper half of the bin, including the roof, and resulted in an ELA of approximately 7.7 cm^2.

A precise comparison of the predicted and measured rates of CO_2 loss from the two experimental bins could not be done because the effects of the three major factors (wind, temperature changes and chimney forces) could not be separated in the experimental results. In both models, however, the simulated effects of these three factors can be compared separately.

The LBL model predicted a loss due to wind that was 60 times higher for the pilot bin and 5 times higher for the full size bin than the measured total rate of CO_2 loss. The predicted CO_2 loss from the full size bin due to the temperature effect was equal to the measured total rate of loss. In the LBL model, the shielding and the terrain coefficients do not account for the direction of the prevailing wind and depend on a subjective assessment of the surroundings. In addition, an overestimation of the ELA by the pressurization test would cause significant differences between predicted and experimental data.

Predicting the rate of gas loss caused by wind with the BA model was difficult because the model was sensitive to an undefined pressure coefficient. Determination of this coefficient requires wind tunnel tests for each bin. These wind tunnel tests were not conducted and are not practical for a general model. The predicted chimney effect using the pilot bin was 75 times the average experimental gas loss rate. An accurate comparison, however, is not possible without more experimental data. For the full size bin, the predicted gas loss

rate due to the chimney effect was approximately 15 times the experimental gas loss rate. Further experimental studies are being conducted in our laboratory to separate the forces of wind, temperature and chimney effects so that accurate relationships can be developed. This should provide data which could be used to validate the existing models more accurately and provide information for the development of a generalized CO_2 loss model.

ACKNOWLEDGEMENTS

We wish to thank the Natural Sciences and Engineering Research Council of Canada for partial funding of this study. We gratefully acknowledge the contributions of our graduate students, Messrs K. Alagusundaram, C. Bundus and M. Peck, to our research program on controlled atmosphere storage of grains and oilseeds.

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EFFICACY OF PHOSPHINE FUMIGATIONS ON BAGGED MILLED RICE UNDER POLYETHYLENE SHEETING IN INDONESIA

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ABSTRACT

Phosphine (PH_3) fumigation is the major means of pest control in milled rice in government godowns in Indonesia. In the tropical climate, insect infestation is severe and PH_3 fumigation is required every 3 months. The predominant pest species are *Tribolium castaneum* (Herbst) and psocids, chiefly *Liposcelis entomophilus* (Enderlein).

To investigate the efficacy of fumigation practices, six stack fumigation trials were carried out. The standard fumigation enclosure involved a single 0.125-mm polyethylene sheet covering a stack of around 300 t. The polyethylene sheeting was sealed to the concrete floor by weighting it with fumigation chains around the perimeter of the enclosure. The nominal PH_3 dosage was 2 g per t plus an allowance for the walkway space (where adjoining stacks are fumigated in one enclosure) of 5% of the dosage. PH_3 was generated from tablets containing aluminium phosphide. Throughout the fumigation, PH_3 concentrations were monitored using portable electronic meters which can determine carbon monoxide separately. In a 5-d fumigation, the concentration by time products (Ct's) exceeded 150 mg h L^{-1}. Fumigations not in accord with the protocol — involving sheeting in poor conditions, fumigation enclosures with two sheets joined only by overlap, or fumigations without chains — all produced much lower Ct's. PH_3 concentrations have been discussed in relation to the rate of PH_3 release from the aluminium phosphide, the rate of its sorption on milled rice, the rate of its permeation through the polyethylene sheet and the rate of leakage. The PH_3 concentrations recorded suggest that complete kill of both species is possible in this type of fumigation, but reports of *L. entomophilus* strains from Indonesia with enhanced PH_3 tolerance suggest that complete kill is not always achieved in practice. Meters capable of monitoring PH_3 concentrations in the field have now been issued to operational staff and should reduce the frequency of fumigation failure.

INTRODUCTION

In Indonesia, buffer stocks of grain are stored by the National Logistics Agency (BULOG). The major commodity is milled rice, which is stored in bags in godowns.

The storage interval varies with seasonal conditions and generally ranges from a few months to two years. Most of the cosmopolitan pest species are present, and in the tropical environment applied pest-control measures are necessary to prevent major losses (Sidik *et al.*, 1985)

These measures are based on spraying the storage fabric and the outsides of bag stacks with insecticide, plus insecticide fogging of the storage interior and phosphine (PH_3) fumigation at intervals of approximately 3 months. Under these circumstances, the major pest species are *Tribolium castaneum* (Herbst) and psocids, chiefly *Liposcelis entomophilus* (Enderlein). From the field observations, it is not clear to what extent these infestations are due to fumigation survivors and to what extent to reinfestation. A Javanese strain of *L. entomophilus* has been reported to be quite tolerant to PH_3 (Pike, 1994), and PH_3 resistance has also been reported in strains of *T. castaneum* elsewhere (but apparently not as yet from Indonesia).

Despite the importance of duration and PH_3 concentration during fumigation, relatively few data have been published about commercial fumigation under tropical conditions. Fumigations of milled rice in Southeast Asia at an application rate of 2 g t^{-1} — but under unspecified conditions — gave PH_3 concentrations 0.2–0.4 mg L^{-1} over 100 h (Taylor and Harris, 1994). However, the same authors also reported that in Tanzania fumigations of maize under PVC sheeting, with a careful fumigation protocol, gave concentrations of 1.0–1.8 mg L^{-1} over 7 d. The current paper reports the concentrations of PH_3 achieved in fumigations of stacks of bagged milled rice under plastic sheeting within a larger godown.

METHODS

Six stack fumigations starting 29 August 1995 were carried out simultaneously in a typical godown in the BULOG storage complex at Gede Bage near Bandung in Java. The godown was 48 × 30 m. It had a concrete floor and walls to a height of 3 m, a steel frame and galvanised iron walls to a height of 5 m and a galvanised iron roof. The optimal filling capacity was 3,500 t bagged grain in three rows of four stacks each. The milled rice, in woven polypropylene bags each containing around 50 kg, was placed on timber dunnage in stacks typically around 300 t. The dunnage, in the form of pallets, provided an 0.1-m airspace below the stack and enhanced gas distribution. The concrete floor was made of slabs approximately 1.0 × 1.5 m with some surface irregularities. The joints had been filled with bitumen to within around 10 mm of the surface, and in some areas topped off to the surface with cement.

All fumigations were enclosed in 0.125-mm polyethylene sheeting which was sealed to the concrete floor with chains laid around the perimeter of the enclosure over the edges of the sheeting. On one of the experimental stacks, the fumigation chain was not used. Either one, two, three or four adjacent stacks were fumigated in a single enclosure; when required for this experiment, two fumigation sheets were joined by simple overlap of 1 or 2 m held by paper tape.

The application rate was 2 g PH_3 per t plus, where adjoining stacks were fumigated in one enclosure, an allowance of 5% for the walkway space. The PH_3 was generated from PHOSTOXIN® tablets each of which contains sufficient aluminium phosphide to evolve 1 g PH_3. Approximately 20 tablets each were placed on shallow metal trays, and the trays were placed at even intervals around the perimeter of each stack on the floor and, where more than one stack was involved, along the walkways.

Details of the fumigation enclosures are given in Table 1.

TABLE 1
Fumigation enclosures and grain

Enclosure number	Number of stacks	Quantity of grain (t)	Grain moisture (%)	Comments on the fumigation sheets
1	1	269	<14	Single sheet, new
2	3	776	<14	Single sheet, new
3	4	1239	14–15	Two sheets, new, and overlapped 2 m to join
4	2	415	14–15	Single sheet, used, and some patches
5	4	993	14–15	Two sheets, used, and some patches; overlapped 2 m to join
6	1	55	16	Single sheet, used and many patches, floor uneven, no chains

The volume of enclosure 1 was estimated at 347 m^3 (5.7 × 12.7 × 4.8 m) and the volume of milled rice at 321 m^3 (bulk density 0.838), so the unfilled volume, including the space underneath the stack, was 26 m^3. The intergranular airspace was estimated at 143 m^3, so the total gas volume was 169 m^3. The calculated concentration of PH_3 applied was 3.2 g m^{-3}.

The other enclosures will not be considered in detail.

PH_3 concentrations were measured daily throughout the fumigations. Nylon tubing 3 mm in internal diameter connected the metre to the sampling point on the floor, approximately 1 m inside the perimeter of the fumigation sheeting. There were two sample points at separated locations on one side of each enclosure excepting enclosure 1, in which there were three additional sample points (one on each side of the stack in the middle of a bag 2 m from the floor and a third one at the top of the stack in a central bag. At these additional sample points, a short length of stainless steel tubing was connected to the polyethylene tubing so that the steel tubing could be pushed into the bags.

Two similar PH_3 meters based on electrochemical sensors (Bedfont Model EC 80 PH_3 Fumigation Gas Meter, Bedfont Technical Instruments Ltd, England) which respond to both carbon monoxide (CO) and PH_3 were used. One meter incorporated a filter which when activated removed the PH_3. This enabled measurement of PH_3 plus CO and then CO alone. The PH_3 readings for both meters were adjusted by subtraction of the readings for

CO. The means of all readings by the two meters differed by only 0.7%, and the PH_3 levels were therefore calculated as the mean of the results from the two meters. After completion of the fumigation, one meter was check-calibrated in the laboratory using a PH_3-in-air mixture standardised by gas chromatography. The readings agreed within 2% (S. Pratt, personal communication). The PH_3 readings in parts per million were converted to milligrams per litre by division by a factor of 725.

The ambient conditions were measured by a maximum and minimum thermometer set 2 m above the floor on the side of a stack in the centre of godown. They ranged from 22°C overnight to 32°C during the day. The grain temperature was 27°C as measured in bags from the stack perimeter.

An estimate of the rate of PH_3 release was obtained by adjusting the parameters in a mathematical model of the release (Banks, 1991) to obtain a visual fit of the data from the first 4 d of the fumigation in stack 1. The model incorporates an initial phase where the quantity of PH_3 released was described by a power function of time, a transition point termed the crossover time, and a phase where the rate of production was proportional to the quantity of undecomposed aluminium phosphide remaining.

Before the crossover time t_c,

$$m = A_1 a t^n$$

where m = quantity of PH_3 generated from the start of exposure to time t; A_1 = value of m at infinite time; a = constant; n = constant, here 1.15.

After the crossover time t_c,

$$m = A_1[1 - \exp(-k_1(t - t_0))]$$

where k_1 = constant; t_0 = value of t where the exponential function is zero.

The model derives the maximum rate of evolution A_2 from a quadratic equation relating A_2 to the absolute humidity, which for 27°C and 70% r.h. is 18.05 g m^{-3}:

$$A_2 = 4.49 \times 10^{-3}(18.05) - 3.25 \times 10^{-5}(18.05)^2.$$

The maximum rate of evolution is reduced to the rate permitted by the airflow (Q) in $m^3\ h^{-1}$ which brings moisture to the formulation. This airflow was fitted to the data.

The time to 50% release was calculated from the equation:

$$t_{50} = 0.5n/A_2(1 - \exp(-K_2Q))$$

where K_2 = constant, here 146.95.

The total rate of loss of PH_3 was estimated for each day of interest by calculating the PH_3 level each day as a percentage of the PH_3 level measured the day earlier.

The rate of PH_3 loss by permeation through the intact polyethylene sheeting was calculated assuming a first order process.

$$C_t = C_0 \exp(-k \cdot A/V \cdot t)$$

where C_t = concentration at time t (mg L^{-1}); C_0 = concentration at the nominated time of commencement (mg L^{-1}); k = permeation coefficient; A = surface area (m^2); V = volume of gas in the enclosure (m^3); t = time from commencement (h).

The value of k was estimated at 0.2×10^{-3} for surface area in m^2 and volume in m^3 from published data at 25°C for polyethylene sheeting of different thickness (Cooper and Bengston, 1979).

RESULTS

Data on the PH_3 concentration in each enclosure each day are given in Table 2. The PH_3 readings on day 1 were somewhat variable, but there were no systematic differences in readings among the positions (including the one in the centre of the top of the stack); therefore the additional readings on enclosure 1 were not continued after the first day. There was no apparent delay in the penetration of PH_3 into the polypropylene bags. The time to 50% PH_3 release was estimated at 32 h and the release rate (0.016 g h^{-1} per tablet) during the time interval was relatively constant. The time to 90% release was estimated at 66.4 h; 98% had been released by day 4, and 99.7% by day 5 (Fig. 1).

TABLE 2
Mean (n = 4) daily PH_3 concentrations in mg L^{-1}

Enclosure number	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
1	1.55	2.08	2.39	2.41	2.33	2.12	–
2	1.39	1.60	2.24	2.28	2.15	2.01	1.84
3	1.04	1.35	1.55	1.51	1.34	1.28	1.08
4	0.89	0.99	1.05	1.00	0.84	0.70	0.55
5	0.82	0.91	0.74	0.57	0.42	0.28	0.16
6	0.99	0.40	0.14	0.06	0.02	0.02	0.01

The rate of loss due to permeation through the polyethylene sheeting was calculated as 1% per day. The total rate of loss from enclosures 1 and 2 after the fourth day was estimated at 7% per day.

Concentration by time (Ct) products for each fumigation enclosure are given in Table 3. There was a marked difference between the PH_3 concentrations in enclosures 1 and 2 and those in the remaining enclosures; it is significant that the Ct products in the latter did not reach 150 mg h L^{-1}. The concentration of CO was verified as zero at day 1 and reached a maximum of 64 parts per million on days 5–7.

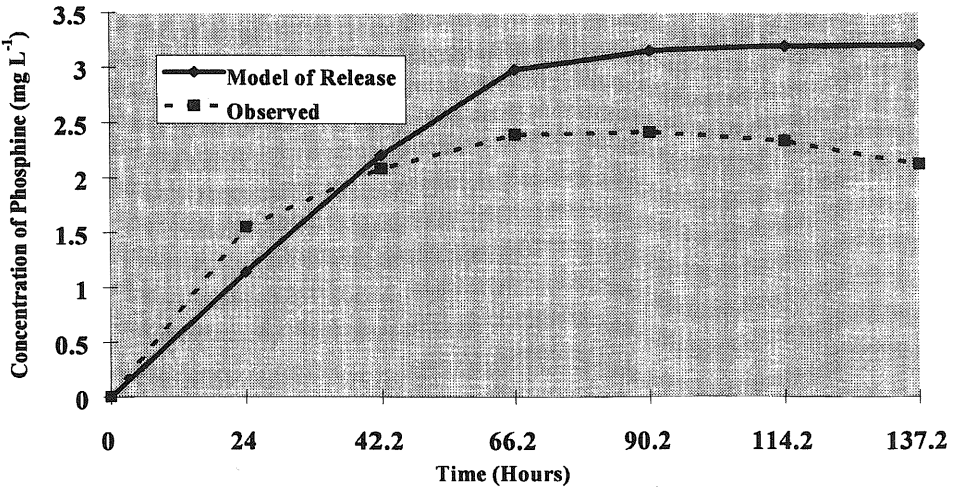


Fig. 1. Observed concentrations of PH_3 in fumigations of milled rice in bags at 27°C and 14% moisture under polyethylene sheeting and modelled concentrations of PH_3 released. The model of release (Banks, 1991) was calculated with the parameters: theoretical maximum PH_3 concentration (total PH_3 (g) in total gas volume (m^3)) 3.2 g m^{-3}, water vapour concentration 18.05 g m^{-3}, Q = 0.002 m^3 h^{-1}, t_{50} = 32.0 h, a = 0.00929, t_c = 42.88 h, k_1 = 0.0626, t_0 = 23.65 h, n = 1.15.

TABLE 3
Concentration by time (Ct) products in mg h L^{-1}

Enclosure number	5 d	7 d
1	229	–
2	212	310
3	145	204
4	103	137
5	76	91
6	38	39

DISCUSSION

Rate of release

The rate of release estimated here (0.016 g h^{-1} per tablet) was significantly lower than the rate of 0.034 reported in an experiment at 25°C and 75% r.h. involving a single tablet in an open petri dish (Ducom and Bourges, 1993). The time to 90% release (66.4 h here) was also significantly below the 25 h reported in that experiment and the 45 h quoted to obtain the maximum release under unspecified conditions in the laboratory (Heseltine,

1973). The current data are nevertheless consistent with the release rate implied in reports of field fumigations of 100 t lots of maize in which peak PH_3 concentrations sometimes occurred at day 4 (Taylor and Harris, 1994). The airflow rate of 0.002 $m^3 h^{-1}$ (25 ml min^{-1}) estimated in the current experiment was well below the rate of 0.005 $m^3 h^{-1}$ used to model release of PH_3 from the tablet formulation (Annis and Banks, 1993). Possibly the technique of placing tablets in groups reduced the rate of release.

Total rate of loss

The rate of sorption, the rate of permeation and the rate of leakage cannot be separated on the basis of field data, but the total rate of loss (7% per day) observed — and estimated for the interval in which PH_3 fumigation had virtually ceased — was at the lower end of the anticipated range. It was achieved in fumigations without a floor sheet, but obviously such a result depends on the presence of a reasonably gastight floor.

Losses due to sorption for specific commodities are influenced by many factors, including temperature, moisture, concentration, previous moisture content and previous fumigations. The rate of sorption is relatively high during the initial hours and thereafter becomes semi-logarithmic (Banks, 1993). Relatively few estimates of the rate of sorption on milled rice have been published, but an apparent first order rate constant for a full system at 25°C and 60% r.h. has been measured as 0.1 per day (Banks, 1990) and, when allowance is made for the filling ratio here, this suggests a possible loss of 9% per day. This estimate should be further reduced since, although no detailed fumigation history was available, it is likely that the rice had been fumigated prior to the current experiment.

A leakage rate of 5% has been suggested for a large well-sealed storage (Annis and Banks, 1993). The location of the enclosures in a godown reduced exposure to wind and sun and thus the rate of loss which might have occurred had they been in the open. There was minimal headspace and this would minimise the effect of temperature fluctuations.

Carbon monoxide levels

Stored dry grain is known to produce significant levels of CO (Whittle *et al.*, 1994), but in the 7-d fumigations measured here, the CO levels were low. These low levels would not significantly affect PH_3 readings made with a meter which did not separate the response of the two gases.

Effect of fumigation enclosure on gas concentration and Ct product

The experiment provided a good illustration of the unsatisfactory results likely to occur when operating procedures may lead to a poor standard of fumigation enclosure. Fumigations involving sheets with holes, joins by simple overlap, or lack of fumigation chains all gave gas concentrations likely to lead to fumigation failure by allowing insect survival. Such fumigations in the longer term also lead to the development of resistance. In contrast, where prescribed fumigation procedures were used, the gas concentrations were above 1 mg L^{-1} from day 1 and throughout the fumigation, and the Ct products were above the 150 mg h L^{-1} which has been quoted as the fumigation target (Annis and Banks,

1993). The concentrations were above 1.7 mg L^{-1} for 5 d, and this has been estimated as the minimum necessary to kill 99% of eggs of *L. entomophilus* (Pike, 1994). This suggests that fumigations carried out according to the standard fumigation protocol are adequate to control all insects. However, the presence in Indonesia of *L. entomophilus* strains with enhanced PH_3 tolerance suggest that complete kill is not always achieved in practice. This is supported by the reports of low gas concentrations in fumigations reported by others (Taylor and Harris, 1994). Meters capable of measuring PH_3 concentrations in the field have now been issued to operational staff and should reduce the frequency of fumigation failure.

ACKNOWLEDGEMENTS

The work was financially supported by the Australian Centre for International Research (ACIAR). This support is gratefully acknowledged.

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INFLUENCE OF GRAIN TEMPERATURE ON EFFICACY OF FUMIGATION IN LEAKY BINS

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ABSTRACT

Hard red winter wheat at 11.1% moisture content (w.b.) and 20°, 25°, or 30°C was fumigated with tablets of an aluminum phosphide fumigant in small cylindrical grain bins of corrugated metal. The bins were provided with sufficient open area to approximate leakage rates commonly encountered in farm and commercial-scale bins of this type, and phosphine concentration profiles were determined over a 10-d period. Leakage and fumigant sorption at each temperature were examined by comparing the fumigant profiles with those obtained in a sealed container fumigated at the same temperatures with and without the same grain. Temperature-induced effects on fumigant profiles were compared with effects on development rates of the most fumigant-resistant stages of various insects. At 20°C, development rates of certain species were too prolonged to insure proper kill. At 30°C, the likelihood of complete kill appeared favorable despite the greater amount of variability. The 25°C condition appeared less variable while providing attributes favorable to a complete kill in most cases.

INTRODUCTION

Much grain stored in Kansas is fumigated with phosphine (PH_3) fumigants in cylindrical bins of corrugated metal. These structures are often sealed only at the ground-level openings (auger tubes, aeration ducts, etc.), whereas the wall joints, roof doors, roof-wall junctures, etc. are not sealed. The grain masses rapidly lose fumigant, with much of this loss occurring at the uncovered grain surface (Skidmore, 1989). Under these circumstances, many factors influence how rapidly PH_3 disappears from the intergranular space. Preliminary tests indicated that grain temperatures above 30°C were associated with the extremely rapid loss of fumigant gas (Reed, unpublished).

Stored-grain insects vary in their susceptibility to PH_3 depending on species, temperature and stage of life (Hole *et al.*, 1977). Within the resistant stages (eggs and pupae), tolerance to PH_3 decreases as the eggs and pupae age (Lindgren and Vincent, 1966). This means that the duration of a lethal concentration may be more important to the success of

the fumigation than either the maximum concentration or the concentration/time product. The recommended strategy for PH_3 fumigations is to maintain a lethal concentration until the very resistant stages have matured into less resistant forms (Winks, 1986). However, this is difficult to achieve in leaky structures, such as metal grain bins. This study was undertaken to determine the relative advantage of manipulating the grain temperature before fumigation.

MATERIALS AND METHODS

A weight of 1.35 t of hard red winter wheat was loaded to within 5 cm of the top of six cylindrical bins of corrugated metal 1.4 m in diameter and 1.2 m tall with wooden lids 2 cm thick. Each bin was fitted with an aeration system consisting of a 15.2-cm PVC tube placed vertically along one wall and extending through the lid which was connected to a 15.2-cm perforated tube on the bin floor. Air was extracted from a controlled-atmosphere chamber and forced through the grain to achieve the desired grain temperature while maintaining the desired moisture content. A thermocouple was placed 40 cm from the bin wall and 40 cm beneath the grain surface to monitor grain temperature. Perforated metal chambers 1 cm in diameter and 23 cm long, attached to PVC gas-sampling tubes, were placed in three locations in each bin. Two were placed at opposite sides of the bin 30.5 cm from the wall and 81 cm beneath the grain surface, and a third was placed 40 cm from the wall and 25 cm beneath the grain surface. The thermocouple and gas sampling tubes were extended to an adjacent room for monitoring during fumigations. The gas sampling tubes were sealed with septa.

A total of 0.15 t of the same wheat was placed in a 216-L sealed metal drum with three gas sampling ports, similar to those described above, at equidistant points along the wall. A rubber stopper covered with paraffin wax allowed the sampling tubes to exit the drum without gas leakage. The drum was placed in a controlled temperature chamber at the same temperature as the grain in the bins.

The entire dose of fumigant was inserted at the center of the grain mass. Tablets of commercial PH_3 fumigant (Weevil-Cide, Research Products Corp.), each with a theoretical yield of 1 g of PH_3 gas, were placed inside a small bag of open-weave cotton. Wheat was placed inside the bags to separate the tablets. Preliminary trials established that a dosage rate of 5 tablets per container (2.7 tablets per m^3), combined with ventilation within the room, produced PH_3 concentration profiles over time similar to those observed in farm bins (Skidmore, 1989), and this dosage rate was used throughout the experiment. In the sealed drum a quantity of fumigant tablets calculated to provide the same dosage was inserted into the center of the volume.

Each sampling line was evacuated immediately before samples were taken. Then 50 ml of gas was extracted through the septa with an airtight syringe. This sample was injected into a conductance cell and the PH_3 gas concentration was determined by the mercuric chloride conductimetric method of Harris (1986). Data were analyzed by SAS software (SAS Institute, 1985).

RESULTS AND DISCUSSION

For convenience, trial conditions are called 20, 25 and 30°C. Observed mean grain temperatures were 19.9 ± 0.04, 25.1 ± 0.05 and 30.1 ± 0.05°C. The mean grain moisture content throughout the trials was 11.1% (w.b.). Mean moisture contents varied as follows: 11.1% at 20°C, 10.8% at 25°C and 11.5% at 30°C.

At 30°C in the sealed empty drum the PH_3 concentration peaked 24 h post-fumigation at 90% of the theoretical yield of 2.7 tablets/m^3 and thereafter remained relatively constant through 10 d (Fig. 1). At 20°C, the concentration increased through 10 d, reaching 91% of the theoretical potential. In the 25°C trial, the concentration reached 95% of the theoretical potential after 48 h and then declined to less than 90%, probably due to slight leakage during the trial. In previous tests, the container had maintained pressure for 10 d.

Sorption and leakage were examined by plotting gas profiles at each temperature in the sealed empty drum, in the sealed drum containing wheat (95% full) and in the leaky bins (95% full). At 30°C (Fig. 2), gas concentrations increased more rapidly in the sealed empty drum than in the sealed drum containing wheat through the first 12 h but did not increase further after 24 h. In the sealed drum containing grain, the mean concentration continued to increase until 36 h post-fumigation. These differences were probably artifactual, being due to the slower diffusion of PH_3 from the center of the drum to the sampling points through the grain than through the empty drum. Although the amount of gas generated was the same with or without grain, the fumigant was more concentrated in the

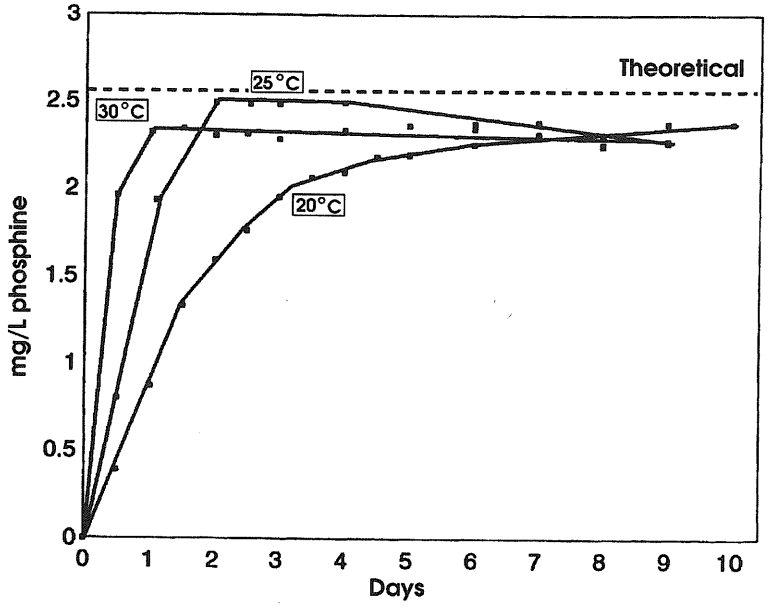


Fig. 1. Phosphine concentrations at three temperatures in sealed drums containing no grain.

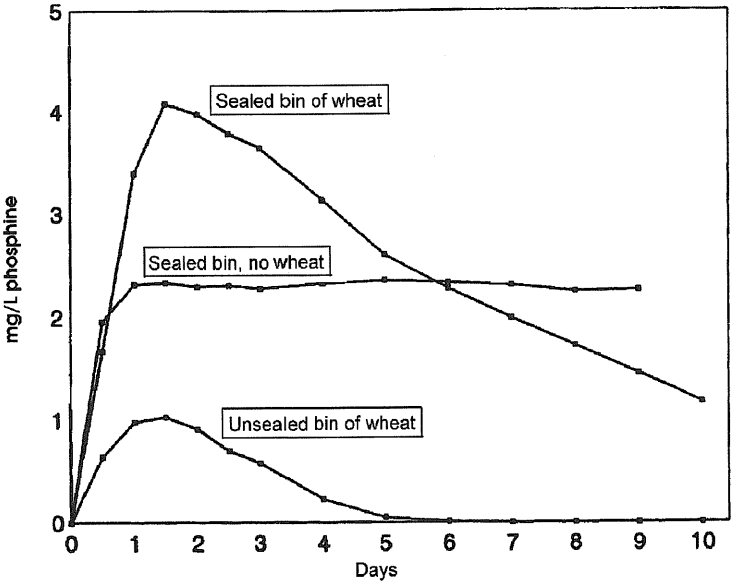


Fig. 2. Phosphine concentrations at 30°C in sealed drums, with and without wheat, and in leaky bins.

drum containing wheat because the wheat occupied approximately 60% of the volume. The reduction in gas concentration in this drum after 36 h post-fumigation reflects the movement of PH_3 out of the void space into the pores of the grain. By the 6th day the PH_3 concentration in the drum containing grain was reduced below that of the empty bin, reflecting sorption of the PH_3 by the grain. Banks (1986) has described and modelled this process. Meanwhile, PH_3 concentrations in the leaky bins were greatly reduced, obviously due to both sorption and loss.

When expressed as a percentage of the theoretical concentration, given the greater void space in the drum without grain, the influence of fumigant sorption is separated from that of leakage (Fig. 3). Curves from the 30°C trial are again shown as the example. The difference between the middle and top line is presumed to reflect diffusion into or sorption onto the wheat. The difference between the middle and bottom curve is presumed to be due to leakage from the bin. In the leaky bin, the maximum concentration achieved represented less than 20% of the calculated amount of PH_3 released by the fumigant preparation.

In the leaky bins, the mean gas concentration at 30°C was greater at the maximum, but it declined more rapidly than at lower temperatures (Fig. 4). These differences reflect the influence of grain temperature on rate of gas generation from the fumigant preparation as well as temperature effects on sorption and loss rates. Maximum concentrations were observed at about 36, 48 and 108 h post-fumigation at 30, 25 and 20°C, respectively.

The mean concentration at these peaks in the leaky bins was expressed as a percentage of the "maximum possible" at each temperature by dividing the concentration observed in

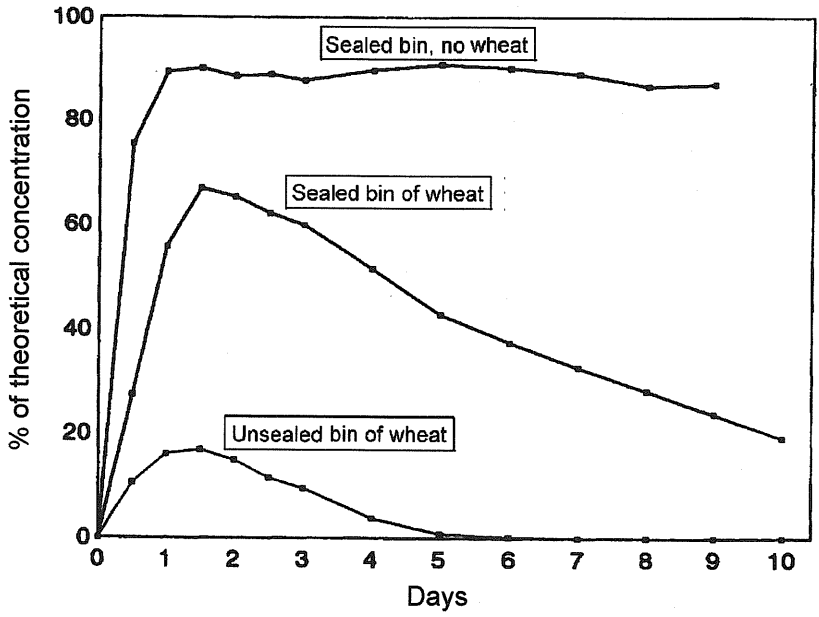


Fig. 3. Phosphine concentrations at 30°C, expressed as percent of theoretical maximum, in sealed drums, with and without wheat, and in leaky bins.

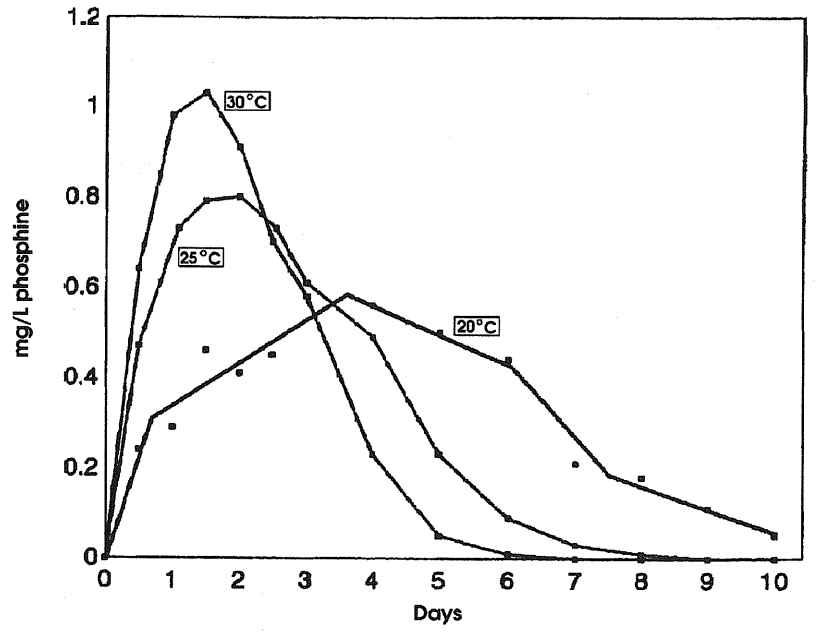


Fig. 4. Phosphine concentrations at three temperatures in leaky bins of wheat.

the leaky bins by that observed at the same sampling time in the sealed drum containing grain. At 30°C, the PH_3 concentration reached 25.1% of the "maximum possible", significantly greater ($P < 0.01$) than the same parameter at the lower temperatures (Table 1). However, the mean within-bin variability, i.e. the standard deviations about the means of the three sampling points per bin over all bins and sampling times, was also significantly greater ($P < 0.01$) at 30°C.

TABLE 1
Mean "percent of possible" phosphine concentrations, given the amount of sorption, and within-bin variability of fumigant concentration at three temperatures

Temperature	Mean "percent of possible" PH_3 concentration	Mean SD within-bin[a]
30°C	25.1 A	0.144 A
25°C	19.8 B	0.084 B
20°C	17.3 B	0.081 B

[a]Means significantly different ($P < 0.01$).

Grain temperature affects the insect's susceptibility to PH_3 and also the longevity of each stage of the insect's life cycle. In order to evaluate the relative advantage of a given grain temperature for fumigation under local conditions (Table 2), the duration of PH_3-resistant stages of certain insects under conditions of the study was calculated from data provided by Hagstrum and Milliken (1988). Hole *et al.* (1977) provided data relative to toxicity of PH_3 at two of the experimental conditions for certain stages of the insects. *Rhyzopertha dominica* (Fab.) is the most destructive insect in Kansas in grain stored under the simulated conditions (Reed *et al.*, 1989). At 20°C, the fumigant-resistant egg would require 28 d to hatch. PH_3 was not detected in the grain at half this developmental period. In contrast, at 30 and 25°C, means of 0.28 and 0.09 mg/L PH_3, respectively, were present at half the egg development period. Hole *et al.* (1977) indicate that 0.046 mg/L PH_3 for 1 d is lethal to 90% of *R. dominica* eggs at 30°C, and 0.044 mg/L for 2 d is required for 90% efficacy at 25°C. Data for 20°C were not given. For several of the other species common to Kansas conditions, either toxicity or developmental time data are not available. *Sitophilus* species, the pupae of which are notoriously resistant to PH_3, are not commonly found under these conditions in Kansas.

In order to minimize variability, this experiment was carried out in a sheltered environment in which the ambient air temperature was the same as the grain temperature and there was a small, constant amount of air movement across the bins. Under field conditions, more variability and greater loss would be expected due to occasional strong winds and chimney effects. In addition to the sorption and leakage effects described here, these uncontrolled variables must be taken into account under practical conditions.

TABLE 2
Development (dev.) times and phosphine concentrations at three temperatures[a]

Species and life stage	30°C		25°C		20°C	
	Mean dev. time (d)	PH_3 conc. at ½ dev. time (mg/L)	Mean dev. time (d)	PH_3 conc. at ½ dev. time (mg/L)	Mean dev. time (d)	PH_3 conc. at ½ dev. time (mg/L)
R. dominica, egg	7.7 ± 0.020	0.28 ± 0.081	12.0 ± 0.08	0.09 ± 0.025	27.8 ± 0.23	0
Cryptolestes spp., pupa	5.5 ± 0.020	0.65 ± 0.114	9.0 ± 0.05	0.36 ± 0.068	15.9 ± 0.08	0.18 ± 0.021
O. surinamensis, egg	4.7 ± 0.010	0.77 ± 0.103	6.6 ± 0.03	0.58 ± 0.065	11.7 ± 0.07	0.45 ± 0.036
S. oryzae, egg	3.9 ± 0.002	0.92 ± 0.080	4.8 ± 0.02	0.75 ± 0.031	9.5 ± 0.07	0.51 ± 0.030

[a]Means and standard deviation.

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METHYL BROMIDE EMISSION CONTROL FROM COMMODITY FUMIGATION

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ABSTRACT

A proprietary process to control methyl bromide (MB) emissions to the atmosphere following commodity fumigation is being developed. The process consists of adsorbing the MB in the vent-stream from a fumigation process on activated carbon (carbon), reactivation of the carbon for further use as an adsorbent, thermal destruction of the desorbed MB and the recycling of its bromide content for reuse in the manufacture of new MB and/or other brominated compounds. Research was undertaken to observe the effects of several factors — temperature, relative humidity (r.h.), MB vent-stream concentration and carbon type — on the amount of MB that could be adsorbed (loaded) on the activated carbon. Tests revealed that for a given type of carbon temperature has the greatest effect on the loading, although r.h. also affects its loading. Loading decreases as temperature and r.h. increase. At a given temperature and r.h., the loading varies significantly for different carbon types. These differences are consistent with the type of pore structure of the carbon, which depends on the raw materials and activation procedures used during its manufacture. Temperature in the carbon column rose in response to the adsorption of MB. The adsorption zone could be followed (by monitoring the temperatures) through the column from the inlet at the start of an adsorption run to column exhaustion or breakthrough at the end of the run. Breakthrough was reached when the MB concentration in the column exhaust stream reached 500 ppm (2 mg/L). Because of the heating of the column, vent-stream r.h. was less critical than first anticipated. The temperature increase due to the heat of adsorption lowered the r.h. on the adsorption zone, decreasing the negative effect on adsorption capacity normally associated with high humidities.

INTRODUCTION

When methyl bromide (MB) was found to be an ozone depletor, the agricultural community increased pressure to modify its application technology so that it might still be used without being emitted to the atmosphere. It has been estimated that non-use of this agricultural

chemical in the state of California alone would result in a loss of 300 million dollars in imports and exports. A great deal of pressure has been brought either to convince other countries to adopt the provisions of the US Clean Air Act (CAA) (which calls for the banning of MB in the year 2001) or to amend it to permit certain uses of MB. If the CAA is amended, it will probably include the proviso that emissions be controlled to acceptable levels. The USDA-ARS has therefore been looking into ways to trap, recycle, incinerate or chemically destroy MB after fumigation and before being emitted into the atmosphere. Several review articles have extolled the virtues of using activated carbon and other materials as sorbent materials to remove volatile organic compounds (VOC's) and other compounds from air and water streams (Parmele *et al.,* 1979; Goldhaar and Graham, 1991; Graham, 1992; Knaebel, 1995). In 1995 the Horticultural Crops Research Laboratory, a part of the USDA Agricultural Research Service, entered into a cooperative research and development agreement with GFK Consulting, Ltd. of San Clemente, CA, to research the potential use of activated carbon to capture MB (following fumigation) and transport it to a central treatment facility where it would be desorbed and disposed of in an environmentally sound manner. We here present the results of the first phase of that research: the adsorption of MB on activated carbon under different conditions.

MATERIALS AND METHODS

The adsorption experiments were carried out in C-200, the bench-scale carbon adsorption column (BSAC), shown in Fig. 1. The C-200 BSAC was modeled after the ventilation conditions used at a 72,000 ft^3 (2,040 m^3) tarpaulin fumigation chamber at the San Diego port facilities. Following fumigation, fresh air was admitted around the periphery of the chamber and the contents evacuated at a rate of 10,000 ft^3/min (280 m^3/min). After a 30-min ventilation, the MB concentration in the chamber was reduced to less than 5% of its original value. The BSAC was designed to treat approximately 1/3,500 of this flow, or 83.4 L/min (2.9 ft^3/min), long enough (30 min) for the chamber concentration to drop to 5% of its original value. The C-200 column had a diameter of 7.6 cm (3 inches) and a height of 99 cm (39 inches). It held approximately 2,300 g of clean carbon. The absolute humidity of the inlet air stream was adjusted in T-110 by vigorously bubbling air through temperature-controlled water. T-120 served to disengage and eliminate any entrained water droplets from the air stream. Following the humidity adjustment, MB gas (99.9%) from a commercial pressure cylinder was introduced to achieve an initial concentration of 16,000 ppm (v/v) (64 mg/L in air). The concentration was gradually reduced to 0 ppm over a period of 30 min to simulate what actually happens during the aeration of a fumigation chamber. A total of 38.5 g MB was added during this period. The average concentration for the period was approximately 3,800 ppm (15 mg/L). When calculating the concentration profile, we assumed perfect back mixing during the ventilation period. The instantaneous MB flow rate was controlled as per both the indications of a rotameter calibrated for MB and the weight of MB lost from the cylinder on a Mettler top-loading balance mode P10N, denoted K-110 in Fig. 1.

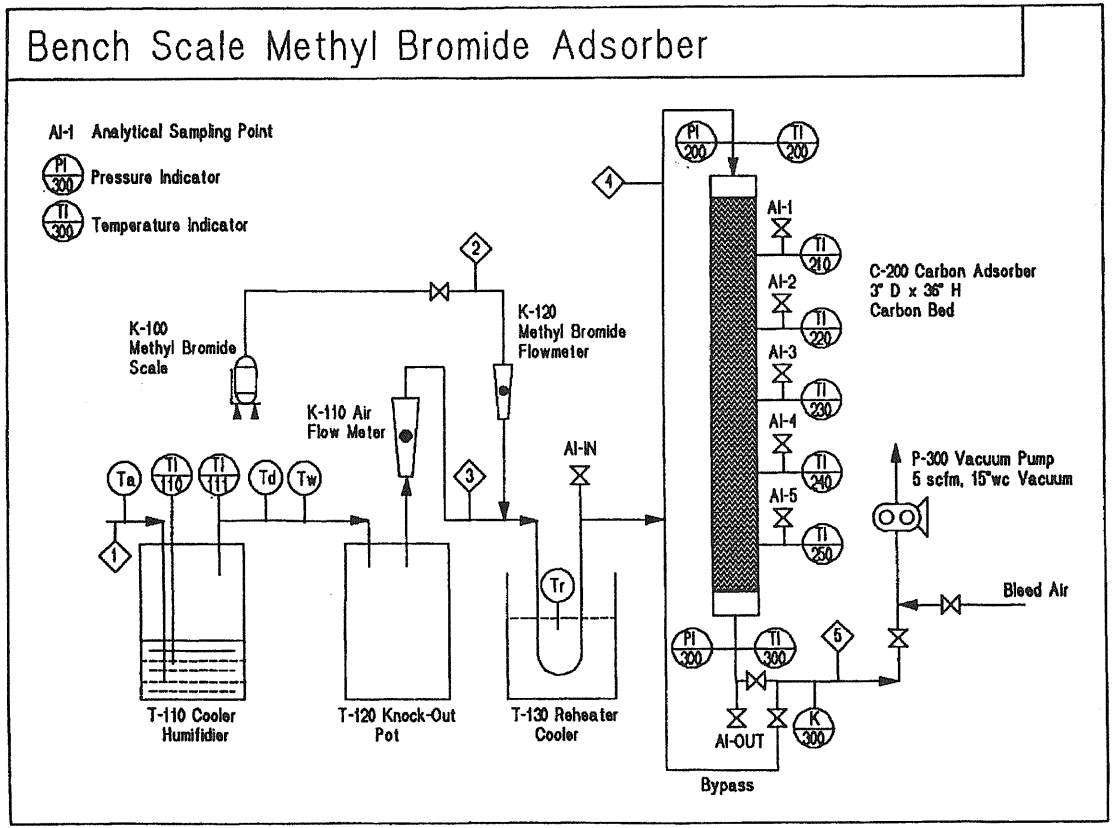


Fig. 1. The design of the bench-scale adsorber used to determine the loading of MB on various activated carbons under various conditions. Tl- markings denote places where temperatures were taken; Al- markings denote places where gas grab samples were removed and analyzed by GLC; Pl- markings denote places where pressure readings were taken to provide flow data.

Following the introduction of the MB into the air stream, the mixture was further heated or cooled in T-130 to achieve the desired column inlet temperature and relative humidity (r.h.). The target inlet temperatures (8, 15 or 25 ± 3°C) were used with r.h.'s of 50 or 95 ± 5%. Temperatures were automatically recorded at positions marked "TI-110, 111, ..., TI-250, TI-300" in Fig. 1. A PC software program from Iotech Inc. (TempBook/66 Data Acquisition System) linked to type "T" thermocouples was used to record all temperatures except those at Ta, Td, Tw and Tr (Fig. 1), which were taken manually with thermometers. Temperatures in T-110 and T-130 were manually adjusted throughout each run to maintain the desired temperature and r.h. in the air stream, as monitored by Td and Tw (dry bulb and wet bulb, respectively). Pressures in the system were measured using simple U-tube manometers filled with water. A typical inlet pressure was –330 mm (–15 inches) of water. A typical pressure drop across the column was 190 mm (7.5 inches) of water. Stream flow through the column was verified by a venturi tube — placed between the column and the pump — attached to a Dwyer incline manometer. Gas grab samples were collected at points named "AI-IN, AI-1, ..., AI-5 and AI-OUT". The pipe carrying the MB was passed through the water bath (T-130) to achieve the proper temperature and r.h. to be tested on the column of carbon.

Since the adsorption of the MB on activated carbon is an exothermic reaction, we used the temperatures at the various thermocouple locations in the column to determine the progress of the adsorption front. The average temperature at a given location increased as long as the adsorption occurred upstream, i.e. the adsorption front was upstream of the location of the thermocouple. Once the adsorption front passed the location of the thermocouple, the temperature started to decrease towards the column inlet temperature (see Fig. 2). The adsorption front at any given location approximately coincided with the peak average temperature at that location. Static pressures at the column inlet and outlet were monitored to determine the pressure drop for both the various types of carbon and the various operating conditions. The procedure also verified the overall integrity of the system since such major leaks as open sample connections were easily detected by the abnormal pressure readings they generated. The air flow was measured with a rotameter (K-110) preceding the adsorption column and, again, following the column using the venturi tube (K-300) coupled to an incline manometer. Grab samples of gas were analyzed on a Hewlett Packard Gas Chromatograph model 5860 equipped with a flame ionization detector and a heated gas injection loop. These grab gas samples showed the progress of the adsorption front through the column as well as verifying the MB "breakthrough" at the bottom of the column. Breakthrough was defined as the moment when MB concentration at the outlet reached 2 mg/L (500 ppm).

The main tool for measuring breakthrough at the outlet of the column was an on-line MTI portable gas chromatograph model P100 equipped with a thermal conductivity detector. This chromatograph monitored the outlet stream every 3 min for the presence of MB. The MTI operated in conjunction with an IBM Thinkpad laptop computer with a 486 Intel chip.

A Gast model R5325A-2 rotary vane pump, capable of pulling the required velocity of air/fumigant mixture through the column bed, drove the whole system by generating a

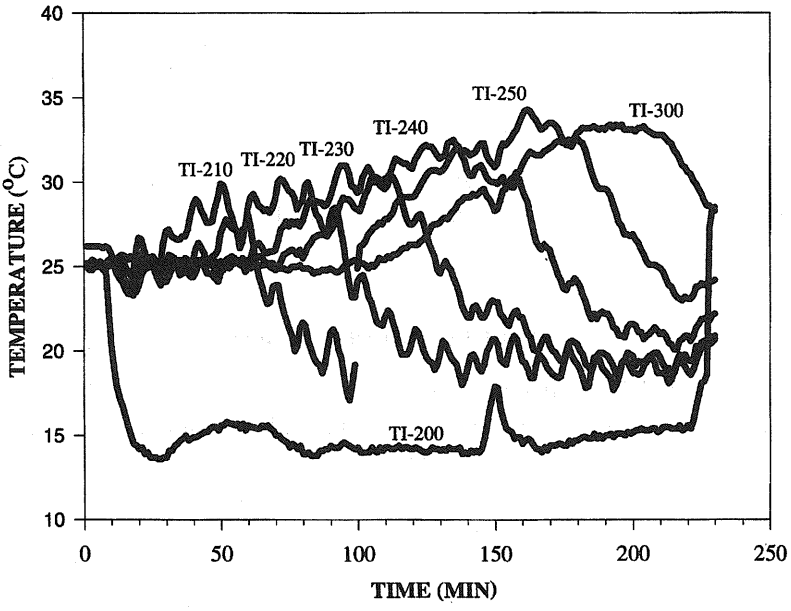


Fig. 2. Temperatures in the column during a typical loading experiment. Tl- designations refer to positions shown in Fig. 1.

vacuum at the end of the column. The pump exhausted directly into a laboratory hood. The entire system, from the preconditioning portion (T-110) to the pump (P-300), ran under vacuum during the testing periods. Working under vacuum was important for the safe operation of the experiments since any leaks only permitted room air to flow into the system, preventing MB from escaping into the room.

In most cases, the column was operated with repetitive 30-min ventilation periods ("runs") until breakthrough was observed at the outlet of the column. During each run, the column was normally charged with 38.5 g MB in 2,500 L (87 ft^3) air. The runs continued until the column outlet concentration reached 500 ppm MB (2 mg/L). The carbon was weighed at the beginning and end of each trial. The weight gain was due to the adsorbed MB plus water (either adsorbed or desorbed during the trial). Most of the trials were carried out during the winter months. We noticed that the moisture content (m.c.) of the clean carbon increased between its initial arrival in the summer of 1995 and its use in the winter of 1995/1996. Fresno usually experiences weeks of cold with high r.h. during the winter, and this winter was no exception. The clean carbon, since it was stored in 55-gal fiberboard drums which are quite permeable to moisture in the air, merely adsorbed water from its surroundings. The amount of water in either clean or loaded carbon was determined by the standard xylene method (ASTM method D-2867-83, reapproved 1988). Following each run, the carbon containing the MB was divided into two equal portions. A polished 2-mm carbon-steel bar (3 × 10 cm) was placed into each portion. Each portion —

with its steel bar — was then placed in a 1-L (quart) Mason jar. The first portion was placed in a room held at 26 ± 2°C, and the second in cold storage at –10 ± 2°C, in order to determine if any corrosion would occur due to the production of hydrobromic acid when MB is hydrolyzed.

Several trials were made at a higher average MB concentration to determine the possible effects of concentration on the sorption. The same amount of MB was introduced in each run during a trial; however, this was done at 10-min intervals instead of 30-min intervals as in the previous trials.

RESULTS AND DISCUSSION

The adsorption of MB by activated carbon for a given trial could easily be followed by observing the temperatures along the axis of the carbon column. Figure 2 shows the temperature profile for one of the typical higher concentration, 10-min-run trials. The gas inlet (TI-200) temperature for this run was held at 15°C. At 15 min into the trial, MB was introduced into the effluent air stream. The temperature did not rise immediately because the thermocouple was located a few cm below the upper surface of the carbon. The temperature at TI-210 was already rising before the temperature at TI-200 peaked after 50 min. The successive thermocouples peaked at approximately 20-min intervals until the bottom position was the warmest, indicating imminent breakthrough of the MB. The temperature variations at each position during the trial followed the 10-min addition intervals used in this example. Temperatures were highest at the beginning of each run when the concentration was high and dropped off as the concentration decreased over the 10-min intervals. The same peaking and fluctuation of the temperatures at each position occurred in the lower concentration trials, when the MB additions were made at 30-min intervals. Figure 3 shows the appearance of interstitial MB from the locations used for the thermocouples. The MB in the interstitial space appeared after the temperature peaked at each location. As the carbon at each position became loaded, the MB in the interstitial spaces at that position increased until it reached the average concentration of each run (in this example, approximately 8,400 ppm). Because we did not have enough channels to record all the positions simultaneously, we unplugged the top thermocouple when the temperature had fallen and plugged in the bottom one to record the later rise in temperature there.

Adsorption capacities under various conditions are shown as % load (g MB/100 g carbon) in Table 1. Adsorption capacity was influenced by all of the variables tested: carbon type, MB concentration, temperature and r.h. Norit carbon, derived from peat, consistently had the lowest MB adsorption capacity; Westates carbon, derived from coconut shells, the highest. The different capacities are probably due to both the different raw materials in each carbon and the differences in the activation process used to produce each of the final products; source and activation process together determined pore size and structure.

Activated carbon is used for the sorption of many products in different applications:

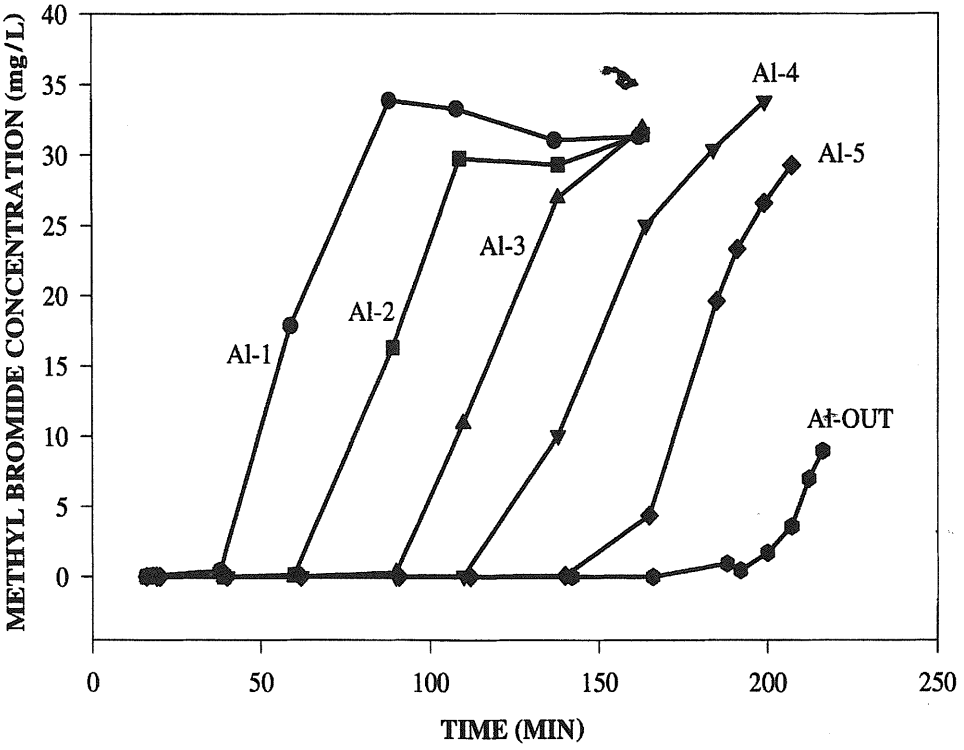


Fig. 3. Concentrations in the column during a typical adsorption experiment. Al- designations refer to those in Fig. 1. The breakthrough curve is shown as Al-OUT.

removal of high molecular weight color bodies from products, cleanup of chlorinated solvents from contaminated ground water and adsorption of low molecular pollutants from water and air streams (Goldhaar and Graham, 1991). The pore structure makes a given carbon more or less suitable for a given application. A significant amount of the pore volume of peat- and bituminous coal-based carbons is in macropores (>500 Å in diameter) and mesopores (20–500 Å in diameter). On the other hand, a majority of the pore volume of coconut-shell carbons is in micropores (<20 Å in diameter). Large molecules, such as color bodies, are preferentially adsorbed in macro- and mesopores, whereas smaller molecules, such as MB, are most effectively adsorbed in micropores. This explains the high capacity of the Westates carbon which has the highest amount of micropores of the three tested (Graham, 1992).

As expected, the adsorption capacity of the carbon was higher in every case when the concentration average was higher (i.e. 34 mg/L in a 30-min run compared with 34 mg/L in a 10-min run). It was also apparent that the adsorber inlet temperature and r.h. play an important role in determining the adsorption capacity of each carbon. Comparing the experiments at any r.h., shows that as temperature decreased from 35 to 8°C, capacity for each carbon

TABLE 1
The loading of MB on three different types of activated carbon under various humidity and temperature conditions

Type of carbon	Average ppm	Inlet temperature (°C)	% r.h.	% load	% pre-treat water	% post-treat water
Calgon	3800	15	50	12.5	–	2.4
Calgon	3800	15	100	10.6	1.6	2.0
Calgon	3800	15	50	13.4	–	0.9
Calgon	3800	25	50	10.5	–	0.8
Calgon	3800	25	75	7.3	–	3.5
Calgon	3800	25	100	7.5	–	3.1
Westates	3800	8	75	18.3	–	1.8
Westates	3800	15	50	15.8	5.4	2.6
Westates	3800	15	100	13.6	–	4.0
Westates	3800	25	50	10.5	6.0	3.9
Westates	3800	25	100	8.8	0.7	5.6
Norit	3800	15	50	12.5	–	1.4
Norit	3800	15	100	6.4	2.4	3.6
Norit	3800	35	50	5.5	–	3.2
Calgon	8500	8	75	18.3	–	0.6
Westates	8500	15	50	22.6	–	1.0
Norit	8500	8	75	12.6	–	3.2
Norit	8500	35	50	8.2	–	1.8

Moisture on the carbon both before and after the adsorption are given for some of the loading experiments.

increased. Likewise, as the r.h. increased for a given inlet temperature, adsorption capacity decreased. As the r.h. approaches 100%, water starts to condense in the pores, occupying space that would normally be available for organic molecule adsorption, thus reducing capacity for organic molecules. Table 1 shows the results of the moisture test for the carbons both before (% pre-treat water) and after (% post-treat water) for several of the trials. At 50% r.h., the carbons lost water in each of the two trials tested for this. Conversely, at 100% r.h., the carbons gained water in each of the three trials so tested.

It is well known that the adsorption capacity of activated carbon decreases sharply as the r.h. approaches 100%. We found much less decrease in capacity at 100% r.h. than expected. This is because the r.h. of air in contact with the carbon is lowered as soon as the adsorption starts due to the heat liberated by the adsorption reaction. At the inlet concentrations used for these trials, sufficient heat was liberated to dehumidify the gas below the critical 100% r.h. figure.

CONCLUSIONS

These experiments were undertaken to find a suitable way for trapping MB from the effluent stream resulting from aeration of a fumigation chamber. We investigated the

effects of temperature, r.h. and average effluent MB concentration on the loading of MB onto activated carbons. The experiments were done over a wide range of temperatures and r.h.'s because commodity undergoing fumigation can be either dry or wet and be stored under either ambient or refrigerated conditions. Durable commodities, such as grain and nuts, do little to increase the r.h. over the period of a fumigation; perishable commodities, such as peaches and plums, may increase the r.h. of the fumigant/air mixture during the fumigation period. Refrigerated commodities, such as imported grapes, if fumigated when the surrounding air is warm and humid, will create a fumigant/air mixture of 100% r.h. in the chamber. The cold commodity acts like a condenser.

From the research we have conducted, it appears that, although higher temperatures and humidities reduce adsorption capacity, at least those carbons derived from coconut shells and coal adsorb sufficient quantities of MB to provide a practical method for minimizing atmospheric emissions from commodity fumigations. For such large chambers as those used in San Diego and Long Beach, a transportable adsorber measuring 6.1 × 2.4 × 2.4 m (20 × 8 × 8 ft) loaded with 8,600 kg (19,000 lb) of carbon would be capable of adsorbing the MB from several fumigations. Assuming a conservative 10% loading capacity for the carbon (10 g MB/100 g carbon), and a 2,039 m^3 (72,000 ft^3) fumigation chamber that uses 130.8 kg of MB to achieve a dosage of 64 g/m^3 (64 oz/1,000 ft^3), each adsorber would be sufficient for at least seven fumigations. This assumes that 95% of the MB charged into the chamber is adsorbed by the carbon. In reality, some MB is sorbed by the commodity during fumigation and only the amount desorbed by the commodity during aeration would be recovered by the carbon. Once the MB has been loaded on the carbon, the carbon can be transported to a reclamation site where it can be desorbed with an appropriate hot gas (air, nitrogen or steam). This process will desorb both MB and volatiles collected from the commodity. The clean carbon would then be ready for reuse at another fumigation site. The MB desorbed during the reactivation process could be oxidized to hydrobromic acid and thus recovered in a convenient form for reuse in manufacturing various brominated chemicals, including MB.

Further experiments will be conducted to verify that the carbon can be recycled by the process herein described and to determine the effect recycling has on carbon's capacity for adsorbing MB. We are planning a series of tests, where volatiles from commodities will be sorbed on the carbon together with MB, to determine the effect of the volatiles on the capacity of the carbon. The sorption/desorption of volatiles and/or MB over many cycles will give a good indication of the practicality of re-using the carbon.

This process provides a procedure which does not interfere with the established fumigation schedules used for quarantine purposes. Because there is no attempt to recycle the MB directly on fumigations following the one from which it was adsorbed, there is no need for concern about the effect of adsorbed commodity volatiles on subsequent fumigations. There is also no need for concern about the disposal of secondary hazardous wastes because 100% of the trapped MB and adsorbed commodity volatiles are thermally destroyed during the bromine recovery process.

ACKNOWLEDGEMENTS

We wish to thank the Great Lakes Chemical Cooperation for providing funds and advice for this research and also Calgon Carbon Cooperation, Norit America Cooperation and Westates Carbon, Inc. for providing the carbon used in the tests. We would also like to thank Janie C. Tebbets and Leah Jost for their technical assistance during the testing.

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SESSION 5

APPLICATION METHODOLOGY OF CONTROLLED ATMOSPHERES AND/OR FUMIGATION, INCLUDING USE OF CARBON DIOXIDE UNDER INCREASED PRESSURE

Chairpersons:
C. Reichmuth, *Germany*
L. Benzing, *Germany*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 255–261

ON-SITE PRODUCTION OF NITROGEN FOR STORED-PRODUCT DISINFESTATION

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ABSTRACT

Treatments with controlled atmospheres for the disinfestation of stored products are often quite costly. This is one of the reasons for the slow acceptance of this technique in commercial practice. Although the costs for structural sealing and treatment supervision cannot be easily reduced, the costs for gas supply, especially in areas remote from industrial gas production sites, could be cut using flexible on-site production systems.

To determine the feasibility of on-site production, a pressure-swing absorption unit (PSA) and a membrane system were tested under semi-practical and practical conditions. It was demonstrated that at about 20°C, a nitrogen (N_2) atmosphere with a residual oxygen (O_2) content of 0.5% in a gastight welded-steel silo bin was sufficient to control all stages of *Sitophilus granarius* in slightly over 6 weeks, and of both *Ephestia elutella* and *Oryzaephilus surinamensis* within 3 weeks. When N_2 with 0.5% residual O_2 and was introduced at a flow rate of 5 m^3/h from below into a 188-m^3 concrete silo bin loaded with grain, ingress of O_2 into the silo produced an O_2 concentration of 3% at the top.

It was concluded that membrane and PSA systems are valuable techniques for the on-site production of hypoxic atmospheres, but that the use of N_2 should be limited to well-sealed silo bins, containers or chambers. Such gastight seals could be achieved using metal sheets, plastic foils or gas-impermeable coatings.

INTRODUCTION

Controlled atmosphere (CA) treatments for the disinfestation of stored products and materials are considered environmentally friendly, residue-free and safe for workers in the surrounding area at doses up to 5,000 ppm for carbon dioxide (CO_2) or higher for nitrogen (N_2). In some fields of stored-product protection, CA's may partially replace toxic fumigants and contact insecticides. The use of CA's, however, is limited to highly gastight enclosures; otherwise, leakage renders the treatment uneconomical.

In contrast to the situation in Australia, as described by Banks *et al.* (1991), in Germany CA treatments with N_2 are not yet in practiced use for stored-product protection. Although sheds and bag-stacks are commonly fumigated with phosphine (and in rare cases with CO_2), N_2 has not yet been registered for use in these treatments. Even though N_2 has been registered for use in grain bins, so far store keepers have been reluctant to use this time-consuming and often rather costly technique. Only in museums are N_2 treatments for pest control of artefacts (display pieces) widely accepted and carried out. This is because the inert nature of this gas means that it does not affect valuable artifacts (Reichmuth *et al.*, 1993; Wudtke *et al.*, 1984).

In this study, a pressure-swing absorption unit (PSA) and a membrane system for the production of a CA (N_2 with low oxygen (O_2) content) from pressurized ambient air were tested under practical conditions for the disinfestation of stored grain in silo bins.

MATERIALS AND METHODS

The welded-steel silo bin used in parts of this study was located within the Institute's Berlin premises. It had a 2-m diameter and a 9-m height, providing a volume of 26 m^3; its holding capacity was 20.5 t of wheat. The silo bin, the PSA unit and the membrane system were located in a building the constant temperature of which was 20 ± 2°C. Grain temperature at the top of this silo was 22°C and at the bottom of the silo 18°C.

Testing the PSA unit

The PSA system was a "Micro 15" from Carbo Tech Co.[1] This apparatus has a maximum output of 1.5 m^3/h and was adjusted to produce N_2 with a residual O_2 content of 0.4–0.5%. The gas was used to fill a 50-L container to a pressure of 5 bar. When the pressure in the container fell below 4.5 bar, the PSA was activated. A pressure tight tube was used to connect the container to the silo bin through a gas inlet at the bottom. A lid was left open at the top of the silo during the first hours of purging. The O_2 content within the grain close to the lid was determined using a Servomex 750 A O_2 analyser. After the initial purge at 720 L/h, the flow rate was reduced to 15 L/h. This guaranteed a slightly increased pressure within the silo, preventing the backflush of O_2-containing air through minor leaks. The complete treatment lasted 49 d.

The test insects were *Ephestia elutella*, *Oryzaephilus surinamensis* and *Sitophilus granarius*. Small wire-mesh cages containing a mixture of eggs, larvae, pupae and adults of these species were placed in the silo bin at three different points (1, 5 and 9 m above the bottom). After exposure times of 3, 4, 5, 6 and 7 weeks, the insects were removed and incubated in a climatised chamber at 25°C/75% r.h. for a further 8 weeks. The insect samples were examined weekly for both emergence and adult mortality.

[1]Mention of a trade name does not imply its recommendation.

Testing the membrane system

The membrane system, a "Prism Alpha" from Messer Griesheim Co., was supplied with pressurized air at 10 bar by a 11-kW compressor, and the air was dehumidified prior to being introduced into the system. Because only a small output was needed, only one of the two membrane compartments was used. The N_2 gas was stored in a 500-L container at pressures of 6–10 bar. Gas was released at 1 bar. When the pressure in the container fell below 6 bar, due to gas consumption, the compressor was activated. The residual O_2 content depended on the gas flow rate; 1% O_2 was obtained when the compressor ran about 60% of the time. The silo was purged from below, and O_2 content was measured at the top. Cages containing eggs, larvae, pupae or adults of *S. granarius* were placed in the silo bin and exposed to the gas for 2, 3, 4, 5 and 6 weeks.

The same membrane unit was also tested in a field trial using a 188-m^3 concrete silo bin loaded with 131 t of barley. Fifty 1-L metal cans with wire mesh lids at top and bottom, containing insect cages with *E. kuehniella*, *O. surinamensis*, *S. granarius* and *Tribolium castaneum* had been placed in the grain when the bin was filled. Measurement prior to treatment determined that the pressure half-life was $Pt/2 \geq 30$ sec. The silo was purged from below with N_2 and 0.5% O_2 at a flow rate of 5 m^3/h for 6 weeks and then at a flow rate of 6.5 m^3/h for an additional 2 weeks.

RESULTS AND DISCUSSION

With the PSA unit, after purging the silo bin for approximately 10 h, a residual content of 0.4–0.5% O_2 was achieved at the silo top. All juvenile stages of *E. elutella* and *O. surinamensis* were controlled within the shortest exposure time (3 weeks). To achieve 100% mortality of all stages of the granary weevil 6 weeks exposure was required. This is comparable to the results of earlier CA treatments of these silo bins with N_2 from cylinders (Adler and Reichmuth, 1989).

The results achieved with the membrane system in the same steel silo bin were similar. The complete control of *S. granarius* egg stages within 2 weeks of exposure was striking, especially in comparison to treatments with N_2 from cylinders. It is difficult to explain this effect unless it was caused by desiccation caused by the high gas-flow rates. Six weeks were insufficient for complete control of pupal stages (Fig. 1). The five survivors from two samples, compared to 126 survivors in the untreated control samples, indicate that 7 weeks of exposure would have been sufficient to achieve 100% mortality in this stage. More survivors were found in the sample placed at the bottom of the silo bin than in that at the top. This may be explained by the protective effects of the lower grain temperature at the bottom.

In the concrete bin, at a flow rate of 5 m^3/h (membrane system), O_2 content of approximately 3% was measured close to the silo top. Increasing the flow rate to 6.5 m^3/h after 6 weeks reduced the residual O_2 content at the lid of the silo to 1.8% (Fig. 2). Control of most insect species in the samples was far from complete. This could be partly attributed to grain temperatures at the surface dropping from approximately 20°C to 12°C

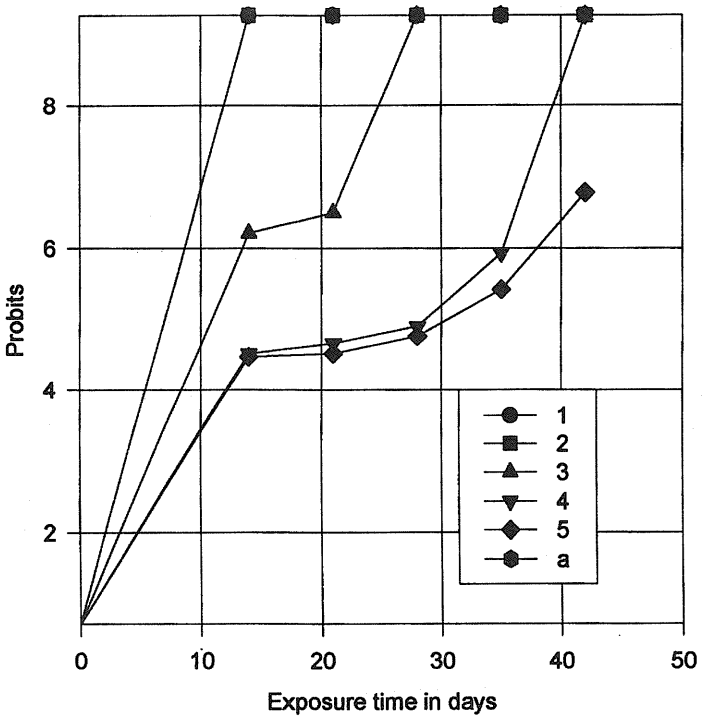


Fig. 1. Response of developing stages of the granary weevil *Sitophilus granarius* (a = adults, 1 = eggs, 2 = young larvae, 3 = larvae, 4 = older larvae, 5 = pupae) to treatment with N_2 from a membrane unit containing a residual O_2 content of 1% at 20°C in a welded steel grain silo bin.

during this time (Fig. 2), but even more to a significant ingress of atmospheric O_2 due either to diffusion or to a leak during the experiment. Since granary weevils survived in the probe close to the silo grain outlet spout, situated below the N_2 inlet, but were controlled in the samples above that point, there may have been a leaky spot in this region.

Running costs of these expensive devices are mainly linked to the cost and consumption of electricity, which is fairly cheap. This technique may therefore be considered for use even in leaky structures, as with fruit storage, overcoming the backflush of air by increased purge. If the structure must be treated several times, or if the sealing procedure is not too expensive, thorough sealing may make this technique more economically feasible. Seals to achieve a high degree of gastightness could consist of metal sheets, plastic foils or gas-impermeable coatings. For large grain storages or warehouses, these systems may be more convenient and more cost-efficient than supplying N_2 by cylinders or tanks (Table 1). The cost aspects are also discussed by Love (1984). Figure 3 illustrates cost-effectiveness in the form of a model. With no seal (0%) there are no sealing costs, but purge costs are prohibitive. With 100% gastightness, only one replacement of the air is

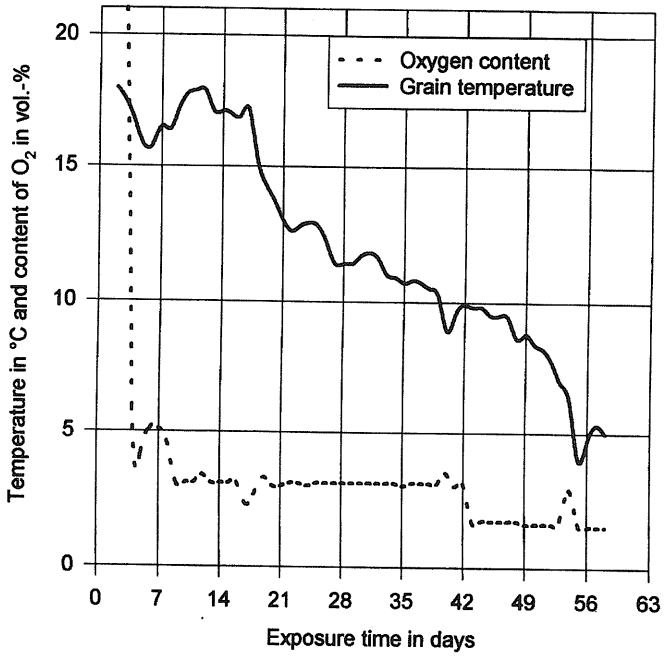


Fig. 2. O_2 content and grain temperature at the grain surface of a concrete silo during treatment with N_2 containing 0.5% O_2 from a membrane unit.

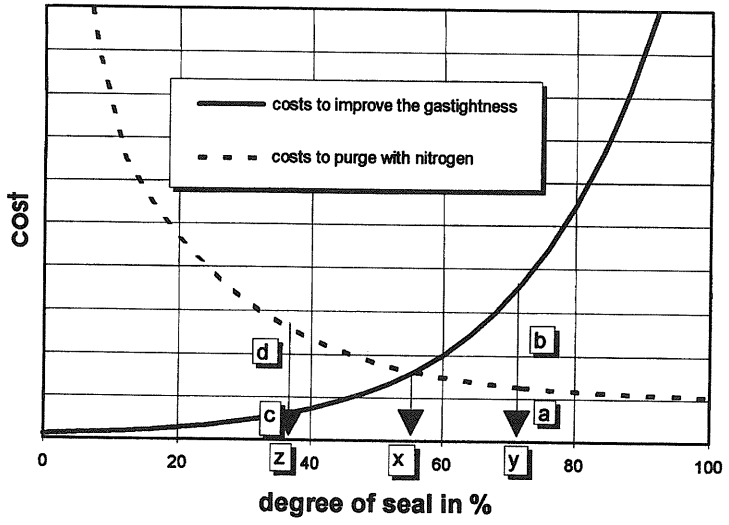


Fig. 3. Model showing the inter-dependency between the degree of seal and the costs of the purge necessary to achieve a low-O_2 atmosphere inside a treated enclosure, and also showing the costs of improving the gastightness of a structure in order to treat it effectively with N_2. The letters a, b, c and d represent costs in any currency unit.

TABLE 1
Approximate costs in Germany for supplying nitrogen for CA treatments

System used	Gas flow rate ($m^3 h^{-1}$)	Rent/year[1] (DM)	Operating costs (DM m^{-3})
Cylinders	0.001–10	180	3.00–3.50
Liquid tanks	1–100	6,000–34,000	0.20–2.50
Membrane or PSA	1–10	6,000–14,000	0.55–1.50
	10–20	20,000–25,000	0.35–0.45
	20–50	50,000–60,000	0.19–0.25
	50–100	60,000–70,000	0.14–0.18
	100–500	150,000–200,000	0.08–0.12

[1]Assuming a 10-year contract and electricity costs of 0.15 DM kWh^{-1}.

required; this is relatively inexpensive but only works effectively in an absolutely gastight structure. For better sealing beyond point x, e.g. at point y, it is more cost-effective to purge with N_2 instead of using the more expensive sealing procedures (a + b). The net saving is the cost of b. At point z it is more effective to seal than to compensate for leakage from the structure with increased N_2 purging. The saving resulting from sealing, quantified as d, can be compared with the costs of the purge, represented by c + d. Point x indicates the break-even point.

In conclusion, both the membrane and PSA units can be regarded as useful devices in providing N_2 for stored-product protection purposes.

ACKNOWLEDGEMENTS

The authors are indebted to Gerhard Schmidt for his full support of the experiments. We also wish to thank Mr Berg of the BEHALA (Berlin), the technician in charge of the concrete silo, who monitored the field trial. The BALM gave permission to use federal grain for the trial.

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THE USE OF ON-SITE GENERATED ATMOSPHERES TO TREAT GRAIN IN BINS OR FLOOR STORES

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ABSTRACT

A system available for on-site generation of low-oxygen (O_2) atmospheres for application to typical storage structures is described. The performance of a propane burner in generating and maintaining an atmosphere lethal to stored-grain pests has been evaluated in three bins and one small floor-store. Under reasonably calm conditions, a flow rate of 4.5 m^3/h was required to maintain an atmosphere of 1% or less O_2 in a bin containing 290 t of grain, compared with flow rates of 11.5 m^3/h for a bin loaded with 810 t of barley and 12 m^3/h for a bin containing 1,150 t of wheat. The 100-t bulk of floor-stored grain was held under a 1% O_2 atmosphere by a maintenance flow rate of 9 m^3/h. The results are discussed in the contexts of sealing problems and the effects on maintenance flow rates and application costs of both bin size and interruptions caused by windy weather or wide temperature fluctuations. A provisional dosage schedule, based on the current and previous work, is presented.

INTRODUCTION

Grain in the UK is stored mostly in either free-standing bolted metal bins or on the floor in barns constructed of either brick or galvanised steel. The proportion of grain admixed with pesticides at harvest has in recent years fallen to less than a third of the total; most grain is dried and cooled to reduce the incidence of spoilage and infestation. Phosphine fumigation of grain is carried out if an infestation is discovered and the nature of the storage structures used can cause gas-retention problems. Modified atmospheres (MA's) offer an alternative to both fumigation and pesticide-admixture strategies for insect control in stored grain. They can be easily integrated into present strategies of drying and cooling and could thus play an important role in the protection of grain, particularly where residue-free *in-situ* treatments are needed (Banks *et al.*, 1991).

Insects are susceptible to both high levels of carbon dioxide (CO_2) and very low levels of oxygen (O_2), although long exposures are required (Annis, 1987; Bell and Armitage, 1992; Banks and Fields, 1995). For MA's based principally on nitrogen (N_2) to work effectively, it is important to maintain the O_2 level below 1%, though Bailey (1955) and Reichmuth (1987) considered 2% O_2 a workable upper limit for the efficacy of MA's against most species. It is also important that the quality of the stored grain not be adversely affected by exposure to MA's. In this respect a N_2-based atmosphere is generally regarded as safer than CO_2. Neither germination nor end-use properties of wheat, rice and barley were affected by continuous storage in N_2 for one year (Ouye, 1984).

Propane combustion is a cost-effective method of producing an O_2-deficient atmosphere. It has the advantage of providing continuous on-site generation of gas, a vital aspect for treatment of most storage structures, but there is a disadvantage: the initial capital cost. Trials have been conducted for some years (Bell *et al.*, 1991, 1993a, b) with a self-cooling combustion unit, and an alternative system is also available in the USA (Storey, 1980a, b; McGaughey and Akins, 1989). Because the UK system does not require an energy-hungry electric compressor to run the cooling system, it is cheaper to operate. The unit needs only a 13-amp electrical supply and is therefore easily used in farm situations. Uncertainties still remain concerning the degree of modification required to render an enclosure suitable for MA's, the required gas supply rate and the full economic potential of the MA's (Banks and Fields, 1995). The results of our recent trials with MA's generated by propane combustion are here presented.

MATERIALS AND METHODS

The sites

Three grain silos of different sizes located on different silo complexes, plus a 100-t floor-store bay, were chosen for the trials. The bins were all constructed from curved sections of corrugated, galvanised steel. The sheets were bolted together using sealant at the joints, and then the sheets were mounted on a concrete base. Each base incorporated a series of aeration ducts (either hexagonal or a set of fingered ducts) fed by a single inlet and an auger. The latter was fed from a central opening and emerged from the silo base diametrically opposite to the aeration duct. The down pipe from the auger to the main conveying system was disconnected. A gastight polythene sheet was wrapped around the auger tube and drive motor and then taped to provide a leak-proof seal. This polythene sheet was then anchored to the side of the silo.

The tested bins were of 290-, 810- and 1,150-t capacity.

The propane burner

A propane-fueled inert atmosphere generation system, originally constructed by Aerogen Ltd., Alton, Hants, and modified at CSL, was used for these trials. The unit burned a calibrated premix of propane and air, with an optimal fuel to air ratio of 1:25 (v/v), in a

closed combustion chamber. The resulting mixture of gases (primarily N_2, water vapour, CO_2 and O_2) was passed via a tightly coiled piping system through a water-based condenser, where it was cooled.

The condenser coolant which contained 50% ethylene glycol was cooled by an absorption refrigerator driven by waste heat from the combustion process. Most of the water vapour was removed as liquid. It was possible to control the amount of cooling required with a secondary heat exchanger fitted in the water-glycol circuit between the refrigerator and the condenser. This exchanger was fan-driven and the air was blown over radiator vanes. The air was sucked, via a large duct, from one side of an open-ended box placed over the warm exhaust draft from the refrigerator. Shutters in the sides of the box provided a means of control over the amount of warm air drawn in by the fan.

The generator was mounted on a road trailer 3 × 1.5 m. It was covered by a tarpaulin stretched over a frame to protect it from the weather and was open at the ends to allow good circulation of the surrounding air. Fuel gas was supplied from two banks of four 47-kg cylinders connected by an automatic change-over valve. The empty cylinders were changed as required to give an uninterrupted supply.

The generator was adjusted to give an output gas stream of less than 1% O_2, 12–13% CO_2 and <40 ppm CO, at about 50% relative humidity (r.h.), at each of the flow rates used in the trials. The maximum output of this low-O_2 atmosphere was about 20 m^3/h. The cool, dry gas mixture was fed to the silo, assisted by a small fan to overcome the back pressure of the grain, via a 5-cm-diameter flexible hose connected to the aeration duct of the silo. For this purpose a plate, featuring a central spigot to receive the flexible hose, was made to fit the dimensions of each duct opening. The output was measured by a flow meter which had to be read manually.

Monitoring the gas constituents and preparations for atmosphere application

All gas monitoring of the generator effluent content and the atmosphere within the enclosures was carried out using 2-mm nylon sampling lines. These were inserted into the grain by interconnecting 1-m lengths of rod in two columns, one at the bin centre and the other 0.5 m from the silo wall below the roof entrance hatch. The lines were located, in a cross pattern determined by the hatch's position, at 1-m intervals from a depth of 6 m to the surface and also at the sides on the surface. Lines in the floor-store covered various points and depths along a transept across the bulk. A line was also attached to the generator outlet and, in some of the trials, to the aeration duct.

Type T thermocouples were inserted into the grain to record temperatures throughout the trials. Attached to rods, they were pushed into the grain as close to the sampling profiles as possible. A further thermocouple attached to the mobile laboratory recorded the ambient temperature. The gas lines and thermocouples, fed out of the silos through the roof hatch, ran down to the mobile laboratory where they were attached to the analytical and recording equipment.

The grain surface was covered with a 70-micron-thick plastic sheet, the edges of which were buried in the grain (at the silo or bin wall) to improve gas retention. Wind was an

important cause of generated atmosphere loss in trials carried out in silos in the open. Wind speed and direction were measured throughout these trials by an anemometer (Vector Instruments, Rhyl, Clwyd) which was mounted on a walkway above the silo or on the silo itself. The floor-store bulk was protected from the weather by being set up in the centre of a large shed, and wind speed was not monitored in this trial.

An automatic sampling system, which could be programmed to move through each of the sampling lines at a pre-set time interval (every 8 min) using a Psion Organiser II (Model LZ64), was used. A complete set of data from each position could be completed every 2 h. The samples were drawn down the lines using a diaphragm pump and were passed through a bank of instruments contained in the mobile laboratory. To allow the readings to stabilise, there was a 2-min delay before the data were recorded. The instruments were connected in series and consisted of a Series 1400 paramagnetic O_2 analyser, a Series 1400 infra-red CO_2 analyser, a Type 1490 infra-red carbon monoxide analyser and a Type 1491 nitrous oxide analyser coupled to a Model 1000D thermal oxidiser (all instruments by Servomex Ltd.). The data from each sample were collated using a HR2300 Hybrid recorder (Yokogawa Electric Corporation, Tokyo, Japan).

Assessment of the MA generation results

Every 2 h during the initial purge, and thereafter at least every 6 h, a record was made for each trial from each line and for each environmental condition. The efficacy of the flow in lowering O_2 levels was then assessed to produce a suitable purge rate and an achievable maintenance rate for each enclosure in relatively calm conditions. The maintenance rate calculation was made by taking a mean O_2 value from the beginning and end of each flow rate period and then subtracting the latter from the former. In order to achieve a more representative result, these calculations were made after the O_2 level of the generator output had been subtracted. These values were then plotted against the flow rate and a regression line fitted. A value for the maintenance rate was identified as the flow rate which produced no change in the O_2 level.

RESULTS

Micheldever (810 t malting barley)

For the initial purge, a rate of 15.6 m^3/h was used. The O_2 levels dropped rapidly, starting at the positions in the centre (1.5% O_2/h) and followed by those at the sides (0.6% O_2/h) opposite the aeration duct entrance and therefore furthest from the point of gas entry. There was no change in the rate of O_2 decrease at the side positions. However, following an initial O_2 decrease, at the centre there was a rapid increase. This increase in the central positions was then followed by another decrease, and over time a large oscillating cycle developed, particularly at the 6-m depth.

The source of this influx of O_2 was the cool, relatively dense night air pushing into the centre of the silo through the auger pipe. The atmosphere within the silo was very warm relative to the ambient air (Table 1). This large differential was caused by the warm,

TABLE 1
Temperatures and wind speed in the trials in free-standing grain bins

Conditions	Location		
	Hursley (290 t)	Micheldever (810 t)	Linton (1,150 t)
Grain temperature (°C)	16.9 (16.2–17.7)	22.7 (22.4–22.8)	9.1 (6.9–9.8)
Ambient temperature (°C)	4.9 (–6.9–12.7)	10.6 (4.3–15.5)	6.5 (–2.8–13.2)
Wind speed (m/s)	4.0 (0.0–17.6)	3.0 (0.0–8.7)	8.0 (0.0–20.0)

post-harvest weather which had given little opportunity for the grain to cool. The lower density of the warm atmosphere within the grain facilitated its displacement. This occurred at night when the temperature difference was greatest. Sealing the auger more thoroughly after the first week removed this problem. However, the atmosphere in the silo had already dipped below the 3% O_2 level by this time and this extended period at the start meant that an accurate purge time could not be calculated.

Lower post-purge flow rates were tried to determine the maintenance rate which would hold the silo at a constant 1% O_2. The results from the O_2-content data at each of the flow rates showed that the maintenance rate for this silo was 10.8–12 m^3/h with a best estimate of 11.5 m^3/h.

Hursley (290 t wheat)

The purge started with a gas flow rate of 15 m^3/h. The smaller diameter and lower height of this silo meant that the MA reached the 6-m centre position almost immediately. However, analysis of the purge revealed that there was a slow decline in the entire silo, and the target of 1% O_2 was not achieved throughout the silo for a further 52 h (6.9 atmosphere changes). This was longer than expected and indicated that there was ingress of air from some source. Again the auger was implicated as the problem area and attempts were made to improve the seal.

After the initial purge further flow-rate adjustments were performed to find a suitable maintenance rate for the low-O_2 atmosphere. This was hampered by occasional increases in the wind speed which affected the collection of results obtained under otherwise calm conditions. Ambient temperature was also a factor which influenced the trial, as below-freezing temperatures were recorded which caused large temperature differentials (Table 1) and resulted in an increase in the O_2 content within the silo, particularly near the grain surface. The final phase of the trial assessed the rate at which O_2 increased in the silo after the flow was turned off. The maintenance rate was estimated as 4.5 m^3/h.

Linton (1,150 t wheat)

The initial purge-flow was 19.2 m^3/h. The 1% O_2 level was reached after 72 h, meaning 2.4 total changes of atmosphere within the silo.

Further changes were made in flow rate, starting with 15.6 m^3/h, to discover a maintenance rate. The lowest flow rate tested was 12 m^3/h. After 36 h, representing 0.75 atmosphere replacements at this flow rate, there was no change in O_2 levels at any point. This indicated that the maintenance rate was lower than this value. However, because subsequently a very high level of wind was experienced during the trial, this was the best estimate of the maintenance rate for this silo (Table 1). As the mean wind speed rose towards 15 m/s, the O_2 level at the sides of the silo rose rapidly.

Sevenhampton (100 t wheat in a small floor-store bay)

The purge to less than 1% O_2 throughout the bulk was achieved within 48 h by a flow rate of 14 m^3/h (6–7 atmosphere changes of the enclosure). The atmosphere was adequately maintained by a flow rate of 9 m^3/h of burner gas containing 0.6% O_2.

Relating the required maintenance flow to silo size

Figure 1 shows the three values for maintenance flows for 1% O_2, produced by the three above-described silo trials, compared with three others from past work with MA generators (Bell *et al.*, 1991; 1993a, b). A regression line (solid line, Fig. 1) fitted to these data gave an R^2 value of 0.824 with a slope of 0.522, as compared to 0.757 ($R^2 = 0.956$) for the above three bins. This would imply that the maintenance flow rate required for the bins can be related to their surface area rather than to their volume. The predicted maintenance rate for a 100-t bin from the regression line was 3.55 m^3/h, and for a 1,000-t bin, 11.8 m^3/h.

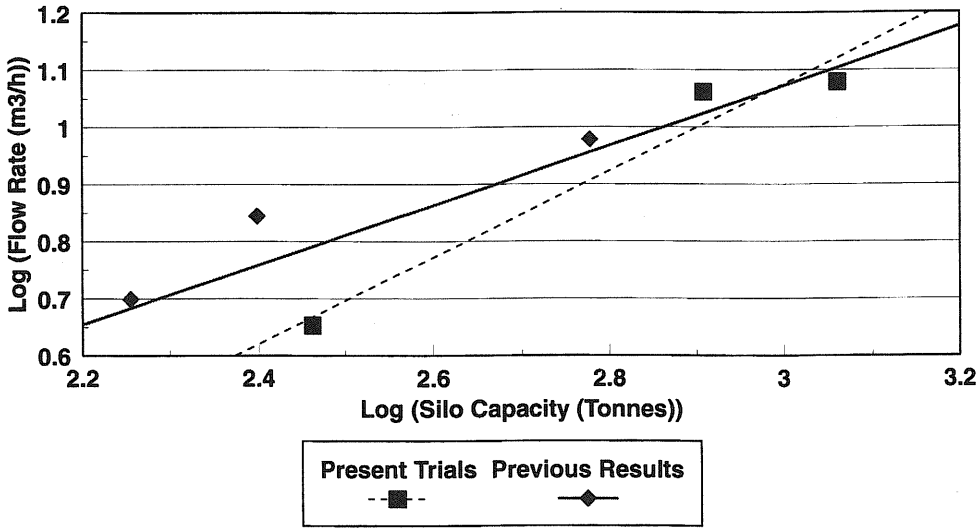


Fig. 1. Relationship between silo capacity and maintenance flow rate to hold a low O_2 atmosphere.

DISCUSSION

It has been shown before that for an efficient purge in MA applications, it is important to have as high a flow rate as possible (McGaughey and Akins, 1989; Bell *et al.*, 1993a). The present results showed that any source of leaks could greatly extend purge times. As far as ingression of air was concerned, the auger inlet to the silo was found to be an area of weakness during the purging operation. The best result was obtained with the 1,150-t bin at Linton, purged at 19.2 m^3/h, which needed only 2.4 atmosphere changes to achieve 1% O_2 at all points.

The results from the floor-store indicated that a low-O_2 atmosphere could be maintained by a flow rate which approximated that necessary to hold an equivalent level in a silo of about 5 times its capacity. Such treatments would of course be more costly than those for bins, but the enclosure volume in this initial trial was increased by the need to sheet over the high grain walls, creating dead spaces amounting to about 25% of the total volume, which reduced purge and maintenance efficiency. In other situations, the volume treated, and hence treatment costs, could be substantially reduced.

Although maintenance flow rates for retaining MA's in grain bins are affected radically by any changes in the prevailing weather conditions, the size of the grain bulk is also important. Because of the relation between volume and surface area, it can be expected that, for maintenance of a low-O_2 atmosphere, doubling bin volume will increase surface area, and hence the flow rate to counteract leakage, by 1.59 times (Bell *et al.*, 1993a). The four-fold difference in volume between the bins at Hursley and Linton indeed gave almost the expected two-and-a-half times increase of the maintenance flow rate needed to maintain 1% O_2. The maintenance rates required as silo size increased from 290 to 810 to 1,150 t represent 0.75, 0.60 and 0.51 changes in atmosphere per day, providing further support for the theory that maintenance flow rates become more economic as silo volume increases.

Comparison with earlier work (Bell *et al.*, 1991, 1993a, b) indicated that some improvement had been achieved in the seal level obtained on the bins at Hursley and Linton; the points lay below the fitted regression line for maintenance flow rates in the bins tested to date (Fig. 1). The outlying value of 7 m^3/h for a 250-t silo was extrapolated from the data generated by that trial (Bell *et al.*, 1993b) which was, in fact, carried out on a similar silo at the same site as the present 290-t silo trial. Therefore, the extrapolated flow rate may have been set too high. However, differences in the effectiveness of joint seals in different silos may alter the maintenance rate attainable even in two silos of similar size.

The gradient obtained for the regression line fitted to data from six bins sealed with differing degrees of success was lower than that expected for a volume surface area relationship, and in fact was intermediate between this value (b = 0.6691) and that expected for a volume linear dimension relationship (b = 0.3346). This provides some evidence that having restricted the potential sites of leakage as much as possible by the sheet edges buried in the grain around the perimeter of the bins, the potential minimum

maintenance flow rates required were more closely related to a linear dimension, such as bin circumference or diameter, than to surface area.

The decreasing number of per-day atmosphere changes with increasing silo size is reflected in the cost per t for the treatment. Taking a treatment purge at maximum flow rate and a 4-week run under the estimated maintenance flow for each silo, the expected UK£ cost per t in terms of propane consumed would be 36, 24 and 17 pence (actual consumption 0.35, 0.90 and 0.94 kg propane/h) for the three sites, in ascending order of tonnage, plus a small, constant electricity cost for running the burner. However, these figures may over-estimate the minimum costs per t because the results for both the Hursley and Linton trials were maximum estimates of the required maintenance rates.

The cost results presented are estimates for calm weather conditions and do not take into account the possible changes in conditions which might cause a rise in the O_2 level. Some interruptions can be withstood without the need to increase exposure times beyond those normally required to produce 100% mortality in certain grain-pest species (Conyers and Bell, 1997), although further work is necessary to define the critical length of such interruptions.

ACKNOWLEDGEMENTS

This work was supported by a grant from the UK Home-Grown Cereals Authority, London.

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PURGING GRAIN BULKS WITH NITROGEN: PLUG-FLOW AND MIXING PROCESSES OBSERVED UNDER FIELD CONDITIONS

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ABSTRACT

Six experimental, full-scale applications of nitrogen (N_2) to grain bulks were extensively monitored. These trials showed that in practice there are often substantial deviations from the theoretical behaviour expected when a gas, such as N_2, is used to displace air from a particulate commodity, such as cereal grain, in store. In the most efficient form of oxygen (O_2) displacement, plug-flow would be expected in the grain bulk, followed by dilution of the headspace by a free mixing process. There was some dispersion in practice as the purging front transited the bulk; most regions lost O_2 by this disperse plug-flow process. Using rudimentary introduction systems, in practice, some areas of grain bulks were not effectively purged by disperse plug-flow processes but instead they lost O_2 by dilution, leading to inefficient use of purge gas. These areas were apparently associated with low pressure gradients and, possibly, also with high levels of grain dust. Dilution of the headspace generally followed the expected logarithmic fall in O_2 content with time.

Purging efficiencies overall were 73–92%, based on the plug-flow/free mixing model, with N_2 usage of 1.2–2.0 $m^3\ t^{-1}$ achieving 1% O_2 atmospheres throughout.

Deviations from ideal behaviour reduced efficiency somewhat, but they were not sufficient to prevent target atmospheres from being achieved in the cases reported.

INTRODUCTION

Storage under nitrogen (N_2)-based controlled atmospheres (CA's) is one of the few means currently available for providing inexpensive, residue-free pest control in dry bulk grain. In some specialised cases, even using liquid N_2 as an atmosphere source, it is already cost-competitive with phosphine fumigation. Part of the export grain terminal at Newcastle, New South Wales, Australia, has used N_2-based CA's routinely since 1993, successfully treating more than 100,000 t of grain. It is to be expected that with the cheaper atmosphere sources now under trial there will be increased interest in CA use, particularly in situations where safety considerations and local regulations place onerous restrictions on fumigation and those where grain protectants cannot be used, such as for market reasons.

There have been a substantial number of studies published (Banks *et al.*, 1980a; Tranchino *et al.*, 1980; Agboola, 1993; Cassells *et al.*, 1994) which describe the experimental treatment of bulk grain with N_2 atmospheres under full-scale conditions. These studies provide a sound basis for the application of CA's in a wide variety of well-sealed grain storage types in a wide range of locations and systems (Banks *et al.*, 1980b).

These publications have tended to concentrate on successes, not failures. They have included little, if any, discussion of the difficulties encountered. However, in general, failures are more instructive than successes. In this paper we have concentrated particularly on deviations from expected behaviour, as it is these deviations that are most likely to result in such operational problems as increased gas usage, incomplete effectiveness or the need for prolonged exposure.

The data on which this paper is based was gathered during the full-scale proving trials carried out in Australia during the 1970's as part of the development program for commercial use of CA's. Summary details of these trials have already been published (Banks, 1979). Only the purge phase of the operation is discussed here, as it is valid to conclude that if an appropriate atmosphere can be created by a certain N_2 application rate, it can be maintained by a rate of addition that is less than the adequate purge rate. The trials were aimed at creating an insecticidal 1% O_2 atmosphere in a number of different structures, but here, for the purpose of illustration, a 2% O_2 level is often chosen as a successful limit. This is the level regarded as the maximum which is lethal to all species of stored-produce insects (Bailey and Banks, 1975).

EXPECTED BEHAVIOUR

The system under consideration here, the addition of N_2 to a grain bulk, is a specific case of addition of a fluid to a packed bed. The latter is a common process in the chemical industry, and the behaviour of the added fluid and its interaction with the fluid already present in the bed can be understood in chemical engineering terms. The mathematics and terms used by Levenspiel (1976) to describe the progress of a step concentration change through a packed bed are used here as one model to describe the behaviour of a N_2 purge passing through a grain bulk. It is not expected that there will be direct displacement of the interstitial air by N_2 (true plug-flow) because a limited degree of mixing occurs at the N_2-air interface. This may be caused by molecular diffusion, due to partial mixing as the N_2 enters the bulks. The mixing leads to a moving zone where the O_2 concentration is less than that in the existing storage atmosphere but substantially more than that in the incoming gas (<0.01% O_2). The process is known as 'disperse plug-flow'. Limited degrees of dispersion give a sigmoid profile for O_2 concentration against distance within the zone. The fall in O_2 concentration with time as the mixing front passes a given point is also sigmoid. Both these profiles, O_2 level with time at a point and O_2 level with distance at a given time, can be transformed to give a linear trend if the percent concentration of O_2 is expressed as a ratio of the initial concentration, normally 21% O_2. This ratio is treated as a frequency of a Gaussian distribution.

An alternative analysis framework was used by McGaughey and Adams (1989) for the application of burner-produced low O_2 atmospheres to small bins. This was not adopted here as we wished our approach to be consistent with the approach used for describing fluid flow through packed beds.

Substantial deviations from this model were to be expected with increasing dispersion, particularly at low O_2 levels. In the limiting case, where complete mixing occurred, the fall in O_2 concentration from an original concentration c_0 to a new value c is an exponential function of time t, following the equation:

$$c = c_0 e^{-kt} \tag{1}$$

where k, the rate constant for the decay is given by:

$$k = \frac{Q}{V} \tag{2}$$

Thus

$$\ln c = \ln c_0 - kt = \ln c_0 - \frac{Qt}{V} \tag{3}$$

and consequently the apparent flow rate, Q, into the region of volume, V, can be found from the slope of the plot of ln c with time.

The rate of upward movement of the N_2 atmosphere can be estimated by observing the time taken ($t_{x\%}$) to reach a set O_2 level ($x\%$) with height z in the storage on a given sampling line. The corresponding apparent input rate (Q) through a cross sectional area (A) in a medium with porosity n associated with observations from a given sampling line is then given by:

$$\frac{dz}{dt_{x\%}} = \frac{nQ}{A} \tag{4}$$

Efficiencies ($E_3\%$, Banks, 1979) are calculated on the basis that the most efficient achievable mode of N_2 purging corresponds to true plug-flow in the grain bulk with complete mixing in the headspace. Thus, the theoretical minimum gas usage V_{th} (V_a = volume added, V_B = bulk volume, V_{HS} = headspace volume and C_0 = concentration of O_2 in air, i.e. 21%) is given by:

$$V_{th} = nV_B + V_{HS} \ln \frac{C_0}{c} \tag{5}$$

and thus,

$$E_3\% = \frac{100 V_a}{nV_B + V_{HS} \ln \frac{C_0}{c}} \tag{6}$$

EXPERIMENTAL DATA

The data discussed here was obtained from seven trials carried out in Australia in cylindrical grain storage bins at Bordertown, South Australia (twice); Cunningar, New South Wales (twice); Sunshine, Victoria; Newcastle, New South Wales; and Balaklava, South Australia. Details of the trials, bin construction, dimensions and contents are given in Table 1.

TABLE 1
Dimensions and loads of the test bins

	Bin construction		Bin dimensions				
Trial	Walls and roof	Floor	Diam. (m)	Height to eaves (m)	Load (t)	Head-space (m^3)	Total storage capacity (m^3)
Bordertown 1	welded steel	concrete (flat)	23.1	19.6	7035	693	9049
Bordertown 2	welded steel	concrete (flat)	23.1	19.6	5220	2365	9049
Cunningar 1	concrete	concrete (conical)	10.9	30.5	2040	430	2930
Cunningar 2	concrete	concrete (conical)	10.9	30.5	2040	430	2930
Sunshine[a]	welded	concrete (flat)	13.9	19.0	1809[a]	601	3055
Newcastle	bolted steel	concrete (flat)	8.2	9.4	294	72	421
Balaklava	welded steel	concrete (flat)	23.1	19.6	6474	812	9049

[a]This trial carried out on barley, the others on wheat.

Of the structures used, only the Newcastle bin had been constructed specifically for use with fumigants or modified atmospheres. All the other structures required some sealing work (sealing for doors, removal of ventilators or filling of the wall to floor joint) to increase their level of gastightness. The existing fitments had to be modified to take the pipe work for the introduction of N_2. In the Bordertown 1 trial, a special distribution system was constructed for the N_2 purge. This was designed to give an even application of gas across the floor of the bin. All other trials either used the existing ductwork, which had been fitted for aeration, or introduced the gas directly into the bulk through the bin wall via a shielded inlet, as described by Banks and Annis (1977). In all cases a substantial level of gastightness was achieved, with the bins used at Bordertown 2, Sunshine and Balaklava exceeding the standard for bins suitable for N_2 use given by Banks and Annis (1977).

Liquid N_2 was supplied to the site by cryogenic tanker. The liquid was vaporised and brought to ambient temperature with forced draught heat exchangers before being introduced into the test bins. The input flow of N_2 was measured using either a rotameter-type system (Elliot Automation 101 Metric Series) or an Anubar in-line pitot tube system. The total gas usage was calculated both from weighbridge readings and by integration of the flow measurements. Gas volumes are given at average grain temperature and ambient atmospheric pressure for the site. Concentrations are given in % v/v.

Before grain inloading, the bins were rigged with both 3-mm o.d. semi-rigid nylon tubing, for gas sampling, and copper-constantan thermocouples, for temperature measurement. Each bundle of lines, designated below by a capital letter, contained a number of

lines terminating at different heights in the storage, giving a 1–3-m separation of sampling points. These points are designated below by height in the bin. Thus, Sunshine A, 4.0 refers to the sampling point on line-bundle A at 4.0 m above the floor in the Sunshine trial. Heights in the Cunningar trials use the base of the cylindrical wall as zero. The distribution of sampling lines for each trial is shown in Fig. 1.

Gas samples were taken with a small diaphragm pump and analysed for O_2 concentration with Bacharach Model K525 or Beckman Model 715 electrolytic oxygen meters.

Grain was loaded into the test bins under normal commercial conditions. However, the bins were not fully filled as this would have tended to overstress the fixings of the cables supporting the sampling lines where they were attached to the roof. The grain surface was raked flat for the Newcastle trial but was left as it fell in the others. Generally, the larger-than-normal headspace in the bins accentuated the deviation from ideal behaviour and reduced the observed purging efficiency.

RESULTS

Movement of the nitrogen atmosphere

Two modes of O_2 removal from positions within the grain bulk, as detailed below, were noted during the trials. The mode followed was decided by the fit of the trend of O_2 concentration with time after one of two transformations.

Mode 1 — The diffuse plug-flow model. With this mode the O_2 level, transformed as a frequency, was an approximately linear function of time for values between 20.8 and 1% O_2 (e.g. for Gaussian frequencies of 0.05 to 0.99).

Mode 2 — The free mixing model. With this mode the O_2 level was found to be a semilogarithmic function of time for values between 20.8 and 1% O_2.

The fall of O_2 concentration was rapid for most points. Often, this fall from >19% O_2 to <3% O_2 occurred within the sampling interval used, and therefore data adequately defining the shape of the O_2 concentration-time curve was not obtained. Where this was so, it has been assumed that the disperse plug-flow model (Mode 1) is appropriate. However, with increasing dispersion, the rate of O_2 displacement lessened, allowing enough observations to be collected for comparison with those expected from the postulated models. In a few cases neither of these models obviously held.

Typical curves obtained for O_2 displacement following Mode 1 are given in Figs. 2 and 3. Examples of the few instances of curves giving behaviour more consistent with Mode 2 are given in Figs. 4 and 5. Figure 5 also shows the most extreme deviation from the ideal plug-flow behaviour observed in any of the trials. In this case a number of points close to the bin wall purged very slowly (10.2, 12.7 and 15.2 m), remaining at >10% O_2 when the remainder of the points in the grain were <1% O_2. The sampling line with the aberrant points was also equidistant from each arm of the Y-shaped gas introduction duct, giving no horizontal pressure gradient to help disperse the O_2.

In Fig. 5 it can be seen that some points give O_2 reductions consistent with Mode 2, albeit with a very slow rate of change. This rate is independent of N_2 introduction rate. A few other lines show behaviour inconsistent with either mode.

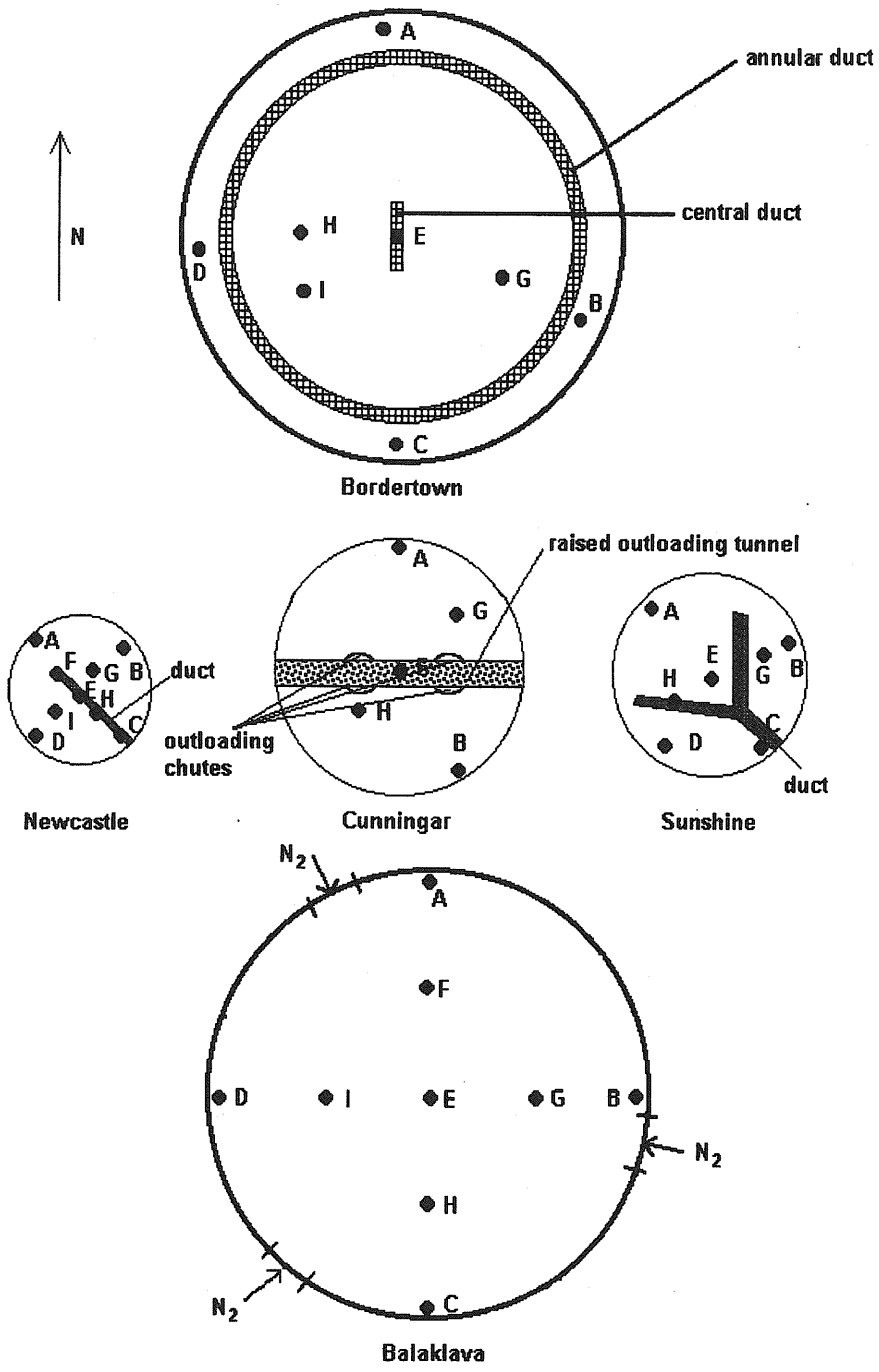


Fig. 1. Distribution of the sampling lines for O_2 analysis.

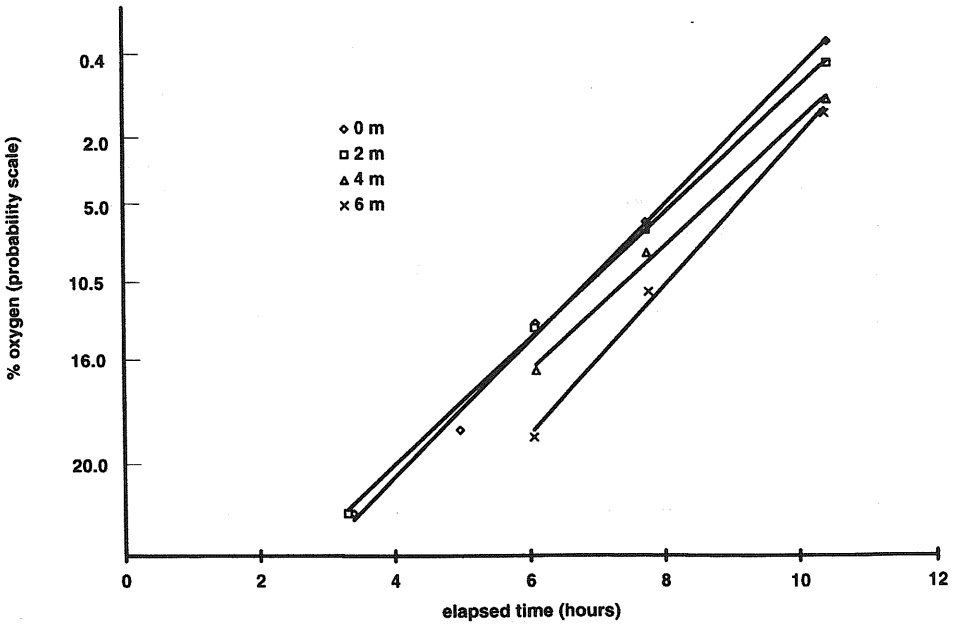


Fig. 2. O_2 concentration fall with time transformed as for Mode 1 in Newcastle trial, line D.

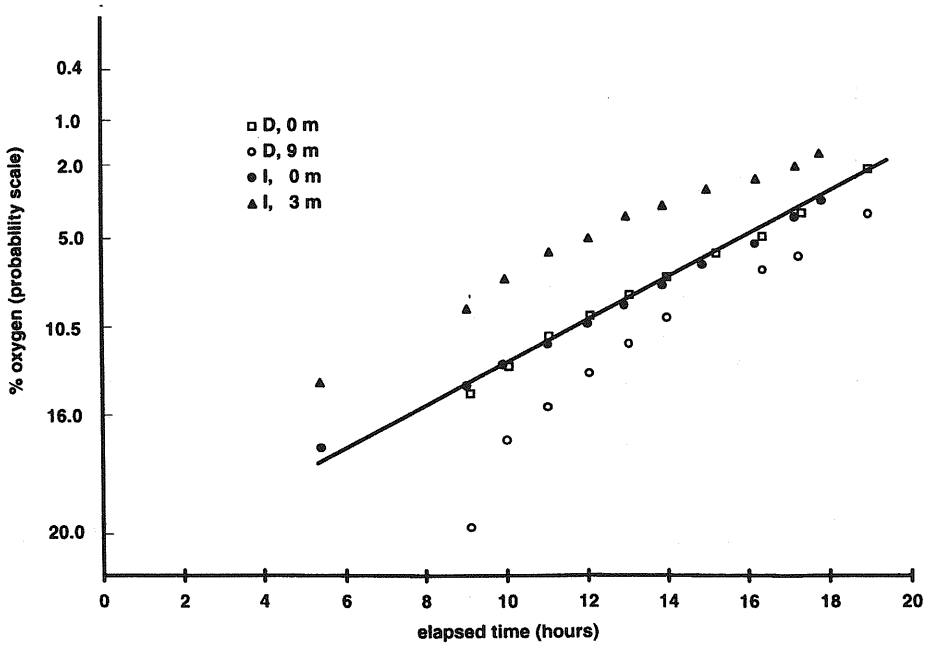


Fig. 3. O_2 concentration fall with time transformed as for Mode 2 for some points in the Balaklava trial.

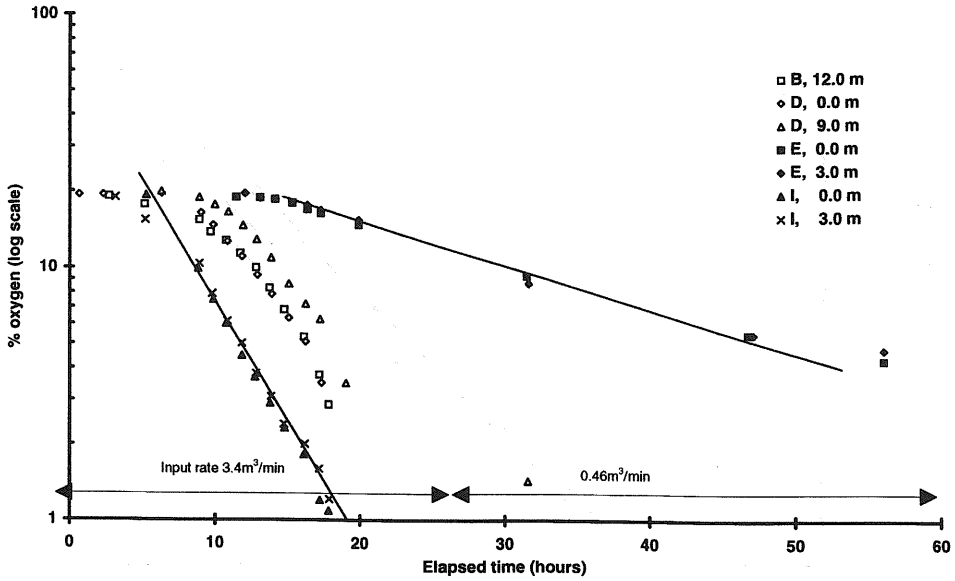


Fig. 4. Variation of O_2 concentration with time for points from the Balaklava trial, transformed as appropriate for Mode 2 and showing variation in N_2 purge rate.

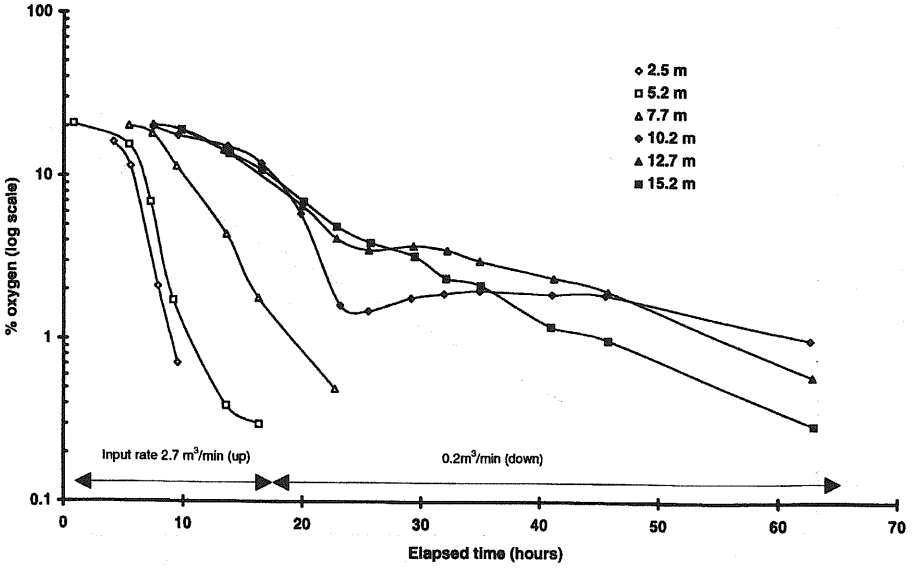


Fig. 5. Variation of O_2 concentration with time for line A, Sunshine trial, transformed as appropriate for Mode 2.

Similar, very slow flow-independent purging, consistent with Mode 2, was observed in one other trial (Fig. 4). Again this appears to be associated with the lack of a horizontal pressure gradient to displace the O_2-containing atmosphere. In this case, the points of concern are in the centre of the bin, on the floor at points equidistant from the N_2 inputs and at three points equally spaced around the bin wall. The modes of O_2 removal for all points sampled in the grain bulks are summarised in Table 2.

Even amongst points that conform to the relatively efficient Mode 1, there is a range of slopes that apparently correspond to different rise rates of the N_2 in different parts of the bin (i.e. the purging front is not level). Table 3 shows face velocities and

TABLE 2
Modes of purging for 193 individual points in seven trials

Number of points	Mode of purging
164	Mode 1 — disperse plug flow
24	Mode 2 — free mixing
5	Mode undefined — neither model holds well

TABLE 3
Nitrogen face velocities and corresponding equivalent gas flow rates for the sampling lines with displacement consistent with Mode 1

Trial	Line	Observed rise rate ($m\ h^{-1}$)	Equivalent input rate ($m^3\ min^{-1}$)	Actual average input rate ($m^3\ min^{-1}$)
Cunningar 1	A	2.14	1.26	2.2
	B	1.66	0.98	
	E	4.44	2.62	
	G	1.85	1.09	
	H	2.27	1.34	
Cunningar 2	A	3.65	2.16	
	B	2.85	1.68	
	E	9.12	5.39	3.2
	G	4.82	2.85	
	H	7.35	4.34	
Sunshine	A	<0.46	<0.58	
	B	2.23	2.82	
	C	2.36	2.98	
	D	2.13	2.69	2.7
	E	10.00	12.65	
	G	6.50	8.22	
	H	10.30	13.02	

Cunningar trials are with wheat, Sunshine trial with barley, hence the different conversion factor for rise rate to equivalent input rate (porosities 38 and 50%, respectively).

equivalent purging rates from several trials. There can be a substantial difference in rise rates in various parts of the bin. It can be seen that within individual trials there was a wide variation in rate for different lines. There is clearly scope for improving efficiency by better application and gas distribution systems. Whether improvement is justified, economically, depends on the balance between the costs of the improvement and the savings in N_2. In all the trials discussed here, or reported in the scientific literature to date, the target level of O_2 has been achievable with the ductwork as used.

Removal of oxygen from the headspace

As expected, in all trials the loss of O_2 from the headspace conformed with Mode 2 (displacement by free mixing). Changes in the rate of gas input were directly reflected in changes in the rate of headspace pull down (Figs. 6 and 7).

Given the volume of the headspace, the flow rate of N_2 entering the headspace can be calculated from the slope of the semilogarithmic decay curve. Table 4 gives a comparison of the calculated rate derived from the rate of loss of O_2 from the headspace with the average input rate. With one exception, the calculated rate was always less than that observed. This can be ascribed either to incomplete mixing and venting of O_2-poor gas or to the entry into the headspace of gas containing significant O_2 amounts rather than pure N_2. The latter possibility is supported by the observed breakthrough times (Table 5). In all but one case, the beginning of the loss of O_2 concentration in the headspace (breakthrough) was observed earlier than was calculated on pure plug-flow bands. In the single exception, Cunningar 2, the calculated and observed breakthrough times were the same. Moreover, the calculated and actual input rates were very similar.

The close correspondence with a free mixing model for O_2 pull down in the headspace makes the extrapolation to target O_2 levels quite reliable. Thus it is possible, given an observed O_2 level of, say, 3% in the headspace, to predict with a high degree of certainty the extra N_2 addition needed to bring the level to 1%. With the few exceptions noted above, the bulk in these trials was already purged to about the target level before the N_2 was added to the headspace. This extrapolation can therefore be used with confidence to estimate the total N_2 usage and purge times needed to create the target atmospheres.

Purging efficiencies

Table 5 gives observed or extrapolated purging data for the systems to reach 1% O_2 throughout in the six trials. Under these conditions, with larger than normal headspaces, N_2 usage was between 1.2 and 2.0 $m^3\ t^{-1}$, with efficiencies E_3 of 73–92%. Other trials, for example Cassells *et al.* (1994) and Banks *et al.* (1980a), have shown similar purge-consumptions and efficiencies.

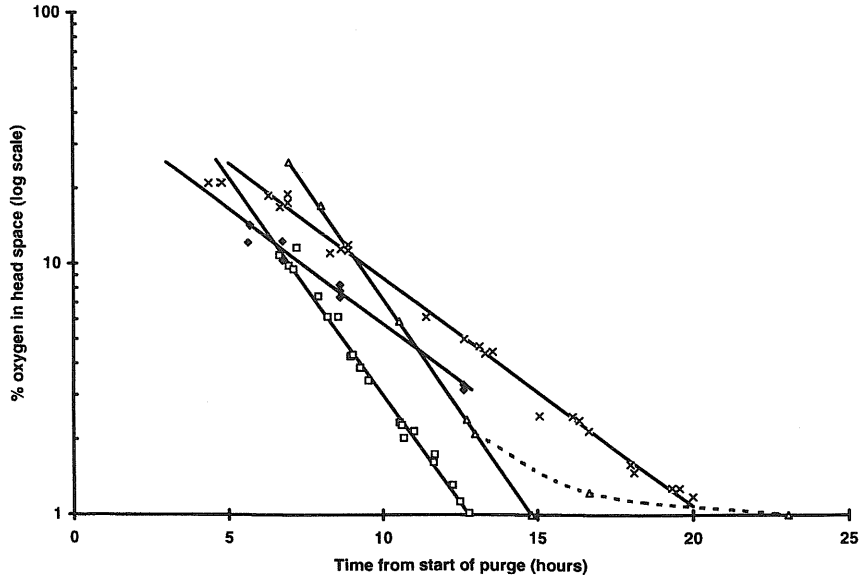


Fig. 6. O_2 concentration decay in the headspace during purging for Cunningar 2 (□); Bordertown 1 (Δ), displaced by + 2 h); Bordertown 2 (♦) and Sunshine (×) trials.

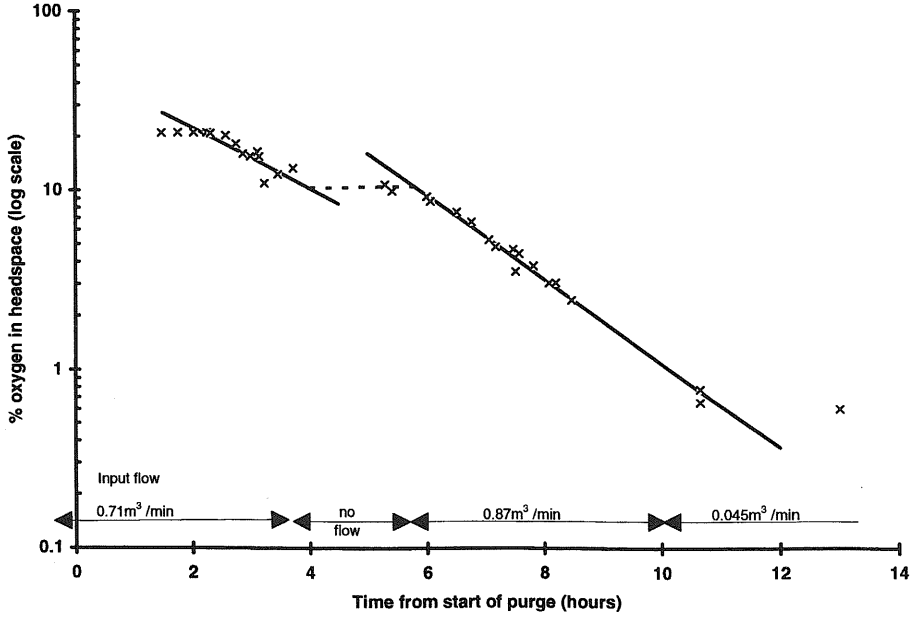


Fig. 7. O_2 concentration decay in the headspace during purging in the Newcastle trial, showing changes in N_2 input rate.

TABLE 4
Efficiency of purging and N_2 usage

Trial	Filling ratio	Headspace O_2% at end of purge	Efficiency of purging (to 1%)	Time required to give 1% O_2 in headspace (h)	N_2 usage calculated for this period (m^3)	N_2 usage ($m^3\ t^{-1}$)
Bordertown 1	0.92	1.1	76	16.2[a]	9170	1.30
Bordertown 2	0.74	3.2	73	18.4[a]	10390	1.99
Cunningar 1	0.85	3.9	84	20.5[a]	3470	1.70
Cunningar 2	0.85	1.0	92	12.8	2430	1.19
Sunshine[b]	0.71	1.2	89	20.4	3340	1.85
Newcastle	0.83	0.7	88	10.2	430	1.46
Balaklava	0.91	2.1	75	141.8[a]	9150	1.41

[a]Extrapolated time on the basis of a semilogarithmic O_2 decay curve. [b]This trial with barley, all others with wheat.

TABLE 5
Comparison of N_2 input flows and usages to breakthrough from regions in the headspace applied flows and intergranular volumes

Trial	Average input rate ($m^3\ min^{-1}$)	Calculated[a] rate ($m^3\ min^{-1}$)	Expected break-through time assuming plug flow (h)	Observed break-through time (h)	Actual N_2 usage to break-through (m^3)	Inter-granular volume[d] (m^3)
Bordertown 1	4.7[b]	5.1	12.5	5.6	1580[b]	3515
Bordertown 2	9.4	8.3	4.6	3.9	2200	2610
Cunningar 1	1.9[c], 4.7	0.57, 2.40	8.3	5.6	803	950
Cunningar 2	3.1	2.9	5.1	5.1	979	950
Sunshine[c]	2.7	2.1	7.6	6.1	988	1227
Newcastle	0.71[c], 0.87	0.47, 0.65	3.1	2.2	92	132
Balaklava	3.4[c], 0.46	1.2, 0.16	16.9	7.4	1541	3459

[a]Calculated from rate of O_2 depletion in headspace. [b]Flow rate measurement approximate only. [c]Flow rate changed during course of purge. [d]Volume without headspace.

CONCLUSIONS

With N_2 added rapidly to the base of a bin, as here, most regions of a grain bulk will purge by disperse plug-flow.

The headspace will lose O_2 by a process giving a semilogarithmic fall with time, though at a slightly lower rate than that predicted by a simple free-mixing model.

With rudimentary gas-introduction duct work, such as that used in these trials, the N_2 purging front is not horizontal. Some areas of the bulk may purge much more rapidly than others.

In a few extreme cases, apparently associated with low horizontal pressure gradients, some regions in some bulks were observed to lose O_2 only very slowly, and that by a process consistent with free mixing rather than disperse plug-flow. This behaviour may be rate-limiting with regard to achieving the target atmosphere throughout a system.

Even with rudimentary gas introduction systems, purging, 70% on the basis of plug-flow in the bulk and free mixing in the headspace, is quite efficient.

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A NEW PHOSPHINE RELEASING BAG PRODUCT BASED ON MAGNESIUM PHOSPHIDE FOR SHORT-TERM FUMIGATION

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ABSTRACT

A new magnesium phosphide-based product for the protection of stored products by phosphine (PH_3) fumigation has several advantages when compared with the usual products. The metallic phosphide formulation is contained in bags made of specially impregnated paper (Tyvek) which are permeable only to water vapor. After complete decomposition of the metal phosphide, the fine dust remains in the bags so no residues can reach the stored, fumigated commodities. The addition of zeolite, a water-absorbing sodium-aluminum silicate, ensures that when the tin is opened for use no uncombined PH_3 can be detected in the packed product. In addition, all danger of self-ignition is precluded. Furthermore, because this adjuvant delays the development of PH_3 at the beginning of the decomposition, there is enough time for the fumigant to diffuse without causing any hygienic risk to the worker who inhales the generating gas. In contrast to similar products based on aluminum phosphide, under normal conditions (20°C/60% r.h.), the new product, "Detia Gas Ex-B Forte," decomposed completely within 48 h. The residue in the bags contained only traces (0.1%) of magnesium phosphide. Because of its prompt decomposition, this product is especially suitable for short-exposure fumigations. This formulation is also remarkable in that it enables fumigations to be carried out at low temperatures (below 5°C) without greatly prolonging the exposure time. Consequently, short-term fumigations in cold weather or in chilled containers are also possible. Using this magnesium phosphide product in bags might open new operational areas for fumigation with PH_3. Good results have already been achieved with the quarantine treatment of bananas, red peppers, mangos, etc.

INTRODUCTION

Considering the three most important aspects in the development of a fumigant formulation for phosphine (PH_3) release, namely storage, application and disposal, raises the possibility of problems at all three stages. When the development of a new PH_3-releasing bag formulation was initiated, the first priority was to achieve greater safety during storage of

the formulation, during application and during retrieval or disposal (when risk should be eliminated entirely). The second objective was to develop a PH_3 product that decomposes faster than other PH_3 formulations presently available on the market. The new formulation was designed to reduce exposure times for commodities during transport and in storage, particularly with regard to container shipments that demand shorter fumigation times.

STORAGE OF THE FORMULATION

Frequently low PH_3 concentrations (in the ppm-range) can be measured outside tubes or tins containing PH_3 formulations. These low concentrations may be attributed to leakage along soldered lines and around tube caps and tin lids.

Recently, part of an American harbour was temporarily closed because of unacceptably high concentrations of PH_3 in an area surrounding one of the transport and storage containers. Both manufacturers and research institutes have repeatedly found up to 1,000 ppm of PH_3 remaining in fumigant packaging. This is not surprising, since the volume of a tube or a tin is very small. If a tube of 9.5 cm^3 contains 1,000 or 2,000 ppm, this means that an employee or technician within about one cubic meter of it could be exposed to a few ppm PH_3 when opening it. However, a special ingredient in the newly developed bags has, for the first time, enabled the manufacture of a PH_3-free package.

This was made possible because when PH_3 is released from the metal (magnesium) phosphide, it is absorbed by a specially added ingredient, sodium aluminum silicate. This silicate, known as zeolite, is a naturally occurring substance, and zeolite mines exist in northern Italy among other places. Zeolite has a very high affinity for PH_3, immediately adsorbing the PH_3 molecules that develop from the metal phosphide.

In the new bag formulations there are two different types of zeolite molecule. One type has small pores which temporarily adsorb PH_3 molecules, and the other adsorbs H_2O molecules. This assures that water vapour molecules combine with the zeolite and are therefore unable to react too rapidly with the magnesium phosphide to form PH_3. It also assures that the first PH_3 molecules to develop are initially retained in the zeolite molecular filter. The packaging itself thus remains PH_3 free.

PROTECTION AND SAFETY FOR FUMIGANT OPERATORS

This unique property of sodium aluminum silicate also protects the fumigant operators. This is due to the delayed emission of PH_3 from the new bag when compared to the bag currently in use, without sodium aluminum silicate (Fig. 1). This figure shows emissions during the first 2 h, about the period of time normally needed for applying the fumigant. From this it is clear that there is a marked delay in the decomposition of the formulation with the zeolite ingredients. This delay is normally about 20 min before the first PH_3 concentrations — in very small quantities — can be measured. It continues for up to about 6 h, after which the efficacy of the silicate is diminished and the curves of decomposition approach each other. This delay in decomposition would be an important contribution to the safety and protection of the workers applying the fumigation bags. It should be noted

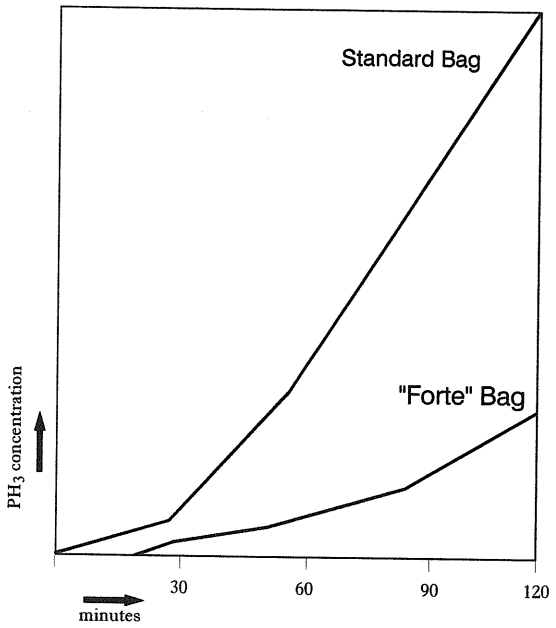


Fig. 1. Rates of initial PH_3 evolution from the standard and from the new "Forte" fumigation bags.

that the MAC-value, or TLV, for PH_3 is almost universally between 0.1 and 0.3 ppm, and such values can easily be reached when tubes or tins containing liberated PH_3 are opened.

RATE OF PHOSPHINE PRODUCTION

Magnesium phosphide is known to decompose faster than aluminum phosphide. Furthermore, the rate of metal phosphide decomposition within a fumigation bag is dependent upon both the temperature and the humidity gradient between the outside and the centre of the bag. Therefore, the reaction can be speeded up by making the bag thinner. The standard 6-mm thick bag can be compared with the 3-mm thickness of the new fast-decomposing bag, and clearly decomposition in the standard bag is much slower. The standard bag contains 34 g of phosphide mixture and generates 11.3 g PH_3, whereas the new magnesium phosphide bag contains only 9 g of phosphide mixture and generates only 3 g PH_3. An additional factor influencing the rate of reaction is the ratio between surface area and weight of the bag. The standard bag is 9×9 cm, with a surface area of 162 cm^2, whereas the new bag is 5×9 cm, with a surface area of 90 cm^2. The weight-to-surface-area ratios of the standard and new bags are 0.21 and 0.1, respectively. This smaller ratio also contributes to a much faster decomposition of the new bag.

Rates of PH_3 concentration build-up over a 48 h period are given in Fig. 2. At 20°C, differences in humidity (between 60% and 75% r.h.) do not significantly affect the rate of PH_3 release.

Magnesium phosphide also has the remarkable property of decomposing rapidly, even at low temperatures. Figure 3 shows decomposition at 3°C and 32% r.h. Even at such low temperatures, considerable decomposition is reached after about 3 d. However, fumigations at such extremely low temperatures should be the exception since it is well known that stored-product pests reduce their metabolic activity drastically at low temperatures.

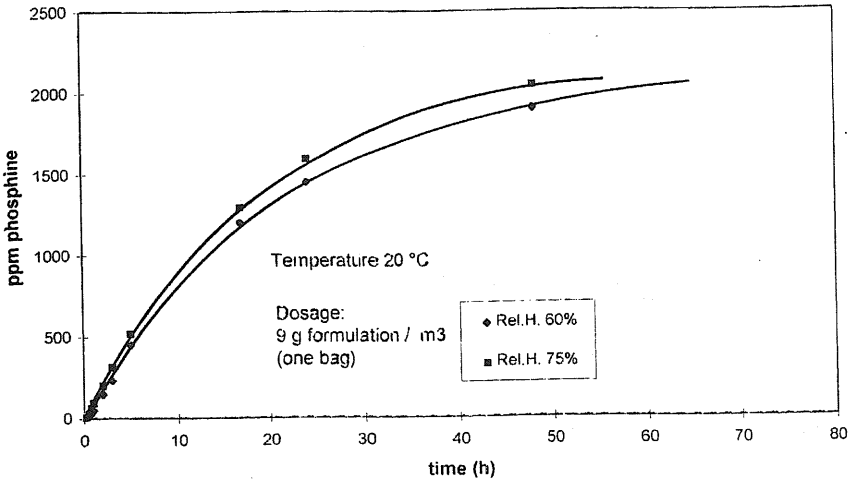


Fig. 2. PH_3 concentration build-up over a 48 h period at 20°C.

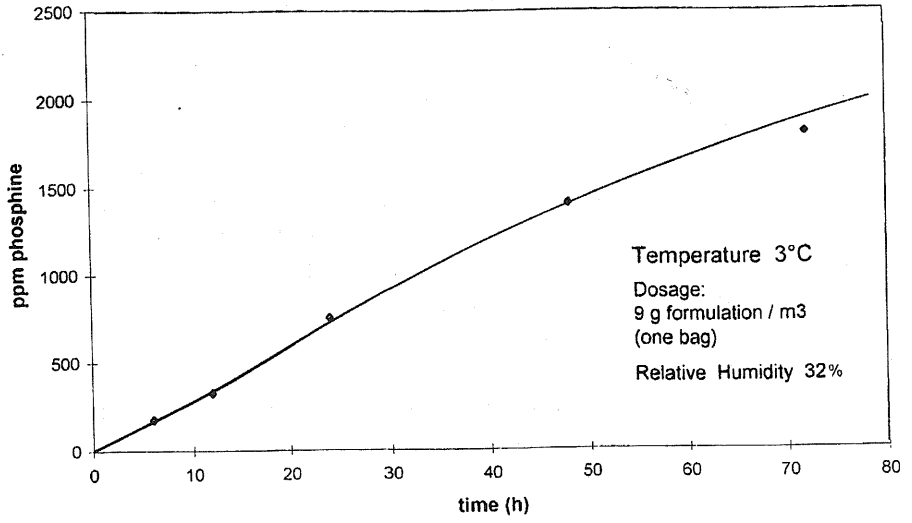


Fig. 3. PH_3 concentration build-up at 3°C.



Fig. 4. Application of the bag chains.

Their respiration is reduced and thus only small amounts of PH_3 are inhaled. Consequently, often only the most susceptible stages, particularly adults, are killed; developing stages, especially when they are inside commodities, survive.

DISPOSAL

Disposal of the decomposed materials has recently received special attention and an increasing number of countries have enacted laws or regulations for handling the waste products. In this important respect also, the new improved bags have decisive advantages. Because the chemical reaction of aluminum phosphide with water to produce PH_3 and aluminium hydroxide only reaches 98% completion, 2% of the original aluminum phosphide remains in the technically spent bags. Magnesium phosphide, on the other hand, decomposes faster and farther than aluminum phosphide. Due to the special adjuvents, in the new magnesium phosphide bags the decomposition rate is further improved, leaving a residue of no more than 0.1% Mg_3P_2.

This new bag is called "Gas-Ex-B forte." It is at present being registered in Germany, Italy, Greece, Austria and Japan for use in the control of stored pests in spices, tobacco, teas, cocoa, dried fruits, seeds, flour and raw coffee. The recommended dosage is 3 g/t or 2 g/m^3. The bags are available in chains, facilitating their use in containers, store-rooms, etc. (Fig. 4).

TREATMENT OF FRESH FRUITS

Preliminary tests carried out with this new fumigation bag for quarantine treatment of various fresh fruits were quite successful. In a survey of literature conducted by the manufacturer, it can be seen that even with high dosages (some with relatively long exposure times) and at different temperatures, the tested fruits were not damaged. These tests are, however, preliminary and they must be confirmed by further studies (Table 1).

TABLE 1
Fresh fruit fumigation with PH_3: preliminary experimental findings

Fruits	Dosage (ppm)	Time (h)	Temperature (°C)	Fruit damage
Grapefruit	500	48–72	15	no
Oranges	1000	72	20	no
Avocados	500	72	21	no
Mango	500	48	21	no
Papaya	2800	24	12	no
Bananas	1000	72	10	no
Grapes	800	72	4	no

The newly developed PH_3 bag product based on magnesium phosphide can possibly replace some of the present methyl bromide (MB) applications. This is of particular importance in those countries where in the near future MB will be partly or completely banned.

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 293–303

ACTIVE FUMIGATION SYSTEMS: BETTER WAYS TO FUMIGATE GRAIN

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ABSTRACT

Although for many years phosphine (PH_3) has been used to fumigate grain in unsealed storages with static methods, such usage is not an acceptable option in any on-going fumigation strategy. In these methods, the source of PH_3 is simply added to the fumigation enclosure or admixed with the commodity, and the gas generated is allowed to permeate through the commodity naturally. At best, such methods will lead to an illusion of success by killing the mobile stages; at worst, they will promote the selection for PH_3 resistance. Other methods, such as "trickle-flow" or regular "top-up" of concentrations in storages that are not sealed, are equally unacceptable.

The development of SIROFLO®, an active fumigation technique designed for both sealed and unsealed storages, has brought into focus the question of fumigation strategies and the problem of whether or not some storages should be sealed. The choice of fumigation strategy can now be between static methods in sealed storages (other than vertical silos) and active methods in storages that are not gastight. An understanding of both the behaviour of gases and the factors that give rise to gas loss is of primary importance in examining this question. Other factors that will influence the choice include the geometry of the structure and the costs of the available options.

A new manifolded recirculation system called SIROCIRC™, that uses the engineering principles of SIROFLO® and achieves balanced airflows in silos that are less than gastight, is described, together with data obtained from recent trials. The relative merits of the alternative strategies are discussed.

INTRODUCTION

The fundamental requirement of fumigation is the provision an adequate concentration of fumigant for a sufficient period of time, a time that, in principle, will achieve a dosage or concentration × time product (Ct) that will kill all stages of all species present. This is a simple concept, but it is difficult to achieve, largely because most structures in which fumigations are carried out are not gastight. Although all recommendations for fumigation clearly state that the enclosure should be gastight, the great majority of fumigations

worldwide are conducted in structures or enclosures that fall well short of the standard of gastightness that ensures dosages sufficient to kill all stages of all species present in an infested commodity (Winks *et al.*, 1980). Tablets or pellets that produce a peak phosphine (PH_3) concentration in about 1.5 d, and decay to zero in about 5 to 7 d, are used for many if not most PH_3 fumigations around the world. These parameters, however, are typical of concentration profiles in quite leaky storages in which the most that can be expected is a cosmetic fumigation giving the illusion of success. The susceptible adults and larvae may be killed, but the more tolerant eggs and pupae survive; within a relatively short time, the infestation returns to its prefumigation level. At this stage the fumigator, farmer or store manager, simply fumigates the commodity again, in the process selecting the population for resistance to PH_3. This constitutes the greatest threat to the continued use of this valuable fumigant (Winks, 1986; Winks and Ryan, 1990).

The most obvious approach to sound fumigation practice is to achieve a standard of gastightness that will retain the gas long enough to kill all stages of insects, including the more tolerant eggs and pupae. Banks and Annis (1984) among others have done much both to define standards of gastightness aimed at achieving this objective and to describe how such standards may be achieved in a variety of storages. However, these standards are not always sufficient. In themselves they do nothing to ensure adequate distribution of fumigant throughout the fumigation enclosure, and thus, in some situations, they fail to eliminate pockets of low concentration. This is because of the forces that give rise to gas loss, most importantly air ingress, and the forces that influence distribution.

This paper describes the forces that affect gas behaviour and fumigant distribution in grain storages and the benefits of active fumigation systems; it also describes a new fumigant recirculation method.

FORCES THAT AFFECT FUMIGATION OF GRAIN

The principal forces that give rise to air ingress are the chimney effect and wind. The forces that influence distribution are convection, diffusion and sorption. In this paper the chimney effect, wind and convection are discussed. Molecular diffusion is a minor force in terms of gas loss and distribution (Banks and Annis, 1984), and although sorption can be very significant in the fumigation of some commodities it is outside the scope of this paper.

The chimney effect

The "chimney effect" occurs as a result of temperature differences between the grain and the atmosphere surrounding a silo (Winks and Russell, 1994). In a silo in which the intergranular air is at a higher temperature than the air surrounding the silo, the lower density of the intergranular air creates a potential for the air within the silo to rise. This potential generates a pressure difference between the inside and the outside of a silo and in this case, with warmer intergranular air, the pressure at the base of the silo would be negative with respect to the outside of the silo. If the intergranular air was colder than that surrounding a silo, a positive pressure would be generated at the base of the silo.

To measure, or even observe, the maximum chimney pressure, a silo needs to resemble a bucket, either upright or inverted. In this case, there is measurable pressure but no flow, i.e. no ingress of air. In a real silo with cracks, etc., around the base and at the top, the temperature difference between the grain and the atmosphere surrounding the silo will determine the chimney potential, and the size of the cracks will determine the amount of flow into and out of the silo, i.e. the amount of air ingress. In turn, this determines the size of the pockets of low concentration in which insect survival can occur.

An example of the chimney effect is evident in data obtained by the pest control staff of the NSW Grain Corporation during the attempted fumigation of a number of sealed vertical bins at a site in southern New South Wales. PH_3 was "dumped" from cylinders into the base of the bins with fan assistance to drive the gas to the top of the bins; the fans were then shut off and the balance of the contents of the cylinders added to the base of the bins to try to compensate for the chimney effect. It is clear from Table 1 that there was either little or no gas in the bottoms of these bins within a few days, and the fumigation would thus have failed.

The sealing would need to be absolute to stop the ingress of air altogether by sealing cracks, etc. This is virtually impossible to achieve. Anything less for a given temperature difference will simply determine the size of the pocket of low concentration. When the grain temperature is consistently greater than (or less than) the temperature of the surrounding atmosphere, there will be a constant and significant ingress of air. There is thus a high probability of insect survival in the affected zones, either at the top or bottom of a silo depending on the direction of the chimney. An alternative to storage-sealing is application of positive pressure equal to the chimney effect (Winks and Hunter, 1994). The site referred to in Table 1 has been equipped with SIROFLO® which, because of the positive pressure it generates, has overcome the problems evident from the earlier data.

TABLE 1
The influence of the chimney effect on PH_3 concentrations (ppm) at the bottom of sealed bins at Wallendbeen following the dump method with cylinders of PHOSFUME

Bin no.	Day 3 (6 May 1991)	Day 4 (7 May 1991)	Day 6 (9 May 1991)
3	2100	1500	0
4	1350	1200	0
5	5[1]	1	0
6	0[1]	4000	0

[1]More gas added.

Wind

In substantial structures such as fixed silos, sheds, squat bins, farm bins, etc., wind is a relatively minor force in the context of fumigant loss or air ingress. It is more significant in temporary structures, such as bag-stacks and bunkers. From numerous field trials of SIROFLO® in which wind speed and wind direction were monitored, it would appear that wind would be a problem only if it causes air disturbance in the head space and turbulence in the surface layers of grain (Winks and Russell, unpublished data). This disturbance or turbulence is not difficult to prevent or overcome.

Convective distribution

Without the imposition of mechanical systems, air movement occurs within a grain mass as a result of thermal convection driven by temperature gradients throughout the grain mass (e.g. Nguyen, 1985). Simply put, warm air rises while cold air sinks. When grain is loaded into a silo, e.g. from trucks, it is to be expected that the loads will vary in temperature and this may lead to layers or pockets of different temperatures within the grain mass. These differences may be sufficient to induce movement of air within localized cells in the grain mass. In addition to this, the variations in temperature near walls and roofs will also induce convective movement of air within the grain mass. In sheds and squat bins it is commonly found that during the cool night hours the air in the grain adjacent to the walls will drop in temperature and, because of the higher density, will start to move downwards. At the same time the warmer air in the center of the grain mass, with its lower density, will start to move upwards. In a sealed bin the warm air rising out of the surface of the grain will be cooled against the roof above and drawn back into the grain in the area adjacent to the walls. This creates efficient convective movement of the air within the bin and relatively rapid distribution of fumigant. However, in a bin that has a leaky roof this same process is simply a variation of the chimney effect and it will cause loss of fumigant from the headspace through the roof, draw cool air from outside the silo down the walls of the structure and thus rapidly dilute the fumigant concentration.

Convective distribution can be eliminated or substantially reduced if the temperatures within the grain mass are evened out and the differences eliminated or substantially reduced. Cases have been documented in Australia which clearly demonstrated this phenomenon. In both cases the grain in large squat bins had been aerated which evened out the temperatures, and when fumigant was added to the headspace it largely remained there.

While most of the time convective distribution can be expected to provide the mechanism for effective distribution in sealed sheds, squat bins and farm bins, it cannot always be relied upon. The only methods currently available that can provide both consistent fumigant distribution and an acceptable probability of success are those based on active or forced distribution systems. In vertical storages, forced distribution is the only viable approach to effective fumigation. All other methods provide a lower probability of success and, usually, little more than a cosmetic fumigation and effective selection for resistance.

ACTIVE FUMIGATION SYSTEMS

The term "active fumigation system" is used here to describe any system in which another process is superimposed on natural gas distribution processes in order to improve distribution of fumigant or to overcome the ingress of air, which causes reduction of fumigant concentration, into the enclosure. By contrast, "static fumigation systems" are those in which the fumigant is simply added to the enclosure; the success of the treatment thereafter depends on natural processes, such as convection and diffusion, to distribute effective concentrations of fumigant throughout the enclosure. Static fumigation processes include both 'one-shot' and 'multiple-shot' (or top-up) techniques as well as those using simple 'trickle flow' from cylinders.

Active fumigation methods include recirculation and SIROFLO® (Winks, 1992). SIROFLO® is a pressurised distribution system aimed at overcoming the chimney effect. It has been implemented over the last 8 years in a wide range of storages, ranging from small farm bins to large horizontal or vertical storages, in Australia. While SIROFLO® is effective in poorly sealed units, including open-topped bins, and can overcome the chimney effect, conventional recirculation (Fig. 1) is effective only in sealed bins. Even then, success depends on the way the system is operated. For example, if conventional recirculation is carried out by operating the fans only to mix the fumigants, whereupon they are switched off, there is a high probability of the chimney effect's producing pockets of low concentra-

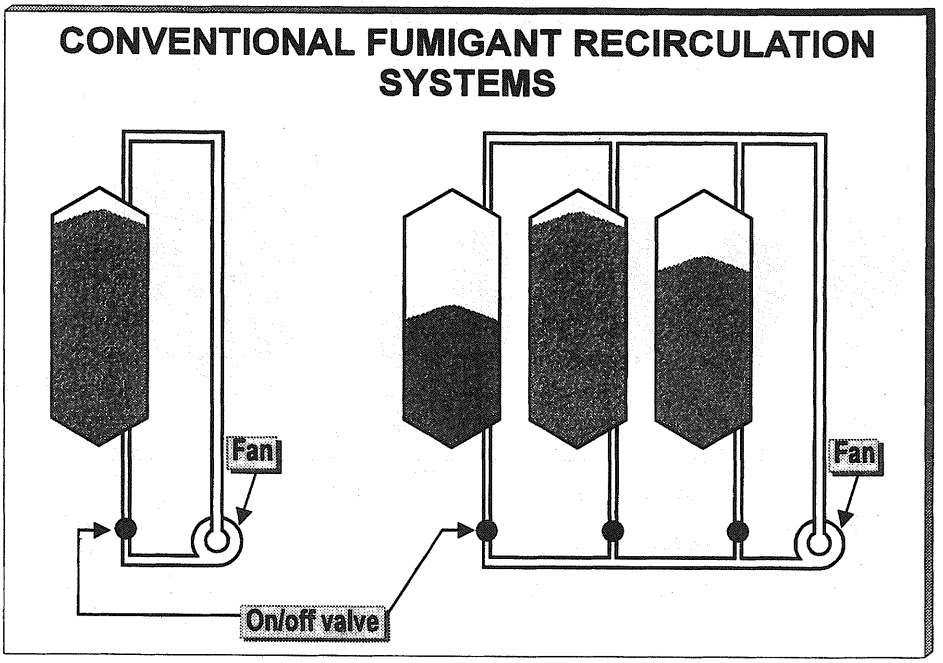


Fig. 1. Conventional fumigant recirculation in single bins or multiples of bins manifolded together.

tion in different parts of the bin, which part depending on the relationship between the grain temperature and that of the surrounding atmosphere. In sealed bins conventional recirculation can be effective if it is based on a continuous low-flow system. This implies flows, or recirculating fans, that will generate only low pressure differentials within the bin. If large fans, such as those traditionally used with methyl bromide recirculation systems (Bond, 1984), i.e. fans that produce an air change in a few hours, are used, the excessive pressures generated within the bin are likely to lead to fumigant loss and air ingress.

SIROCIRC™

A new recirculation method, called SIROCIRC™, has been developed. It employs the engineering principles of SIROFLO® and is designed for use in manifolded systems in which multiples of capped bins in any combination are fumigated concurrently (Fig. 2). Earlier methods of recirculation with manifolded systems (Fig. 1) are capable of achieving balanced airflows only in very symmetrical systems in which there are uniform back-pressures through each bin.

It seemed possible that the new method would allow for a degree of leakiness in addition to achieving a balanced flow through multiples of bins. To examine this

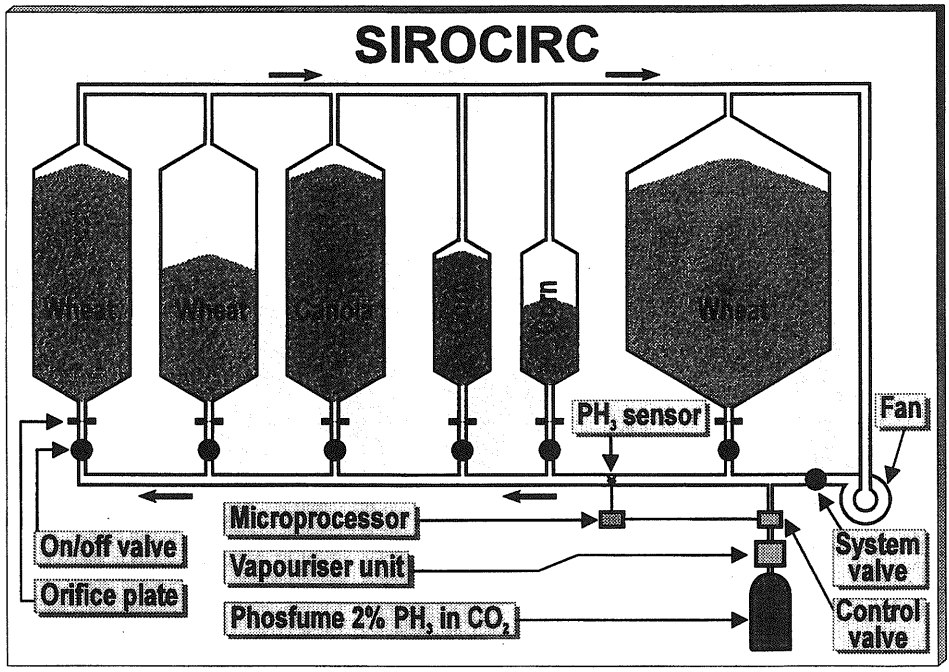


Fig. 2. SIROCIRC™, an active fumigation system, applies a balanced air flow to multiple bins manifolded together. Bins are not constrained by size, shape, commodity or bin-fill ratio.

possibility, a number of trials were carried out in three 50-t riveted-steel bins, purchased as gastight units, in which a pattern of leaks was established and the effects evaluated.

MATERIALS AND METHODS

During these trials temperature and pressure were monitored automatically at a number of locations within each of the bins and, in addition, ambient temperature together with wind velocity and direction were recorded. In the latter part of these trials, a PH_3 concentration was established and its decay rate monitored using a gas chromatographic method with an automated sampling procedure which took samples from all points within the grain of each bin and compared the response of a flame photometric detector to those with the response to samples drawn from a cylinder containing a reference PH_3 concentration. The detector responses for PH_3 were stored electronically and graphically on a chart recorder, and the electronic records were subsequently extracted into a Microsoft Excel spreadsheet.

For the most part, the input of PH_3 into the fumigation system was controlled using a newly-developed micro-processor control unit. The aim of these trials was both to examine how effectively a concentration could be maintained throughout these bins with different patterns of leaks and to examine the distribution of these concentrations through the bins. Leakiness was established progressively by ranging from no leaks in any of the three bins to the worst pattern of leaks tested (a 148-mm diameter hole in the top plus a 100-mm hole in the bottom of each bin).

The concentration of PH_3 was monitored in the inlet duct, the head space and at three depths within each of the bins. In addition, in one of the bins, PH_3 concentration was monitored at several locations around the periphery about 150 mm in from the wall.

RESULTS

PH_3 concentrations in all trials were found to be evenly distributed throughout the bins. The results obtained for the worst pattern of leaks are shown in Figs. 3 and 4. The fluctuations observed at points within the bins mirrored the fluctuations in the inlet concentration. Comparing the concentration profiles for sampling points within the grain shows that the distribution throughout each of the bins was remarkably constant and, more importantly, that the leaks created had little or no effect on either the concentration or the distribution of the PH_3. The leaks did, however, affect the rate at which PH_3 was introduced into the system. Even with the worst-case situation, however, there was only 72.3% of the usage rate for SIROFLO®. The usage rate for other leak patterns decreased with the magnitude of the leaks; with no introduced leaks in the bins, it dropped to 9% of the SIROFLO® rate.

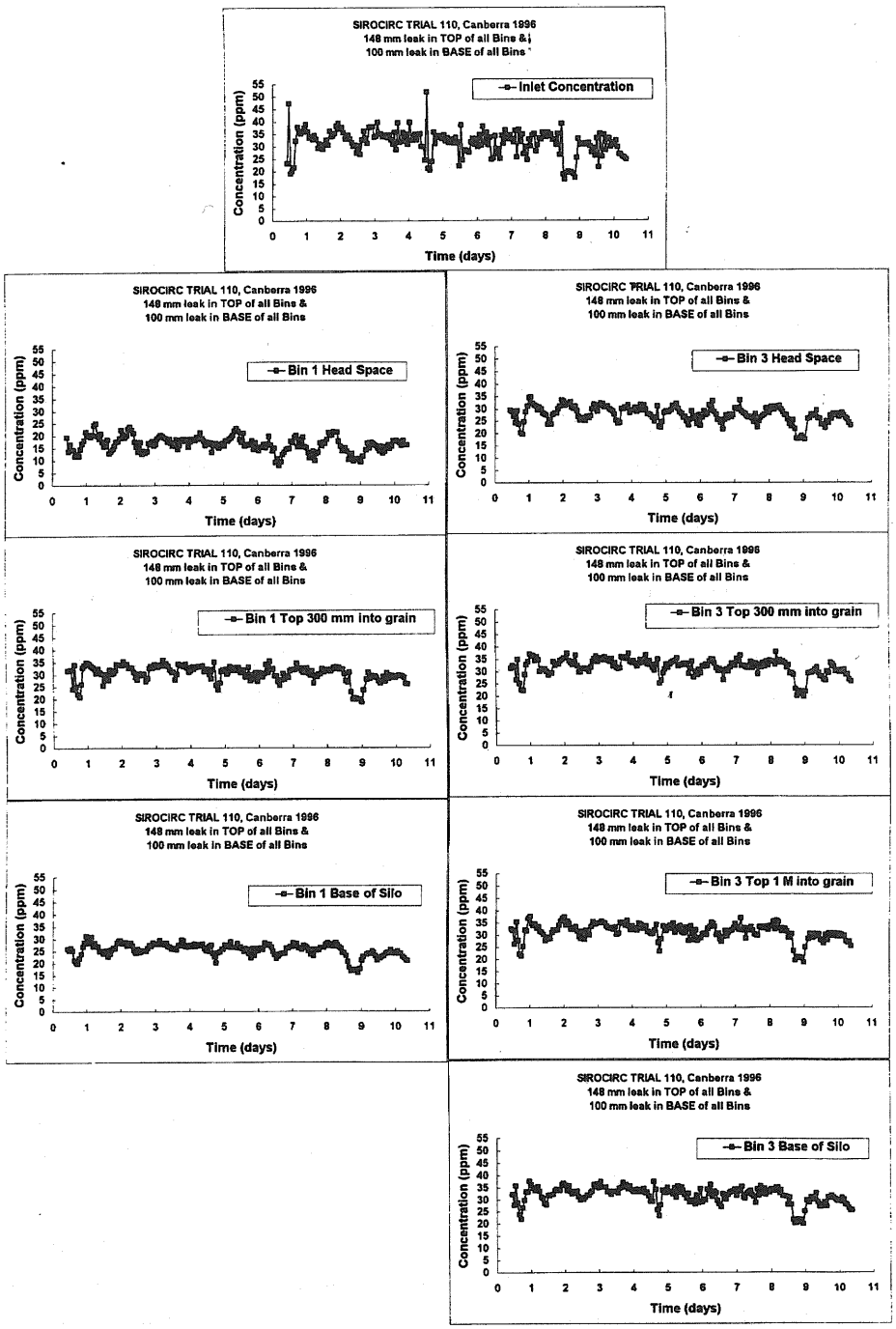


Fig. 3. PH_3 concentration profiles in bins 1 and 3, SIROCIRC™ Trial 110, Canberra, 1996.

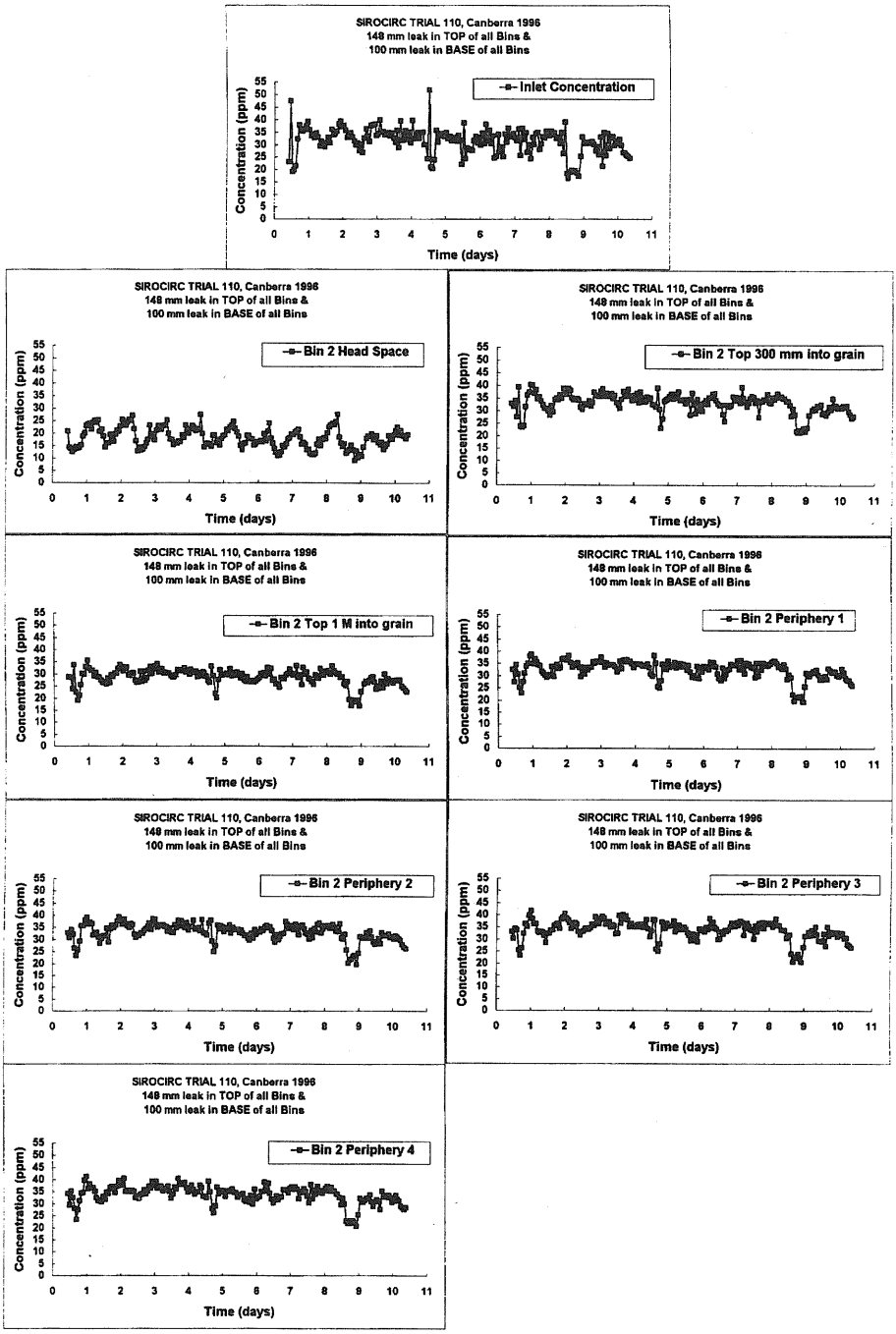


Fig. 4. PH_3 concentration profiles in bin 2, SIROCIRC™ Trial 110, Canberra, 1996.

DISCUSSION

SIROCIRC™ appears to provide an effective recirculation system in capped bins that fail to meet the gastightness standard. Compared with SIROFLO®, a substantial saving in gas can be achieved. In a 2,000-t vertical bin, the lowest usage rate achieved in these trials was equivalent to a cost of less than A$0.03/t. A further benefit of the system is that it substantially reduces gas emissions into the environment. It should be noted, however, that even with SIROFLO®, which is a flow-through system, emission levels are substantially below those currently set by any known environmental protection agency.

SIROCIRC™ has now been installed in a 25,000-t silo block at the GRAINCO grain export terminal at Gladstone, Queensland.

CONCLUSION

Because of the importance of the chimney effect on the effectiveness of fumigation and other gas processes, there is little doubt that active fumigation systems offer a higher probability of achieving efficacious fumigations in a wide range of storage structures. Moreover, this may be achieved in structures that are less than gastight with SIROFLO® and SIROCIRC™.

Static fumigation processes have an acceptable probability of success only in gastight structures, and then only in bunkers, sheds, squat bins and low-profile farm bins. In structures that are not gastight, and in all vertical silos, static fumigation can not be recommended as a means of achieving efficacious fumigation, and any such usage may significantly threaten the long-term usefulness of PH_3 by increasing selection for resistance.

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GASEOUS PHOSPHINE — A REVITALISED FUMIGANT

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ABSTRACT

Phosphine (PH_3)-generating formulations, commercially available for some fifty years, have made significant contributions to grain protection. A patented non-flammable PH_3 formulation, PHOSFUME™, both overcomes the hazard of flammability associated with quick release of gaseous PH_3 from metallic phosphide formulations and offers controlled dosing of PH_3. The PH_3 formulation is a liquefied gas mixture of PH_3 and carbon dioxide which satisfies a wide range of concentration and exposure time (Ct) options by being dispensed through innovative equipment. PHOSFUME™ has been adopted by the Australian bulk grain authorities, who have successfully treated in excess of 15 million t of grain using both traditional fumigation procedures in sealed storage and flow-through fumigation (SIROFLO®) in non-gastight structures. International interest is intense. A number of countries are actively planning to adopt this fumigation technique, which would enable them to expand the use of traditional PH_3 fumigation applications.

Because of its ability to instantly deliver high concentrations of PH_3, an investigation has been initiated to test the practicality of the use of PH_3 as a replacement for the ozone-depleting methyl bromide in disinfestation treatment. The investigation aimed at identifying and eliminating contaminants formed by PH_3 reacting with atmospheric oxygen. It uncovered new aspects of phosphorus chemistry and led to the development of the recently patented multi-component non-flammable compressed gas formulation which reduces costs for both the production and dispensing of the liquefied gas mixture. This investigation supports the use of gaseous PH_3 as a revitalised fumigant.

INTRODUCTION

Although the use of residual pesticides has resulted in a high standard of insect disinfestation, international consumers now demand that stored products be both insect- and residue-free. This is resulting in the replacement of liquid insecticide grain protectants by fumigant gases. Gaseous phosphine (PH_3), with its proven track record, low cost and residue levels of less than 1 ppb (Winks *et al.*, 1995), is an ideal fumigant, except that it is highly flammable. BOC Gases has patented a non-flammable PH_3 mixture, PHOSFUME/

ECO_2Fume. PHOSFUME™ is marketed as an alternative to traditional problematic insect control methods. Grain protectants pose a residue problem. Metallic phosphide tablets pose problems of operator exposure and safe disposal. And methyl bromide is an ozone depletor.

The safe, accurate and controlled metering of the universal grain fumigant, PH_3, has revolutionised stored-product disinfestation. PH_3 fumigation is a proven cost-effective alternative to residual pesticides. PH_3 is, however, an extremely flammable gas, and existing commercial metallic phosphide formulations do not eliminate this hazard. PHOSFUME™ is patented (US Patent No: 4,889,708) and its fumigant non-flammable gas mixture of 2% w/w PH_3 in carbon dioxide (CO_2), marketed by the international BOC Group, is easily, accurately and safely dispensed into all types of grain storage by using gastight pipelines.

BOC Gases has supplied some 600 t of PHOSFUME™ to date. It has been used to fumigate in excess of 20 million t of grain. The cost of a 21-d fumigation with PHOSFUME™ (0.3 g PH_3/t) is 15 US¢/t. A major use of PHOSFUME™ is in conjunction with SIROFLO® in unsealed storages. The PHOSFUME™/SIROFLO® combination can be applied directly to grain in any storage type, and it obviates the need for "turning" the grain for spraying with insecticide. The cost of turning is dependent upon storage type and the capacity of the machinery, but it can be as high as US$3.50/t. A hidden cost is the need for empty storage to receive the grain.

TRADITIONAL PHOSPHINE GENERATING PRODUCTS

PH_3 generated *in situ* from metallic phosphide formulations has made a significant contribution to grain hygiene for over 60 years; the original patent was issued in 1934.

PH_3 is an extremely flammable gas with explosive limits of 1.6–100% in air, and existing commercial formulations generate gas mixtures with a PH_3 composition which is usually in excess of 50% PH_3. The phosphide formulations are designed for slow release of the PH_3 gas which is generated by reaction with atmospheric moisture. This slow release allows the PH_3 gas to diffuse into the commodity being fumigated, minimising the risk of an explosive mixture.

Occupational health and safety concerns about metallic phosphide formulations exist in two areas: the inhalation risk, associated with the formulation's generating PH_3 immediately on exposure to air, and the safe disposal of the "spent" residual powder, which contains ~2% unreacted phosphide.

GASEOUS PHOSPHINE

The predominant component of the smell of acetylene is PH_3. Historically, industrial gas companies have produced PH_3 as an impurity (~400 ppm) in acetylene, with some 100 t of PH_3 generated annually. It is possible to fumigate with industrial, PH_3-containing acetylene even at concentrations below the explosive limit of 2.5% acetylene.

In more recent years, electronic-grade PH_3 (99.9999% pure), together with silane,

diborane, arsine, stibine, etc., has been used in the manufacture of silicon wafers/integrated circuits.

While the necessary purity of fumigation-grade PH_3 is not as high as that of electronic-grade, some impurities are critical. Purification of the PH_3 gas, unlike that of metallic phosphide formulations, ensures that such pyrophoric contaminants as diphosphine (P_2H_4) and white phosphorus (P_4) are removed from the PH_3 gas prior to its being mixed with inert gas. In early investigations (Gallagher *et al.*, 1991), an analytical procedure based on extraction and ^{31}P NMR analysis techniques was developed. The exposure of PH_3 to atmospheric oxygen and moisture can result in the formation of an inert orange-yellow polymer plus phosphorus acids (Gallagher *et al.*, 1995). The reactivity of PH_3 disguises its reported natural abundance. It is the cause of the reported ignitions of marsh gas which result in the "will-o-the-wisp" flickering lights observed in marshlands at night (Toy, 1976); it evolves from sewage treatment (Devai *et al.*, 1988); and it has been detected in animals and humans at significant levels (Gassmann and Glindermann, 1993).

About twenty years ago (Ryan, 1976), BOC Gases approached the Gosford Postharvest Laboratories (a joint CSIRO/NSW Department of Agriculture research laboratory) to request consideration of gaseous PH_3 mixtures for fumigating fruit for the control of fruit flies. This project was resurrected in 1983 when BOC Gases supported the CSIRO Division of Entomology flow-through fumigation research project which culminated in the SIROFLO® process.

FLAMMABILITY

Extensive flammability testing has been conducted by BOC Gases and by WorkCover Australia (Londonderry Occupational Safety Centre). The systems studied included PH_3–CO_2, PH_3–N_2 and PH_3–CO_2–N_2. This research resulted in two patent applications (Ryan and Latif, 1989; Ryan and Nguyen, 1995 (provisional application)), one for a non-flammable liquid mixture of PH_3 and CO_2 (2 w/w% PH_3/CO_2) and one for a non-flammable gaseous mixture of PH_3, CO_2 and N_2 (2.4 v/v% PH_3–60% CO_2–N_2).

This research showed that the PH_3/N_2 system could not contain more than 2.0% v/v PH_3 in N_2. It would thus be necessary to replace one cylinder of the existing 2.6% v/v PH_3 in liquid CO_2 mixture (PHOSFUME™) with approximately three cylinders of the 2.0% v/v PH_3 in N_2 mixture; i.e. a ~300% increase in the number of cylinders would be required. The PH_3/60% CO_2/40% N_2 system could contain 2.5% v/v PH_3, and with a cylinder filled to 163 bar it would be necessary to replace two cylinders of the existing 2.6% v/v PH_3 in liquid CO_2 mixture (PHOSFUME™) with approximately three cylinders of this mixture; i.e. a ~50% increase in the number of cylinders would be required.

HAZARD RANGE

The estimate of the hazard range for non-flammable PH_3 mixtures of 200 ppm, based on the NIOSH/OSHA IDLH (**I**mmediately **D**angerous to **L**ife or **H**ealth) concentrations, a

range within which a worker could not escape without symptoms of impairment or irreversible effects, was 20 m or less from the release point under all foreseeable circumstances (Hill, 1988).

APPLICATIONS

Structures/flour mills

Fumigation Services & Supplies carried out fumigations using PH_3, CO_2 and heat to fumigate flour mills (Mueller, 1994). This technique, which requires quick release of PH_3 (~100 ppm), has achieved good results. Concerns about corrosion of electrical circuits, since PH_3 reacts with copper, could be overcome.

Grainco Queensland developed innovative techniques for the fumigation of wheat in sealed storages (Ryan, 1992). In concrete/steel vertical silos and bunker/pad storages, the Grainco technique ensured quick release of PH_3, achieved peak PH_3 concentration some four times that resulting from metallic phosphide formulations, ensured rapid distribution and resulted in entomologically effective concentrations. Fumigations at dosages as low as 0.3 g/m^3 PH_3 were successfully performed.

Recirculation fumigation

The Western Australian Cooperative Bulk Handling (WACBH) authority used PHOSFUME™ at their ~1.5 million-t capacity Kwinana Grain Terminal. WACBH injected PHOSFUME™ into the forced air recirculation system of their 2,200-t vertical storage. A capillary tube restrictor ensured that the PHOSFUME™ was dispensed at a uniform rate over the ~5 h required for the air blower to change one complete volume. The 2-d saving in time achieved by using PHOSFUME™ is critical at a seaboard terminal.

SIROFLO®

Fumigants have been used worldwide for many years, but to be effective they should be carried out in hermetically sealed (gastight) storages. The pressure standard for gas-tightness requires the decay of externally applied pressure from 500 Pa (50 mm water gauge) to 250 Pa (25 mm water gauge) in filled storages in not under 5 min (SCA Technical Report, 1980).

An exception to the requirement for appropriately sealed storage for gaseous grain fumigation is the CSIRO flow-through SIROFLO® fumigation technique. The CSIRO Division of Entomology patented SIROFLO®, the flow-through fumigation technique (Winks, 1986). It is a positive-pressure continuous-application technique designed for structures that are not gastight but can be effectively sealed in critical areas. It is based on the continuous introduction of PH_3 (~30 ppm) from a PH_3 source, such as cylinders of PHOSFUME™, into an air stream which provides positive pressure within the storage. This positive pressure and the continuous flow ensure SIROFLO®'s independence from either leaks or the influence of high wind, and together they maintain a minimum PH_3 concentration over the fumigation exposure period (~4 weeks).

Levels of PH_3 in the workspace environment are less than the TLV (0.3 ppm) because of the low levels specified by the SIROFLO® technique. In addition to providing improved safety, SIROFLO® controls PH_3-resistant strains which succumb to the gas provided that the exposure periods are long enough (Winks and Ryan, 1990).

In the 1995/96 grain season, it is expected that over 10 million t of grain will be treated with SIROFLO®. The capital cost of SIROFLO® has been found to range between US$1/t for vertical silos and US$3/t for horizontal storage. Fumigation costs for large storages vary from US$0.20 for vertical silos to US$0.80 for horizontal storage. Although the horizontal sheds have higher treatment costs, their capital cost is about half that of vertical silos; e.g. a 7,000-t horizontal storage costs US$300,000 (~US$40/t). A major cost of large vertical silos is the grain-handling equipment.

Continuous Dose

This technique using PHOSFUME™ was initially described in a UK report (Bell *et al.*, 1991) as a "continuous flow system". The difficulty of controlling gas flow, experienced in the UK study, can be overcome using LoDOSE™ regulators. The requirement for a low-cost fumigation method, with "nil electrics" equipment, makes this technique attractive for on-farm fumigation.

CONCLUSION

PHOSFUME™ provides a controlled PH_3 source that allows concentrations to be adjusted during a fumigation to compensate for unforeseen air ingress. This adds an order of sophistication to grain fumigation. Combined with the non-flammability and Occupational Health and Safety benefits, PHOSFUME™ yields significant advantages over alternatives. The new LoDOSE™ regulator provides the long-term continuous flow that is required for techniques such as SIROFLO®.

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AN INVESTIGATION INTO SAFE DISPOSAL OF PHOSPHINE-GENERATING FORMULATIONS

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ABSTRACT

Recommended methods for disposal of phosphine (PH_3) formulations require immersion, combustion or burial. All can pose safety hazards to workers. Although burial has been recommended, no clear operating procedures have been defined.

This investigation examined the efficacy and occupational safety of the burial of PH_3-generating formulations. Partially spent and unspent blankets, pellets and tablets were buried in soil at two depths. PH_3 levels generated by the buried formulations were monitored both within and immediately above the burial site. Above the burial site, PH_3 levels fell below the 0.3 ppm Threshold Limit Value (TLV) within 25 d.

INTRODUCTION

Aluminium phosphide (AlP) formulations are used to produce phosphine (PH_3) gas for fumigation of stored products. The AlP reacts with water vapour in the air to generate PH_3 gas, leaving a residue of aluminium hydroxide ($Al(OH)_3$) powder. If, for any reason, the fumigation is interrupted before the reaction of the AlP is complete, fumigators are faced with occupational health and safety problems in disposing of the partially spent formulation. Even when AlP is apparently fully reacted (or spent), care is required when disposing of the residue (AFHB/ACIAR, 1994; Friendship, 1989). Several authors have noted that up to 5% of the AlP can remain as unreacted material occluded in fully spent $Al(OH)_3$ residue (Banks, 1991; Rosebrook, 1972; Winks, 1970).

Disposal directions are usually given by manufacturers, either on the label or in product manuals (Anon., undated; Anon., 1988). However the instructions vary considerably with

respect to both content and detail. The methods fall into two categories: dry methods involve either burial or incineration and wet methods deactivate the AlP or $Al(OH)_3$ with a detergent in water mixture.

In general, disposal of an unspent AlP formulation requires that it be allowed to stand in the open (protected from the rain) until the material is inactive and then treated with detergent in water and/or buried. Unfortunately, there are no guidelines indicating either when the formulation can be safely treated with detergent in water or the relative quantities that can be safely handled and treated. More importantly, it is neither safe nor practical to allow a formulation to generate PH_3 in the open without taking appropriate precautions concerning worker safety and environmental protection.

A range of AlP PH_3-generating formulations from several manufacturers had been accumulating at the Stored Grain Research Laboratory since the mid 1970's. The need to dispose of this material presented an opportunity to test burial as a method of disposal. Burial of spent residues is one normally accepted method of disposal, and it was considered that, provided certain conditions are followed, this method should provide a worker-safe and environmentally responsible method for disposal of unspent formulations. In this test unspent AlP was specifically included in order to obtain maximum PH_3 levels and observe their decay to safe levels.

MATERIALS AND METHODS

This investigation was undertaken in New South Wales at the GrainCorp, Parkes sub terminal. A secure location was selected within a bunker site (33°, 10 min, 53 sec S; 148°, 12 min, 54 sec E), where two rows of 15 holes approximately 1 m apart were dug using a mechanical posthole digger fitted with a 300 mm diameter auger. One row of holes was dug to a depth of 500 mm, the other to 250 mm.

The following formulations were placed into these holes: blankets (either rolled up as they were packed in their containers or unrolled), pellets or tablets (Table 1). The formulations were covered with approximately 50 mm soil; then a bottomless 460-ml chamber connected to the surface by a nylon tube (3 mm i.d.) was placed on the soil, providing an airspace from which PH_3 generated by the formulations could be sampled. The chambers were constructed from plastic bottles perforated by six holes (approximately 4 mm diameter), four at the sides and two at the top (Fig. 1). The space around and above the chambers was then filled with soil excavated from the hole, and excess soil was heaped over the hole at ground level. The soil replaced was not tamped down or compacted in any way. Controls, in which no AlP formulations had been buried, were prepared in the same manner.

Underground PH_3 levels, generated by the reaction of the buried AlP formulations, were monitored by sampling gas concentrations through the nylon tubing attached to holes at the tops of the chambers. PH_3 levels were also monitored at two locations (Fig. 2): immediately above the burial site at ground level (at 14 points between the two "columns" of holes and midway between the 15 rows of holes) and above ground level (at

TABLE 1
Depth and contents of two rows of holes on a 2 × 15 m grid

Hole number	Row F (0.5 m)	Row B (0.25 m)
1	Celphos blanket	Control
2	Quickphos blanket	Partially spent pellets
3	6 × 100 g Quickphos tablets	Partially spent tablets
4	2 × 1667 Celphide pellets	Mixed pellets
5	Celphos blanket	Quickphos tablets
6	6 × small Quickphos blankets	Celphos blanket
7	5 × 100 g Quickphos tablets	Small blankets
8	2 × 1667 Phostoxin pellets	4 × 100 g Quickphos
9	Celphos blanket	Celphide pellets approx. 2 flasks
10	Quickphos blanket	Quickphos blanket
11	Mixed pellets approx 2 flasks	6 × small Quickphos blankets
12	5 × small Quickphos tablets	Pellets
13	Partially spent blanket	Pellets
14	Partially spent blanket	Quickphos blanket
15	Control	Celphos blanket

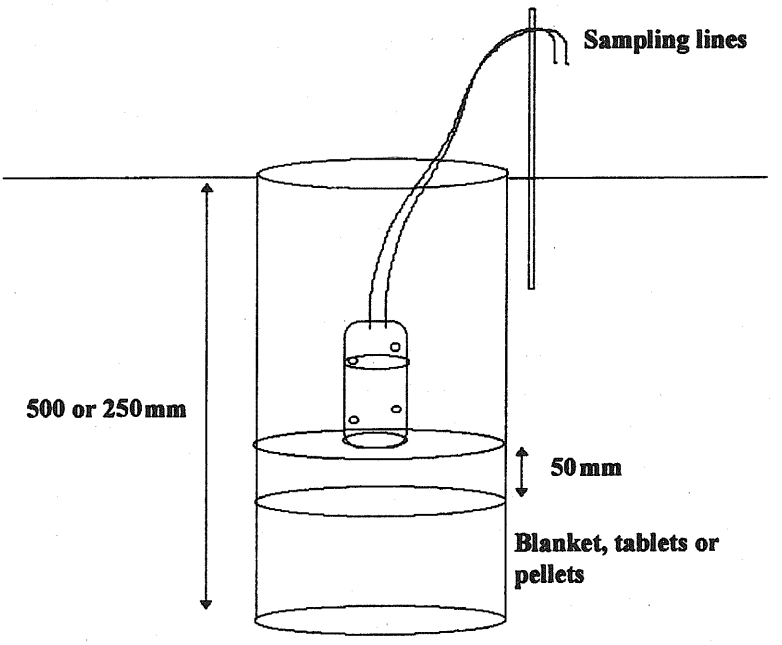


Fig. 1. Scheme of disposal hole and sampling layout.

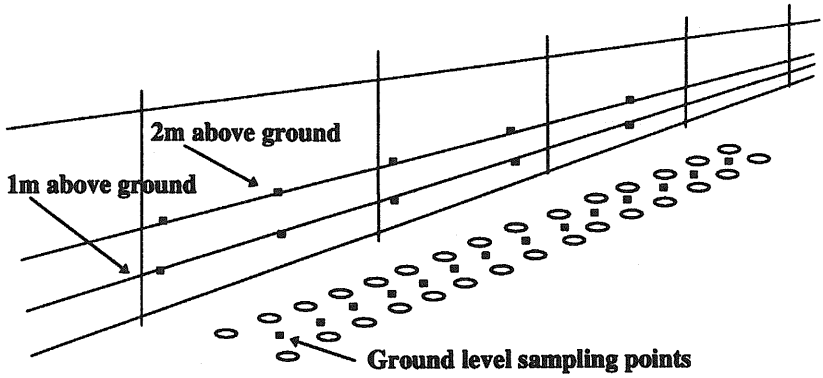


Fig. 2. Schematic of above ground sampling positions relative to disposal holes.

heights of 1 and 2 m along the border of the site in a position generally downwind and 1–2 m from the burial site).

PH_3 levels inside the disposal holes were determined using a calibrated gas analyser (BEDFONT™ Phosphine Monitor, fitted with a CITICEL® electrochemical sensor). Each chamber was sampled until a maximum reading was obtained. The instrument was allowed to return to zero before the next sample was measured. Above-ground sampling was carried out with a workspace monitor (Amahsco GEM) also fitted with a CITICEL® electrochemical sensor. Both instruments were calibrated against reference standards.

Samples of the soil in which the AlP formulations were buried were taken for classification.

RESULTS

The mean (n = 14) ground level PH_3 concentrations (measured at different times after the AlP formulations were buried), compared with mean readings taken at 1 m (n = 5) and 2 m (n = 5) above ground, are shown in Fig. 3. Windspeed on the day of measurement and total rainfall over the 3 d preceding measurement are also shown.

The mean (n = 14) below-ground PH_3 levels measured in the chambers at various times after burial are shown in Fig. 4, with the data for the formulations buried at 500 and 250 mm shown separately. The upper limit of detection in the Bedfont detector used for the measurements in this work was 2,000 ppm, so concentrations above this value could not be quantified.

For the first 2 weeks after burial, high (>2,000 ppm) concentrations of PH_3 were observed in the control holes, indicating that PH_3 can diffuse laterally at least 1 m through the soil. For this reason, comparisons between the different formulations are not possible, and samples will need to be buried much farther apart in any future work.

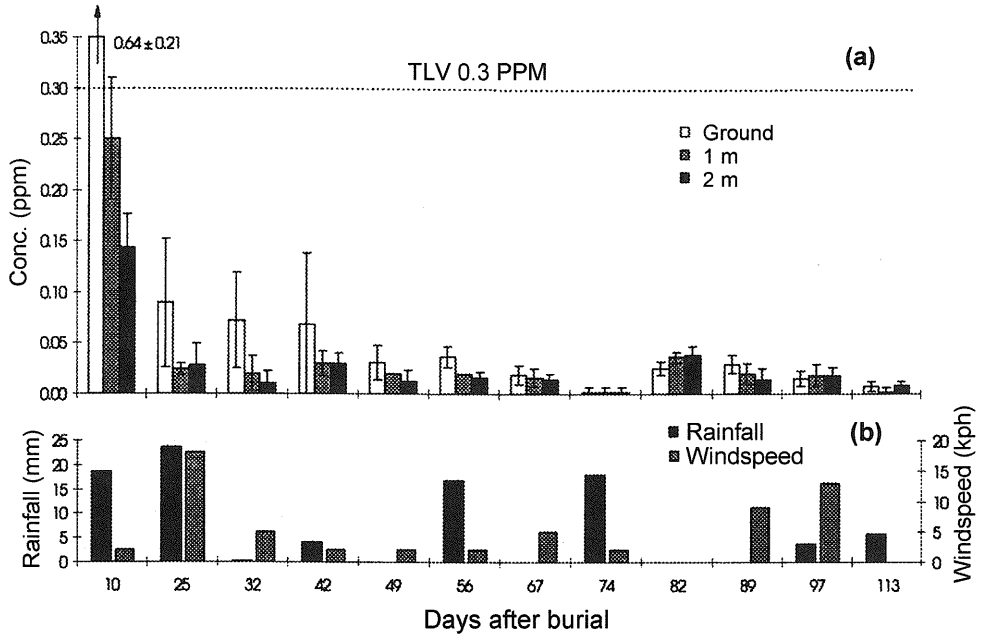


Fig. 3. Mean phosphine concentrations (±SD) above the test site (a) compared with windspeed on day of measurement and total rainfall for the previous 3 days (b).

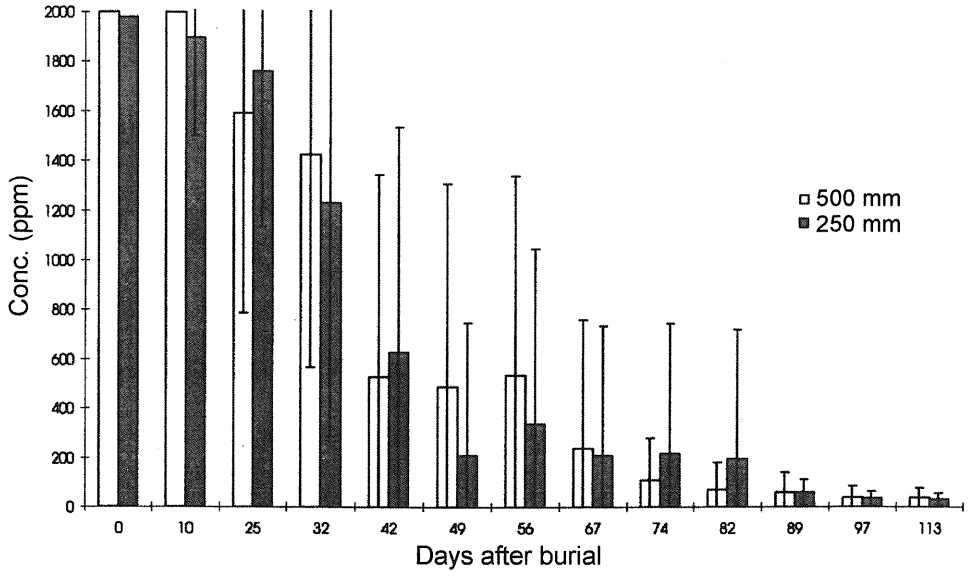


Fig. 4. Mean below-ground phosphine concentrations (±SD), sampled from formulations buried 250 mm and 500 mm deep.

The soil was classified as either a Dermosol (Isbell, 1994) or a Euchrozem Gn 4.12 (Northcote, 1979). These are clay soils, strongly structured and moderately deep red to reddish brown. In eastern Australia they are usually used for cropping or intensive grazing on improved pastures (Hubble *et al.*, 1983; Northcote *et al.*, 1974).

DISCUSSION

PH_3 levels measured above the burial site during the first 10 d after burial exceeded the 0.3 ppm TLV. Thereafter, no PH_3 was detected above the TLV (0.3 ppm) from ground level up to 2 m above ground level at any point across the site.

It had been expected that PH_3 levels would be affected by weather conditions at, or immediately prior to, time of measurement. In fact, these effects were not consistent. Only in some instances was there either an apparent decrease in PH_3 levels with increased windspeed or higher PH_3 levels after rain during the previous 3 d.

PH_3 levels measured below ground also appeared to be largely independent of rainfall during the observation period. From Fig. 5 it can be seen that PH_3 levels below ground decayed exponentially and could therefore be expected to reach safe levels after about 6 months. This is not to say that the same result can be expected with burial in different soil types. In the present case the soil had a high clay content; although friable and porous when dry, it could be expected to become less porous when moist, which would restrict

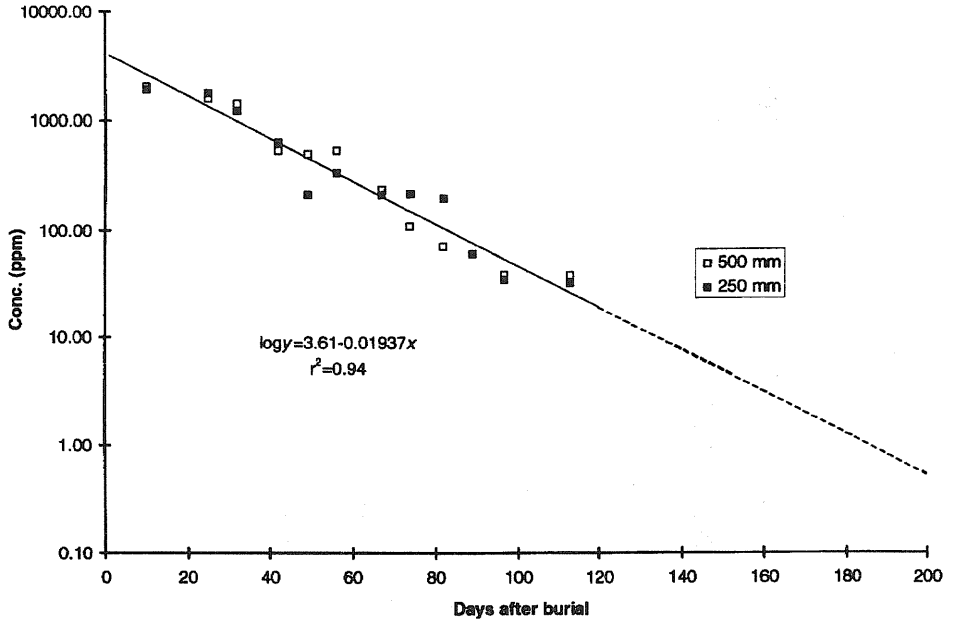


Fig. 5. Mean below-ground phosphine concentrations (ppm, log scale) showing rate of decay during the test period.

the movement of both gas and water. Disposal in sandy soils may produce different results; it would still be reasonable to expect an exponential decay but this would perhaps proceed at a faster rate.

This study is continuing, with monitoring to determine the length of time which must be allowed to pass before it is safe to unearth the buried formulations. Any future studies should include measurements of soil moisture before and during the burial period and the effect of different soil types, as well as possible effects of rain in higher rainfall areas.

CONCLUSION

If spent residues are buried as dry powder (AFHB/ACIAR, 1994), it is very likely that no PH_3 above 0.3 ppm (v/v) will be released into the environment.

In the case of unspent or partially spent AlP formulations, the work reported here indicates that PH_3 emissions immediately above the burial site remained above the TLV on day 10 but had fallen below it by day 25. Burial of these formulations resulted in lower localised levels of PH_3 in the environment than could be expected had the formulation been allowed to decompose in air. However, where this form of disposal is used it is esential to monitor PH_3 emissions to ensure that the site is safe before anyone enters the workspace. Both soil type and initial soil moisture content may also influence the rate at which PH_3-generating formulations decompose. For this reason, access to such sites needs to be controlled in accordance with local regulations.

ACKNOWLEDGEMENTS

We wish to thank Rex Veal and the staff at the GrainCorp Parkes sub terminal for their interest and assistance in preparing the site for this work. We are also grateful to Steven Torrens, of the Parkes Post Office, who provided the meteorological data. Stephen Pratt (SGRL) calibrated the sensors used to monitor PH_3 levels.

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ECONOMIC FEASIBILITY OF PHOSPHINE RECIRCULATION SYSTEMS IN SEALED SILOS AT US GRAIN ELEVATORS

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ABSTRACT

A gas recirculation, or closed loop fumigation (CLF), system for phosphine (PH_3) fumigation using a low volume blower/piping system per tank was patented by James Cook of Houston, TX, in 1980. In 1991, two large grain storage tanks (2,000–10,000 t/tank) were manifolded to one blower in order to make CLF more effective. Research on the manifolded PH_3 recirculation (MPR) system design concept was expanded in 1995, through an EPA grant, to include concrete silos. In an effort to make concrete CLF systems as cost effective as those for 2,000–10,000-t steel tanks, MPR designs for three to seventeen silos manifolded to a single blower (forming a 1,500 to 8,000-t fumigation unit) were developed in 1995.

Economic data in cost/unit volume for sealing, plumbing and blower equipment vs. fumigated storage volume for five concrete silo installations are compared to those for representative steel tank MPR systems. Installation and operating costs for representative steel tank MPR systems are reviewed.

BACKGROUND

"Closed Loop Fumigation" (CLF) was originally known as a recirculation process developed for methyl bromide (MB) fumigation in the US and other major grain producing areas. Reports cite recirculation of MB as early as the 1920's. James S. Cook of Houston, Texas, developed the J-SYSTEM®, a low airflow fumigation recirculation process, for use with phosphine (PH_3). Cook received US Patent No. 4,200,657 on this process on April 29, 1980.

The CLF system was designed to improve fumigant distribution and to reduce both worker exposure and the incidence of fumigation failure. It involves the use of a simple, low pressure, low volume centrifugal blower which draws the fumigant/air mixture from the head space of a sealed structure and pushes the gas into the base of the structure, forcing it to flow upward through the grain and back to the head space in a closed loop cycle.

FUMIGATION SAVINGS

CLF systems minimize worker exposure to fumigants. They also increase fumigation efficacy through better gas distribution. The most measurable economic benefits of MPR systems are that they use lower amounts of fumigant and also reduce labor and grain turning expenses.

The most common method of fumigating grain stored in concrete silos is by distributing PH_3 pellets into the grain with automatic pellet dispensers while the grain is being turned (moved from one silo to another). However, unless there are other sound management reasons for turning the grain, the extra electricity needed to turn grain, the additional shrink (0.25–0.50% per turning cycle) and the labor to monitor turning must all be considered as part of the fumigation costs.

DEMONSTRATION PROJECT

In the fall of 1995, Oklahoma State University initiated a demonstrational project focused on PH_3 recirculation systems. In this project, 17 recirculation systems were installed in participating country elevators for use during the 1996 grain handling season. Five of these demonstrational units involved the installation of MPR systems in units of sealed concrete silos. Oklahoma State University supplied almost all of the MPR piping systems, including blowers, for all cooperating elevators. Each elevator manager was responsible for the construction/wiring costs of installing the system. The size (total amount of manifolded silo space) of the MPR systems ranged from 1,590 to 7,950 t. Based on actual material cost and projected installation cost, the total installation cost, projected to range from US$1,481 to US$1,895, averaged US$1,768.

The MPR system represents the first practical system for fumigating concrete grain silos without turning the grain. Elimination of grain turning can generate substantial savings in fumigation expense. The use of MPR systems also allows managers to reduce the amount of PH_3 used while still obtaining results equal to, or better than, those with conventional methods.

This continuous recirculation, which distributes the gas more uniformly throughout the grain mass, generally allows PH_3 use to be reduced by 25% or more. A summary of the potential cost savings of MPR systems is shown in Table 1.

CONSTRUCTION COSTS

Construction costs of the five representative concrete silo MPR systems are summarized in Table 2. These installations involve manifolding 3–17 concrete silos together to form 1,590- to 7,950-t sealed fumigation units. These systems used 0.5–1.5 HP low-volume centrifugal blowers to recirculate the gas from the headspace of one silo into the base of each silo. All outside vents and silo openings were sealed while the existing inter-silo vents were maintained, except where MPR fumigated silos were intermixed with non-fumigated silos. In that case, both exterior and interior vents on the fumigated silos

TABLE 1
Cost reductions obtained by using Manifolded Phosphine Recirculation (MPR) versus automatic dispenser and turning (conventional)

Component	System	
	Conventional (US$/t)	MPR (US$/t)
Labor (sealing)	0.0755	0.0755
Labor (turning)	0.0377	–
Fumigant[1]	0.1877	0.1405
Supplies and overhead	0.1132	0.1132
Fumigation cost	0.4152	0.3303
Grain turning electricity[2]	0.1510	–
Grain turning shrink	0.2831	–
Total cost, fumigation and turning	0.8492	0.3303
Projected savings		0.5190

[1]Based on a 25% reduction in the amount of phosphine needed for the MPR system.
[2]Electricity for operation of the MPR is ignored since it is projected at less than US¢0.01/t.

TABLE 2
Average installation costs of MPR systems in Oklahoma country elevators[1]

Component	Cost (US$)
Blowers (1 HP each)	594
Ducting materials (PVC pipe)	392
Hardware	184
Installation labor	598
Misc. hardware (rubber boots, bolts, screws, etc.)	428
Total installation costs	1,768
Total installation cost per t	0.396

[1]Does not include a minor amount of elevator personnel labor.

were sealed. The inter-silo vents allowed a group of silos to function as a single large fumigation unit, substantially reducing the CLF system cost per t. As the table indicates, the total installation cost was US$1,768, or approximately US$0.40 per t. The range of construction costs for the three separated MPR silos was projected as US$0.23 to US$0.91 per t.

ECONOMIC ANALYSIS

The most important aspect of any new technology is how the adoption of the technology will affect a grain-handling firm's profitability. Three common measures of the attractiveness of an investment are the payback period (PP), the net present value (NPV) and the internal rate of return (IRR).

The PP measures the time required for the savings in annual fumigation costs to equal the original cost, without regard to either any interest costs or any alternative uses of the investment funds. The PP indicates how long it will take to recover the invested funds, but it does not measure overall profitability.

The NPV reflects net savings over the life of the project. These savings balance the installation cost against savings over the 20-year life of the system and include an inferred interest charge on the original cost. Since NPV measures the total net savings over the life of the system, a positive NPV means the CLF system should be an attractive investment.

The IRR measures the financial return, just as the interest rate measures the return on funds in a bank account. The IRR converts the annual savings in fumigation costs into a rate of return on the original investment. As long as an elevator manager can borrow funds at a lower rate than the IRR on the fumigation system, the MPR is a good investment. Results of the investment analysis for the MPR system are summarized in Table 3. The comparative measures in tonnes for a PH_3 recirculation system in a steel grain bin are provided for comparison.

TABLE 3
Summary of costs and benefits of closed loop fumigation systems

Component	Probe and tarp in corrugated steel bin (5,300 t)	MPR systems (5,700 t)	
		Ignoring grain turning savings	One grain turning eliminated
Total construction cost (US$)	1,358	1,768	1,768
Construction costs per t (US$)	0.2563	0.3099	0.3099
Cost reduction per t (US$)	0.0566	0.0566	0.0566
Payback period (years)	4.5	5.5	0.6
Net present value at 8% interest (US$/t)	0.30	0.2460	4.79
Net present value at 8% interest (US$/t) over 20-year life	1,587	1,404	27,310
Internal Rate of Return (%)	21.65	17.55	167.48

As Table 3 indicates, the MPR system provides an investment return similar to that of conventional recirculation systems installed in steel bins. A grain elevator manager can expect to recover the costs of a MPR system in less than 6 years, even if grain turning cannot be completely eliminated. The present value of the MPR investment to the elevator (net expenses of US$1,403) is equivalent to a 17.6% return on investment. Firms which turn grain from one silo to another for the sole purpose of fumigation can reap an exceptionally high return on their investment in MPR technology. When a grain turning can be eliminated, the MPR system should more than pay for itself in a single fumigation.

CONCLUSIONS

Manifolded Phosphine Recirculation systems represent an improved technology for fumigating grain stored in sealed concrete silos. The initial results from the six demonstration projects in grain elevators located in Oklahoma in the USA indicate that MPR systems will allow managers to reduce the amount of PH_3 used by 25% without any loss in effectiveness. The MPR technology can be installed for a modest investment (approximately US$0.30/t). Analysis indicates that the MPR technology is thus an attractive investment. In situations where MPR systems can completely eliminate grain turning, managers can pay for a MPR system in under 1 year.

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CONTROL OF PESTS AND QUALITY ASPECTS IN COCOA BEANS AND HAZELNUTS AND DIFFUSION EXPERIMENTS IN COMPRESSED TOBACCO WITH CARBON DIOXIDE UNDER HIGH PRESSURE

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ABSTRACT

In the food processing industry, such raw agricultural products as cocoa beans, hazel nuts and tobacco require quick disinfestation prior to storage. The disinfestation method must not cause any decrease in quality or any build-up of chemical residues. Exposure to carbon dioxide (CO_2) under pressure of 20–40 bar for a few hours is a recently developed and effective control method for this purpose.

Experiments with caged pest insects (developmental stages and adults) of 12 species (*Lasioderma serricorne, Oryzaephilus surinamensis, O. mercator, Tribolium castaneum, T. confusum, Cryptolestes turcicus, Trogoderma granarium, Corcyra cephalonica, Ephestia elutella, E. cautella, Plodia interpunctella* and *Sitotroga cerealella*) were carried out on 1 t of bagged product in a 3-m^3 chamber. At about 10°C under 20 bar of CO_2 the lethal treatment period was slightly longer (3 h) than at 20°C. At 20°C and 30 and 37 bar, complete control was achieved within 1 h and within 20 min, respectively.

The components and possible alterations of aroma in cocoa beans and hazelnuts were tested, as were resistance to deterioration, triglyceride composition and crystallisation behaviour. The quality of the cocoa beans had not changed following these treatments. Similar results occurred with hazelnuts, excepting the tendency of treated ones to turn rancid earlier than untreated ones. In the centre of compressed tobacco, a slight delay in even distribution of the gas was observed.

INTRODUCTION

Both growing public awareness of insecticide residues in food and discussion of a ban on the use of methyl bromide encourage the search for alternative methods of pest control. The

necessity of finding ways to protect stored products is especially acute for such high value commodities as confectionary and sweets, both quantitatively and qualitatively. In addition to actual economic loss, another critical factor in the food processing industry is possible damage to the reputation of the manufacturer.

To address this problem, Stahl *et al.* (1985) and Stahl and Rau (1985) described a new process, using carbon dioxide (CO_2) under high pressure, for residue-free pest control. According to follow-up studies, the quality of the treated products was not affected detrimentally (Gerard *et al.*, 1988a, b, 1990). Furthermore, using CO_2 under high pressure has the added practical advantage of requiring extremely short lethal exposure times, ranging from minutes to only few hours (Reichmuth and Wohlgemuth, 1994; Prozell and Reichmuth, 1990, 1991).

MATERIALS AND METHODS

High pressure chamber

All experiments were conducted in a 3-m^3 high-pressure chamber (CARVEX) connected to a tank of liquid CO_2 placed on a balance (Fig. 1).

Prior to its injection into the chamber, the CO_2 was warmed in the regulator. Pressure regimes for the different experiments were adjusted as required (Table 1), and at the end of each exposure period the pressure was released within about 8 min.

The exposure time included the time needed to obtain the final pressure, the time held at constant pressure and the time needed to release the CO_2 (Fig. 2). The temperature in the chamber was recorded during exposure.

Insects

Experiments were undertaken using all developmental stages of a mixture of 12 insect species, all important pests in the confectionary and tobacco industries (Table 1).

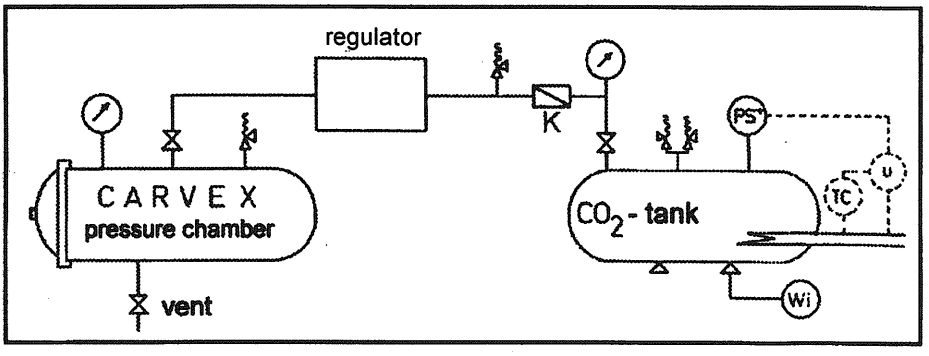


Fig. 1. Pressure chamber of about 3 m^3 designed to hold CO_2 at 20 and 37 bar, liquid CO_2 supply tank and regulator unit (Wi = balance, PS+, u, TC = instrumentation to adjust temperature and pressure in the tank, K = valve (after Gerard *et al.*, 1990).

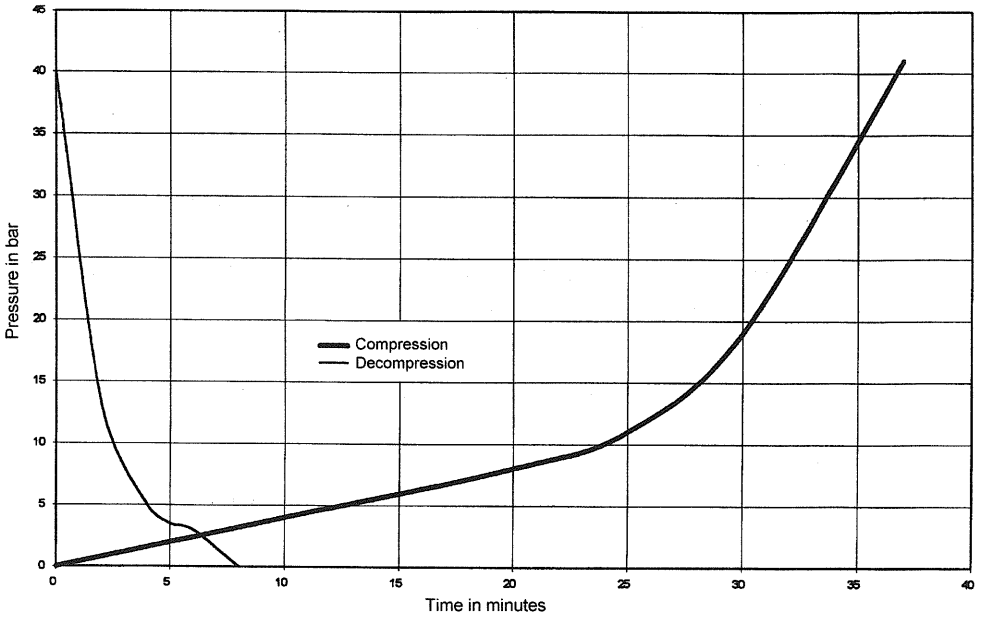


Fig. 2. Time required for compression and decompression in experiments at 37 bar; in other experiments, when the pressure was lower, the corresponding times for pressure build-up and decrease were shorter.

Adult insects of each of the following species: *O. mercator, C. turcicus, T. granarium, C. cephalonica, E. elutella, E. cautella, S. cerealella* and *P. interpunctella* were exposed together with the developmental stages. They were introduced in tubular, stainless-steel wire-mesh cages (length 10 cm, diameter 1 cm) closed with stoppers.

For the beetles *Oryzaephilus surinamensis*, *Tribolium castaneum*, *T. confusum* and *Lasioderma serricorne*, trials were carried out using eggs, young larvae, larvae, pupae and adults in separate cages.

At the beginning of each trial the chamber was loaded with cocoa beans and the cages were then placed among the cocoa beans in three areas: the front, middle and back of the chamber. The chamber was then closed and pressurized with CO_2. Tests were carried out at 10 and 20°C.

Following treatment, the pressure was released and the cages removed. The samples were then held at 26°C and 75% r.h. For the following 14 weeks, they were observed weekly for survivors.

Quality control of cocoa beans and hazelnuts

Several trials were performed to determine any possible changes in the quality of treated cocoa beans and hazelnuts. At all experimental pressures and exposure times, the composition and changes in several quality parameters were tested.

Aroma analysis was done by gas chromatography (Ziegleder, 1991). Organoleptic assessment of raw and roasted cocoa beans was carried out by a team of six experienced

persons and using chemical analysis. The aroma of hazelnuts was analysed by gas chromatography. Amadori compounds in raw cocoa (the preliminary stages of aroma) were analysed by gas chromatography (Ziegleder and Oberparleiter, 1996). The storability of hazelnuts was evaluated using an accelerated storability test at 35°C for 3 months. The induction time of extracted hazelnut oil was evaluated by means of the ranzimat test. Hexanal in hazelnuts was determined by headspace-gas chromatography. The gradient of cocoa butter, extracted from cocoa beans, was determined by the isotherme dsc method (Ziegleder, 1990). HPLC-trygliceride determination in oil extracted from both treated and untreated nuts was performed (Ziegleder *et al.*, 1996).

The rate of distribution of carbon dioxide within the commodity

A 1-m^3 tobacco bale (0.8 × 0.6 × 0.5 m) containing a stainless steel tube (3 mm diameter and 1 mm centre bore) (Ulrichs *et al.*, 1997b) was treated with CO_2 at 20 bar. The results of the diffusion measurements into the centre of the bale were recorded.

RESULTS

Insect mortality

All insects in the untreated control samples showed normal development. The results of CO_2 treatment of the insects under different pressures are presented in Fig. 3 and Table 1.

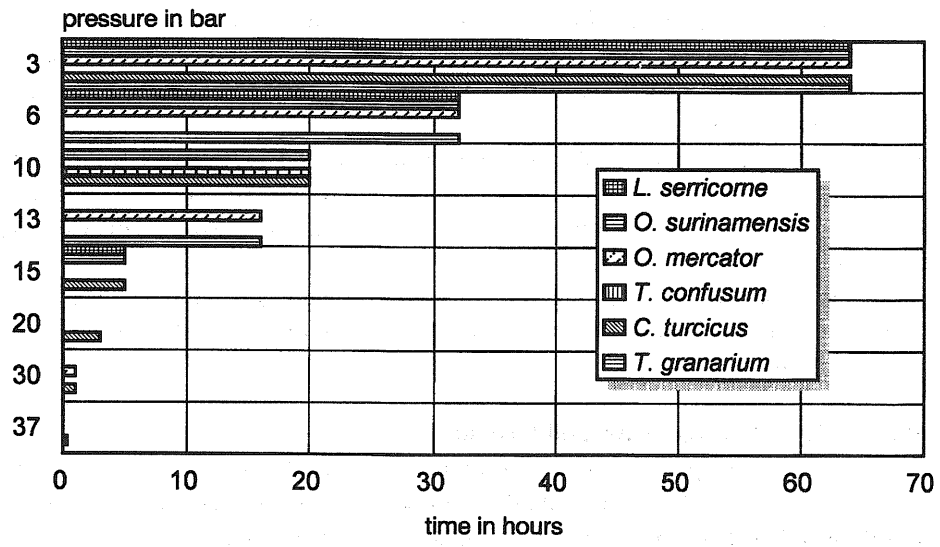


Fig. 3. CO_2 treatments at different pressures and exposure times, lethal to all stages of six stored-product beetles at 20°C.

TABLE 1
Results of CO_2 treatments for different exposure times at 10°C and 20°C of all developmental stages of 12 stored-product insects in a 3-m^3 pressure chamber

Species	Temperature (°C)	CO_2 pressure in bar (Exposure time in h)								
		3 (64)	6 (32)	10 (20)	13 (16)	15 (5)	20 (8)	20 (3)	30 (1)	37 (0.33)
L. serricorne	10	–	–	x	–	x	–	x	0	0
	20	x	x	–	0	–	0	0	0	0
O. surinamensis	10	–	–	x	–	x	–	x	0	0
	20	x	x	–	0	–	0	0	0	0
O. mercator	10	–	–	–	–	–	–	–	–	–
	20	x	x	–	x	–	0	0	x	0
T. castaneum	10	–	–	–	–	–	–	–	–	–
	20	0	0	–	0	–	0	0	0	0
T. confusum	10	–	–	x	–	0	–	0	0	0
	20	0	0	–	0	–	0	0	0	0
C. turcicus	10	–	–	x	–	x	–	x	x	x
	20	x	0	–	0	–	0	x	x	x
T. granarium	10	–	–	–	–	–	–	–	–	–
	20	x	x	–	x	–	0	0	0	0
C. cephalonica	10	–	–	0	–	0	–	0	0	0
	20	–	–	–	–	–	–	0	0	0
E. elutella	10	–	–	0	–	0	–	0	0	0
	20	0	0	–	0	–	0	0	0	0
E. cautella	10	–	–	0	–	0	–	0	0	0
	20	0	0	–	0	–	0	0	0	0
S. cerealella	10	–	–	–	–	–	–	–	–	–
	20	–	–	–	–	–	–	0	x	0
P. interpunctella	10	–	–	0	–	0	–	0	0	0
	20	0	0	–	0	–	0	0	0	0

x = survivors; 0 = no survivors; – = no experiment.

Treatments at 20°C

High pressure treatment at 37, 30 and 20 bar was carried out at 20°C. Treatments of 20 min at 37 bar, 1 h at 30 bar and 3 h at 20 bar all produced 100% mortality of all test insects except *Cryptolestes turcicus*. Also, at 30 bar and 1-h exposure *O. mercator* was not completely controlled.

High pressure treatment was carried out at 20°C for 8, 16, 32 and 64 h. After 16, 32 and 64 h at 13, 6 and 3 bar, respectively, complete mortality in all tests was achieved for the moths (*Corcyra cephalonica, Ephestia elutella, E. cautella* and *Plodia interpunctella*) and

the two *Tribolium* species (*T. castaneum* and *T. confusum*). At 8 h and 20 bar exposure, no survivors of the other beetle species were found.

C. turcicus survived only the 64-h treatment at 3 bar; *O. surinamensis* and *L. serricorne* were controlled at 16 h and 13 bar; and *Trogoderma granarium* and *O. mercator* survived 16 h at 13 bar. Despite delays in the hatching of the eggs, it was concluded that some eggs were not controlled.

Treatments at 10°C

High pressure treatment at 37, 30, 20, 15 and 10 bar was carried out at 10°C. At 37, 30, 20, 15 and 10 bar, and at 20 min, 1, 3, 5 and 20 h, respectively, 100% mortality of the moths was obtained. These pressures and exposures times were insufficient to produce 100% mortality of the test beetles.

L. serricorne and *O. surinamensis* failed to survive 30 and 37 bar at 1 h and 20 min, respectively, and survivors of *T. confusum* were found after treatment at 10 bar at 20 h. Only *C. turcicus* survived all of the tested pressures and exposure times.

Quality control

The quality criteria used for cocoa beans did not reveal any significant changes following the treatments. The treated hazelnuts had a tendency to turn rancid earlier than did the untreated ones. All the other quality criteria of hazelnuts also remained unchanged.

Rate of distribution of carbon dioxide

It was shown that the build-up of high CO_2 concentration in the centre of the tobacco bale did not correspond directly to the rapid increase in CO_2 concentration around the bale within the chamber. Shortly after starting pressurisation, an area of compressed air into which CO_2 did not penetrate remained in the centre of the tobacco bale. After 2.5 h the CO_2 content increased in this area as well.

DISCUSSION AND CONCLUSIONS

Ferguson and Hawkins (1949), Johnson and Quastel (1953) and Carpenter (1954) were the first to describe the toxic action of inert gases under increased pressure. They mentioned narcotic effects after treatment with these gases; presumably, then, the death of treated insects during treatment under high pressure is due to prolonged and intense narcosis. In addition, the destruction of cell membranes during decompression causes severe damage. The mortality results of the experiments presented here are similar to those reported by Prozell and Reichmuth (1990, 1991) on *S. granarius*.

Post-treatment quality analyses of cocoa beans and hazelnuts showed that treated hazelnuts have a tendency to turn rancid earlier than untreated ones; cocoa beans showed no quality change at all.

It is difficult to implement in practice the laboratory findings with CO_2 and high pressure because the laboratory results were achieved in small chambers of 150 ml, where

there was a short time for build-up of pressure and decompression and the temperatures were fixed. Findings by Ulrichs (1994) and Ulrichs *et al.* (1997a) indicated the importance of the decompression speed on mortality, and this was also mentioned by Nakakita and Kawashima (1994).

The findings on the rate of distribution of the CO_2 presented here indicate that it depends on the nature and mass of the treated product. When the air was initially compressed, the centre contained low CO_2 concentrations insufficient to control the insect pests (Prozell and Reichmuth, 1991). Later the CO_2 concentration increased, and four phases of penetration which follow the classical transport phenomena (Bird *et al.*, 1960) can be described: the filling of the substrate pores, followed by the balancing of the pressure; the diffusion of CO_2 into the interstitial spaces, balancing out differences in gas content; the diffusion of oxygen out of the pores; and lastly even mixing by means of diffusion.

With compressed products or commodities with small interstitial air spaces, the air within the product is quickly compressed as CO_2 is introduced into the chamber. This is due to the pronounced pressure gradient from the outside of the product to the inside. This compression occurs so quickly that the interstitial air cannot diffuse out from the centre during the short time following the initiation of treatment. After even pressurisation diffusion processes slowly lead to gas exchange within the product. This exchange time must be added to the actual treatment time in carrying out effective pest control.

The time required to obtain the necessary CO_2 concentration for controlling the insects may be longer for compressed products because more time will be required for the first three phases (Fig. 4). Prior to high pressure treatment, it is necessary to identify the pest and the developmental stages present in the commodity. The treatment time must be adjusted accordingly to obtain the necessary lethal exposure.

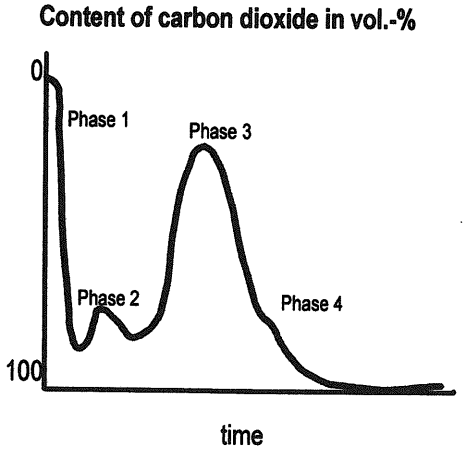


Fig. 4. Change in CO_2 concentration at the centre of a bale of compressed tobacco in a fumigation chamber during treatment with high pressure at 20°C (Modelled after Bird *et al.*, 1960).

In conclusion, unlike treatment with other classical insecticides and toxic fumigants, this treatment can be used as a preventive method to ensure pest-free cocoa beans and other foods without leaving chemical residues. In addition, this method has a low probability of causing change in quality, provided that the moisture content of the commodity is low.

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CARBON DIOXIDE UNDER HIGH PRESSURE TO CONTROL THE TOBACCO BEETLE *LASIODERMA SERRICORNE*

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ABSTRACT

Pressurized carbon dioxide (CO_2) can control most of the important insect and mite pests within a few hours. A rapid increase from atmospheric pressure to 20 bar for several hours and subsequent decrease back to atmospheric pressure within a few minutes reduces the lethal exposure time to under 1 h. The short treatment time makes this method attractive for pest control, and it is especially feasible with high value products. Pressure tight chambers of up to 30 m^3 with CO_2 recapture apparatus to reduce gas emission are used for short-exposure pest control.

The lethal effect seems due to a combination of increased CO_2 solution in the insect tissues, leading to reduction of pH or increase in acidity, and to the rupture of cell membranes following depressurisation.

Laboratory and practical results are presented describing the possibility of controlling the tobacco beetle *Lasioderma serricorne*, which causes severe losses in the tobacco processing industry, at various CO_2 pressures at various temperatures.

INTRODUCTION

Control of stored-product insects with inert atmospheres at atmospheric pressure requires days or even weeks, depending upon the developmental stage and species, the commodity and, above all, the temperature. In contrast, effective disinfestation of stored products with carbon dioxide (CO_2) under increased pressure lasts only a few hours. Due to the relatively high treatment costs involved, this method is more suitable for such high-value commodities as spices, drugs of plant origin, tobacco, cocoa beans and hazel nuts. Stahl *et al.* (1985a, b) determined for the first time that insects could be controlled within minutes or hours by exposure to CO_2 under 10–50 bar pressure. In experiments by Gerard *et al.* (1988), the tobacco beetle *Lasioderma serricorne* proved one of the most tolerant insect species. The lethal effect is presumably due to the combined effects of pressure and CO_2. In addition to the physiological stress — increased pressure, especially during the process of quick

pressure build-up, and subsequently decreased pressure at the end of the treatment — caused to their cells, the insects also suffered from lack of O_2 and from changes in cellular acidity caused by sorption of CO_2.

The present paper tries to answer several questions concerning the differences in sensitivity of the different developmental stages of the tobacco beetle to CO_2 high-pressure treatment, the extent to which mortality is dependent on temperature, the effect on mortality of the speed of build-up and decrease in pressure, the possibility of delayed mortality among insects surviving the treatment and the correspondence between laboratory mortality results and those of a field trial.

MATERIAL AND METHODS

The experimental high-pressure chamber was originally designed by Dr R. Wohlgemuth, the former director of the Institute for Stored-Product Protection. The 8-mm steel cylindrical chamber has an inner diameter of 55 mm and a height of 80 mm, with a volume of 200 ml. It is sealed with a thick brass screw and a rubber gasket. The temperature in the chamber can be controlled by using water that is heated or cooled in a bath connected to copper tubes which surround the steel cylinder which in turn is embedded in styrofoam. A thermosensor inside the chamber and a pressure sensor in the gas supply tube were used to monitor the experiments.

The eggs, larvae and adults of the tobacco beetle *L. serricorne* were obtained from an established culture reared on tobacco leaves, bran and yeast at 25°C and 65% r.h. at the Institute for Stored-Product Protection in Berlin.

Experiments on eggs were undertaken at 15 and 25°C and at 25, 30, 35 and 40 bar over a range of exposure times. Because the beetles hide their eggs by sticking them on the tobacco, they could not be counted in advance. Therefore, it was only possible to distinguish between complete and incomplete control. For all other results, an LT_{95}, derived from probit analysis of the mortality results, was calculated.

Larvae and adults were examined at 15, 25 and 35°C, and at 15, 20, 25, 30, 35, 40 and 45 bar, over a range of exposure times; additional insects served as controls.

The effect of temperature on adult beetles was investigated at 15, 25, 30, 35 and 45°C and at 20 bar of CO_2.

In further experiments, the speed of pressure change from 1 to 20 bar was varied between 1 and 2 min. In five replications a total of 400 adult beetles were exposed at 25°C to the four possible alternative combinations of slow and quick build-up and decrease of pressure. The treatment period of 5 min at a final pressure of 20 bar was constant. The bioassay was carried out 24 h after treatment by counting living insects.

There were three investigations into delayed mortality during the post-treatment period. Replicates of 100 adults were treated with exposure periods of 5, 10 and 15 min at 20 bar and 25°C, with build-up and decrease of pressure within 2 min. After treatment, both the treated beetles and untreated control insects were placed on tobacco and bran at 25°C and 65% r.h. and checked daily for mortality.

In the commercial scale trial, 12 cages containing insect samples were placed in the centres of three compressed 2-m^3 tobacco bales. They were treated for 17 h at 2 to 4°C with a pressure build-up of 100 min and depressurisation within 15 min. The samples were regularly examined for surviving insects for a period of 10 weeks after the treatment.

RESULTS AND DISCUSSION

Figure 1 shows the relationship between lethal exposure period and CO_2 pressure at two temperatures for the eggs of *L. serricorne*. At 25°C complete control was obtained after 80, 52, 36 and 20 min, at 25, 30, 35 and 40 bar, respectively. For comparison, Gerard *et al.* (1988) report a lethal exposure time of 50 min at 30 bar and about 10°C, in a trial using a 3.3-m^3 chamber, for complete control of all stages including eggs of *L. serricorne*. Eggs of *Plodia interpunctella* failed to survive a 15 min treatment with only 20 bar at 25°C; thus, they seem to be more susceptible than the eggs of *L. serricorne* (Reichmuth and Wohlgemuth, 1994).

As shown in Figs. 2 and 3, the larvae and adults of the tobacco beetle are less tolerant of this treatment. For example, at 25 bar and 25°C, adults and larvae are more sensitive than the eggs (Fig. 1).

Figure 4 shows the influence of temperature on lethal exposure times for adults; 90% mortality was obtained within 1, 4, 15, 24 and 35 min at 15, 25, 30, 35 and 45°C, respectively.

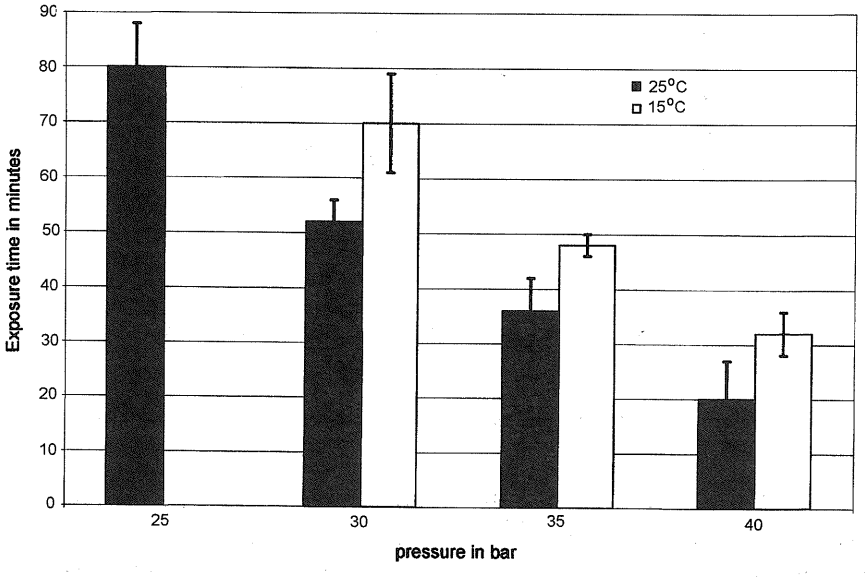


Fig. 1. Exposure time to control the eggs of *Lasioderma serricorne* with CO_2 at 15°C and 25°C under various pressures.

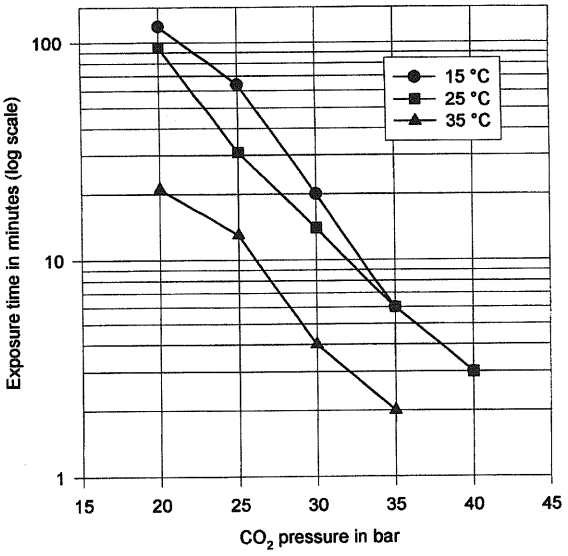


Fig. 2. LT_{95} for various CO_2 pressures to control larvae of *Lasioderma serricorne*.

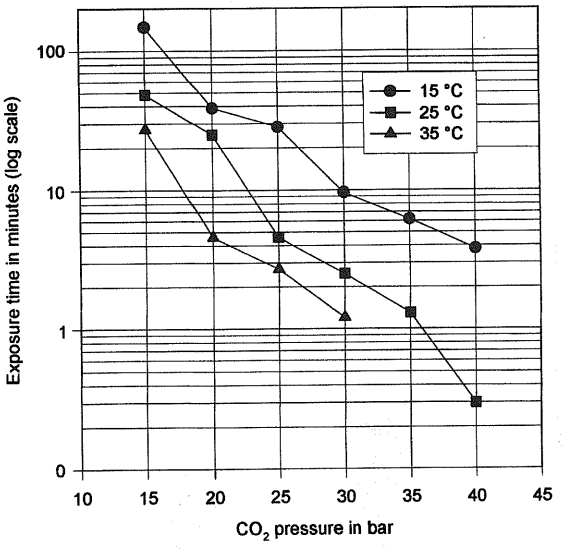


Fig. 3. LT_{95} for various CO_2 pressures to control adults of *Lasioderma serricorne*.

From Fig. 5 it can be seen that the speed of pressure change exercises a strong influence on the mortality of treated tobacco beetle eggs (Ulrichs, 1994). The paramount influence of quick depressurisation has often been postulated (Caliboso *et al.*, 1994; Nakakita and Kawashima, 1994; Ulrichs, 1994; Prozell and Reichmuth, 1992); it was demonstrated here for the first time. In treated products, the egg cells might even burst

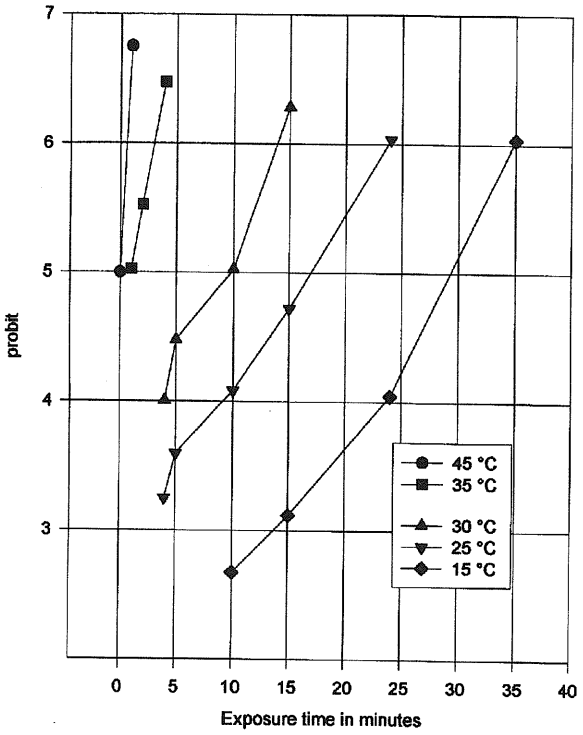


Fig. 4. Influence of the temperature on the mortality of adult *Lasioderma serricorne* during treatment with 20 bar of CO_2.

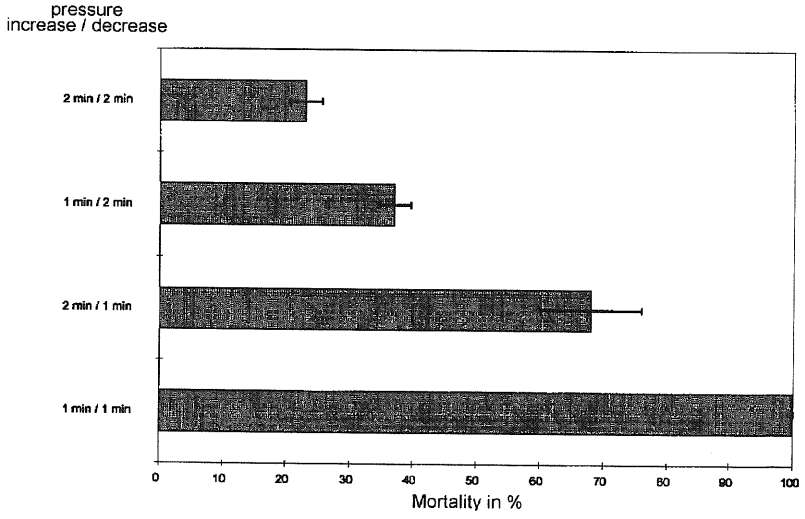


Fig. 5. Effects of different speeds of pressure increase and decrease using CO_2 treatments of 5 min at 20 bar and 25°C on the mortality of adult *Lasioderma serricorne.*

after very rapid expansion (Gerard *et al.*, 1988). With a constant exposure period of 5 min at the set experimental pressure of 20 bar, a difference of only 1 min in the speed of pressure change caused pronounced changes in mortality. Independently of how rapid the build-up of pressure was, pressure decay of 2 min caused significantly less mortality than decay of only 1 min. Rapid build-up of pressure, however, was more effective than was slower pressure build-up.

Figure 6 shows the principal difference in the characteristics of exposure to pressure. Considering CO_2 as behaving like a typical fumigant, it may be expected that when pressure build-up is slow, the insects should be exposed for a longer time. The integral of the corresponding characteristic can be calculated as: 20 bar min (build-up) + (5 × 20) bar min (exposure at 20 bar for 5 min) + 20 bar min (decrease of pressure) = 140 bar min to express a product resulting from pressure × time (Pt-product). In the case of quicker build-up of pressure this Pt-product is reduced to: 10 bar min (build-up) + (5 × 20) bar min (exposure at 20 bar for 5 min) + 10 bar min (decrease of pressure) = 120 bar min.

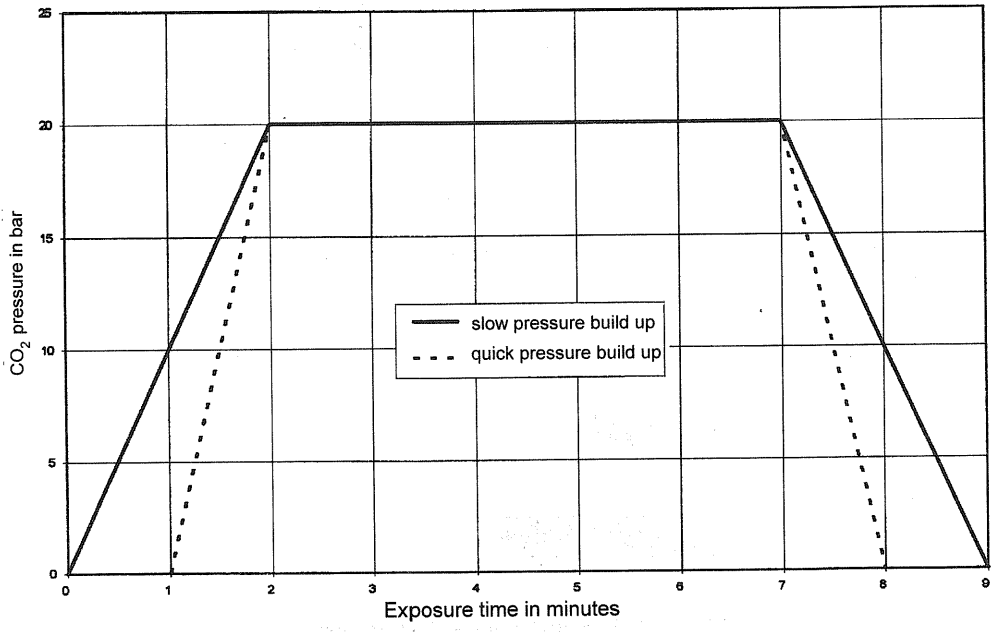


Fig. 6. Model of the treatment with 20 bar of CO_2 for 5 min with 1 min or 2 min pressure change at the beginning and end of the fumigation.

Despite the higher Pt-product, the shorter pressure change time is more effective in obtaining control. The effect of quick depressurisation is known as divers' disease ("the bends") and is caused by the transition of the dissolved gas from liquid to gaseous phase with a concomitent sudden expansion in volume. This internal pressure change leads to the shattering of cell membranes. The rapid entry of the gaseous CO_2 into solution also seems to lead to additional lethal effects.

For practical application, it can be deduced that large-diameter supply pipes will cause more rapid lethal effects when pressure build-up and decay are effected within a shorter time. To ensure success, the chosen exposure time was extended in order to compensate for both slower penetration into the tobacco and lower temperatures.

The results of the field trial with compressed tobacco showed 100% mortality. This supports the feasibility of applying the CO_2 high-pressure disinfestation method to bulky, high-density products.

ACKNOWLEDGEMENTS

The authors wish to thank the Guttroff Company, Germany, for providing support for the field trial.

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RATE OF GAS EXCHANGE DURING TREATMENT OF COMPRESSED TOBACCO WITH NITROGEN OR CARBON DIOXIDE FOR PEST CONTROL

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ABSTRACT

Tobacco suffers from infestation by the tobacco beetle *Lasioderma serricorne* and the tobacco moth *Ephestia elutella*. Most of the control measures tend to leave chemical residues. Nitrogen and carbon dioxide are proposed for use as non-toxic fumigants. The exposure time for total control depends on how long it takes for the gas to replace the oxygen (O_2) and on bale size; for large compressed bales, especially, there must be sufficient time for the inert gases to diffuse to the centre of the bale where the insects may reside.

The diffusion time necessary to achieve complete exchange of the air inside the tobacco bale was determined in laboratory experiments under controlled climatic and fumigation conditions. At room temperature, high concentrations of both gases, with a corresponding decrease in O_2 content, were reached in the centre within about 6 h. For practical application of both gases for pest control, where exposure periods last many days, this relatively short period can be neglected.

INTRODUCTION

The tobacco moth *Ephestia elutella* and the cigarette beetle *Lasioderma serricorne* cause severe damage in the tobacco processing industry (Ryan *et al.*, 1995). Not all stages of *L. serricorne*, regularly introduced into the factories with raw tobacco, are completely controlled during the processing. Cross infestation between uninfested and infested tobacco is a constant threat in the store rooms. The resulting damage to the manufacturer's reputation and his financial losses may both be enormous. Even a single hole bored by an insect through the wrapper of a cigar makes the product unsmokable and renders it worthless.

Due to growing public concern about the protection of the environment, the use of toxic gases and other insecticides is under critical review. Alternative non-toxic fumigants

such as nitrogen (N_2) and carbon dioxide (CO_2) demand a good, hermetically-sealed enclosure around the products as well as requiring lethal exposure periods up to several weeks, depending on temperature, insect stage, and the nature of the commodity (Annis, 1986; Reichmuth, 1986). In these treatments the insects die mainly because of lack of oxygen (O_2) (Adler, 1994a; Friedlander and Navarro, 1979). This procedure does not leave chemical residues.

Raw tobacco is traded in compressed form in bales or in cardboard boxes of about 2 m^3 capacity. Under the most unfavorable conditions, when insects at the centre of the bales have to be controlled, the lethal exposure periods obtained in the laboratory have to be prolonged to allow for the time required for N_2 and CO_2 to diffuse to the centre and replace the O_2.

MATERIALS AND METHODS

Diffusion experiments were carried out in a 2.8-m^3 gastight chamber (Reichmuth, 1979) using compressed tobacco in a European-standard size cardboard box (1,530 × 1,100 × 1,150 mm) weighing 230 kg. A 20-mm diameter stainless steel tubular probe was inserted to the centre of the tobacco. Another concentric tube within the probe sucked gas samples from the centre by continuous purge. The O_2 content was determined and recorded using SERVOMEX and THORAY instruments. The concentric tube was sealed against the outer tube and special holes allowed it to determine synchronically the O_2 content at a depth of 200 mm within the box. The O_2 content in the chamber was also recorded, and the temperatures in the chamber and in the tobacco were monitored. Due to the excellent insulating properties of the compressed tobacco, 15 d of adjustment were necessary to obtain a uniform temperature.

RESULTS AND DISCUSSION

Carbon dioxide purge

Figure 1 shows the slow change and temperature adjustment inside the tobacco. From Fig. 2 it can be seen that after less than 5 h of purging with CO_2, the concentration in the chamber reached 95% after which this level was maintained by means of an automatically switching solenoid valve. A further delay of 8 h was required to obtain this CO_2 level at the centre of the compressed tobacco. Under practical conditions, at 20°C, about 13 h would have to be considered the additional time required for the diffusion process. After 6 h the O_2 concentration was less than 5% (75% CO_2), a lethal atmosphere for stored-product insects (Adler, 1994b; Annis, 1986; Reichmuth, 1986).

In Fig. 3 desorption of CO_2 after treatment can be correlated with the increase in the recorded levels of O_2.

Nitrogen purge

Air contains about 79% N_2. Presumably due to respiration of the tobacco, the initial O_2 content in the tobacco bale was about 12% instead of 21% (the normal value). Purging

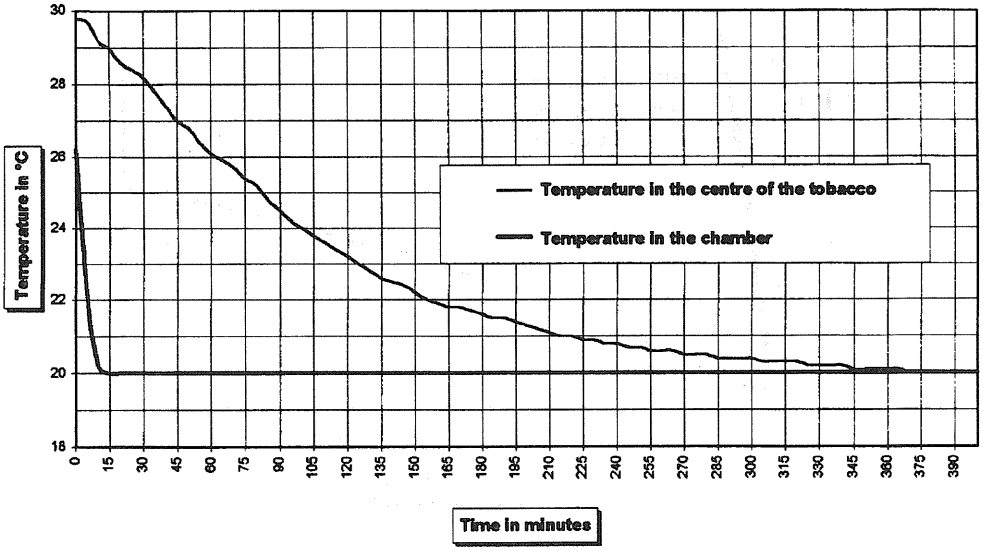


Fig. 1. Time required for temperature adjustment at the centre of a compressed tobacco bale (box dimensions: $1.53 \times 1.10 \times 1.15$ m).

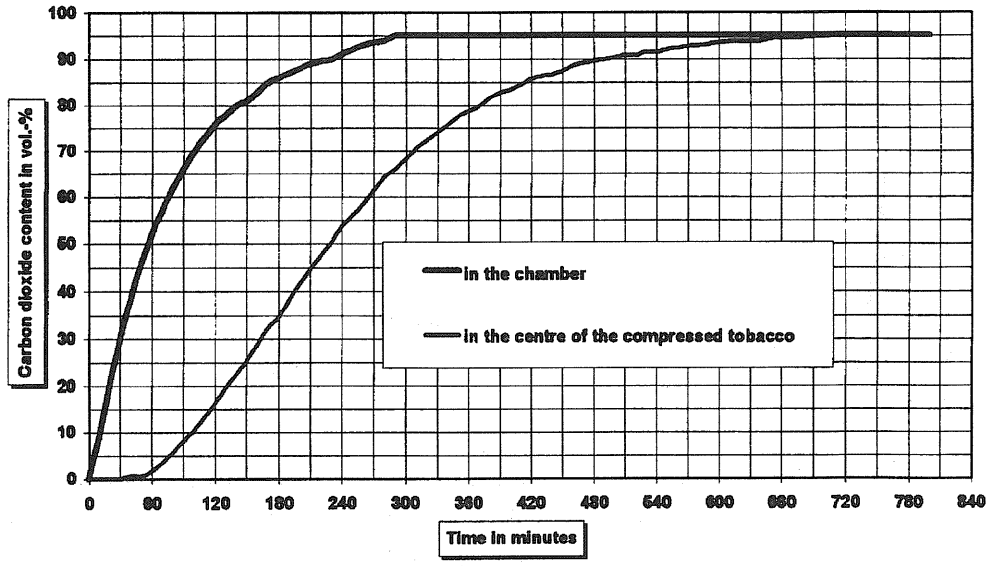


Fig. 2. Build up of CO_2 concentration in the centre of a compressed tobacco bale following the purge with CO_2 to a concentration of 95% with a corresponding final O_2 concentration of 1%.

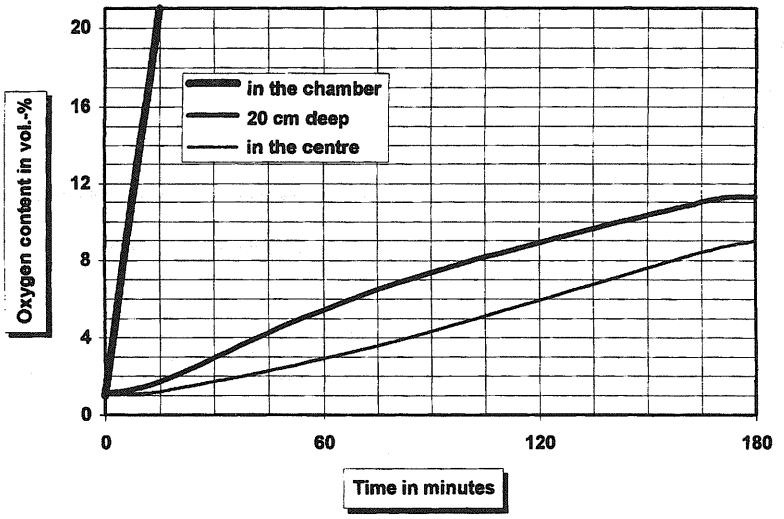


Fig. 3. Increase in O_2 concentration at the centre of a compressed tobacco bale treated with 95% CO_2 following post-treatment aeration of the fumigation chamber.

with pure N_2 produced 99.9% N_2 in the chamber within 2 h, as indicated by the drop in O_2 concentration given in Fig. 4. Although after about 3 h lethal O_2 contents (about 3%) were obtained, the replacement of all the O_2 inside the tobacco bale required about 6 h.

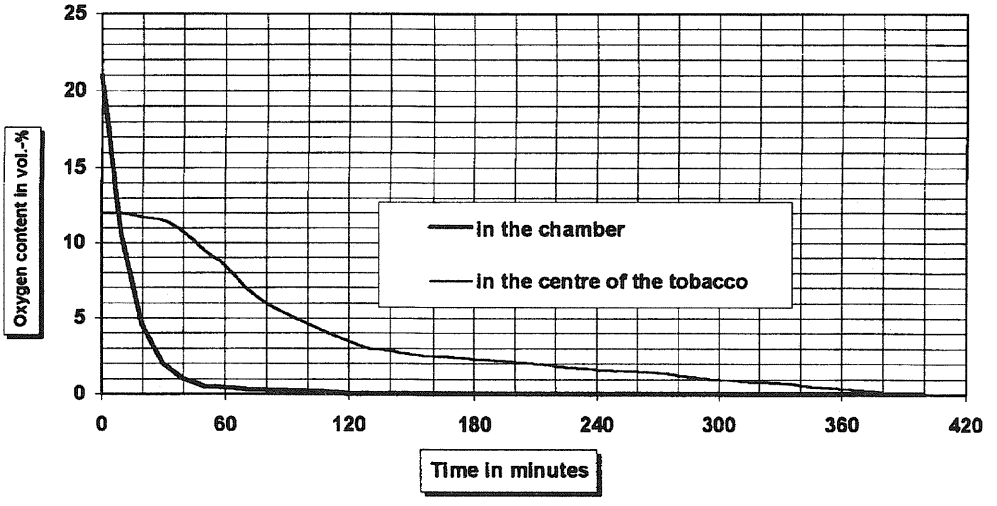


Fig. 4. Decrease in O_2 concentration at the centre of a compressed tobacco bale following a purge of pure N_2.

Direct comparison between the diffusion of the gases is difficult. CO_2 plays an important role by influencing the gas concentration in the chamber by desorption.

CONCLUSIONS

In conclusion, although the diffusion coefficients seem to be quite similar, the type of gas exchange, because air already contains 79% N_2, is quite different. The time needed to obtain lethal conditions for the insects is therefore shorter for N_2 applications. Compared to the necessary lethal exposure periods of many days for both gases (Reichmuth, 1979), neither these small differences nor the few hours required for gas exchange at the centre of this bulky product are significant enough to warrant prolongation of the treatment. This additional time can therefore be neglected when treating tobacco against *E. elutella* or *L. serricorne* with N_2 or CO_2 under atmospheric pressure.

ACKNOWLEDGEMENT

Our thanks are due to Mr Gerhard Schmidt for his excellent technical assistance.

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SESSION 6

SEALING TECHNIQUES AND METHODS OF DETERMINING GASTIGHTNESS

Chairperson:
R.T. Noyes, *USA*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, p. 351

TEST METHODS FOR SEALING LEVELS OF GRAIN STORES: PRESSURE TESTS AND TRACER TECHNIQUES

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ABSTRACT
(Full paper not available)

Verifiable and convenient predictors of fumigation or CA success are needed prior to the actual addition of the gas. Two test systems are in use: pressure testing and tracer gas techniques. There are two versions of the pressure test: the pressure decay (Pt) test and the pressure-flow (PQ) test. The former is quick and simple but can be less informative than the latter. Both suffer from two defects: they must be conducted while the contents of the structure under test are at constant temperature and they give 'worst case' estimates of fumigation suitability. Gasholding will typically be better than predicted, so on some occasions a structure may actually be adequately sealed even though it does not meet a set level. Tracer gas techniques (e.g. with CO) give a more directly applicable test but require several days of intensive monitoring to achieve a result. The various test results can be related mathematically. A pressure half life of 5 min from a Pt test in a full structure approximately corresponds to a gas loss rate of less than 5% per day in grain stores. This is an adequate level of sealing for most fumigation and CA procedures and corresponds to several national standards. As a special case, a decay time of >10 sec is adequate for freight containers. Practical examples of test results and their correlation with success or failure of treatments are given, and the approximate level of test value is discussed with regard to various treatment systems.

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 353–358

PRESSURE TESTS TO DETERMINE NEED FOR SHEETING LOADED FREIGHT CONTAINERS BEFORE FUMIGATION

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ABSTRACT

In Australia, before freight containers loaded with hay may be fumigated with methyl bromide (MB) without being enclosed under sheets or "tarps", compliance with a pressure test standard is required. Containers that fail to meet the standard must be fumigated under sheets. Routine pressure testing of freight containers provides rapid evaluation of the appropriate fumigation regime in commercial practice. It eliminates the need to sheet all containers, increases the number of containers that can be fumigated per day and reduces the average labour requirement per container treated.

Subjective assessment of pressure tests indicates that approximately 99% of new plywood-floored containers and 70% of new plank-floored containers meet the standard, as do more than 80% of old (3–4 years) plywood-floored containers. However, less than 10% of the old plank-floored containers — those which have made 1–2 voyages — meet the standard.

INTRODUCTION

Containerised hay consignments are regularly exported from South Australia to Japan where they are inspected on arrival to determine that they are free from insect infestation. Prior to shipment, the commodity is fumigated with methyl bromide (MB) to ensure compliance with Japanese phytosanitary requirements (de Lima *et al.*, 1994). Preshipment fumigation of containerised hay is a regular part of Australian Fumigation Pty Ltd's (AFPL) business.

The consignments are treated in accordance with the Australian Quarantine Inspection Service (AQIS) standard for MB fumigation (Anon., 1994), which requires that the gastightness of all containers be measured (and recorded) prior to fumigation (unless the containers are fumigated under sheets).

This paper briefly reports on some of the results — and the commercial implications — of pressure testing approximately 6,000 containers over a 3-year period.

MATERIALS AND METHODS

The containers used in the work reported here were all 12.2 m (40 ft) long. None were pre-selected for gastightness prior to loading. The date of manufacture on the compliance plate indicated the age of each container.

Pressure testing was done with a CONTESTOR pressure decay timer (Sharp, 1982; Sharp and Cousins, 1982). Containers were slightly pressurised (to 250 Pa) with air supplied through a specially designed manifold from a bank of compressed air cylinders. When the air supply was turned off, the pressure halving time was measured by the CONTESTOR. All pressure tests were carried out after the containers had been loaded with double-dumped hay.

In the work described here, AFPL applied the gastightness standard established by Sharp (1982) for the in-transit disinfestation of freight containers with carbon dioxide. This requires a pressure halving time from 200–100 Pa ≥10 sec. Containers that could not be pressurised to 250 Pa (the starting pressure for the test) at a set flow rate were deemed by AQIS to have failed the standard (de Lima *et al.*, 1994); they were therefore enclosed under gastight sheets before being fumigated.

Fumigations were carried out using MB at a dose of 44–58 g m^{-3} with a requisite minimum concentration × time (Ct) product of 200 g h m^{-3} after a 24-h exposure period (de Lima *et al.*, 1994). To ensure that the required Ct product had been achieved, gas concentrations were usually monitored only once, at the end of the exposure period. Thus, because a constant MB concentration over the exposure period (based on the reading taken at the end of it) was assumed, the Ct products reported here represent under-estimates.

MB concentrations were determined using a RIKEN™ IF 18 instrument (Gastech Australia).

RESULTS AND DISCUSSION

Since the introduction of the AQIS standard for MB fumigation, AFPL has routinely pressure-tested hay-filled containers prior to fumigation. The age of the container being treated was not recorded in all cases; where it was, it ranged from 3–82 months with pressure halving times from 11 to 85 sec (Tables 1–3). Sharp *et al.* (1986) reported that container gastightness does not deteriorate with age in any predictable way. The more recent work reported here, undertaken over the past 3 years, appears to support this observation.

Estimated Ct products, calculated from concentrations measured in containers that passed the pressure test standard after 22-, 23-, 24- and 27-h exposures, are shown in Table 1. In all cases the required 200 g h m^{-3} Ct product was easily attained. Similarly, most containers that met the pressure test standard had already achieved the target Ct product by 12–15 h after treatment (Table 2).

It should be noted that there is some indication that containers which failed to meet the 10-sec pressure halving standard could achieve the 200 g h m^{-3} Ct target at a pressure

TABLE 1
Pressure tests of hay-filled plywood-floored freight containers dosed with 58 g m^{-3} MB

Owner	Age (months)	Pressure halving time 200–100 Pa (sec)	Concentration after 22–27-h exposure (g m^{-3})	C/C_0	Ct product (g h m^{-3})
NOSU	12	11	13[a]	0.224	>286
NOSU	23	18	15.5[a]	0.267	>341
TRLU	23	31	18.5[a]	0.319	>407
TRIU	41	14	15[a]	0.259	>330
NOSU	58	16	18.5[a]	0.319	>407
TRIU	–	17	11[b]	0.190	>253
OOLU	–	19	15[b]	0.259	>345
TRLU	–	32	12[b]	0.207	>276
TRIU	34	14	15[c]	0.259	>360
OOLU	39	18	11[c]	0.190	>264
OOLU	48	14	12[c]	0.207	>288
OOLU	51	16	13[c]	0.224	>312
OOLU	82	13	14[c]	0.241	>336
POCU	–	24	16[d]	0.276	>432
CAXU	–	15	10[d]	0.172	>270
POCU	–	65	10[d]	0.172	>270
POCU	–	42	10[d]	0.172	>270
POCU	–	15	10[d]	0.172	>270

[a]22 h; [b]23 h; [c]24 h; [d]27 h. C_0 = initial dose 58 g m^{-3} MB; C = concentration after 22–27-h exposure.

TABLE 2
Pressure tests of hay filled plywood-floored freight containers dosed with 58 g m^{-3} MB

Owner	Age (months)	Pressure halving time 200–100 Pa (sec)	Concentration after 12–15-h exposure (g m^{-3})	C/C_0	Ct product (g h m^{-3})
TEXU	7	85	16[a]	0.276	>192
TRLU	15	12	16[a]	0.276	>192
NYKU	23	11	16[a]	0.276	>192
TPHU	57	12	18[a]	0.310	>216
OOLU	–	12	14[b]	0.241	>182
OOLU	–	19	16[b]	0.276	>208
OOLU	–	12	15[b]	0.259	>195
OOLU	–	14	17[b]	0.293	>221
CRXU	–	18	13[b]	0.224	>169
OOLU	3	17	16[c]	0.276	>240
OOLU	3	22	12[c]	0.207	>180
OOLU	5	20	16[c]	0.276	>240
OOLU	10	16	15[c]	0.259	>225
OOLU	34	22	18[c]	0.310	>270

[a]12 h; [b]13 h; [c]15 h. C_0 = initial dose 58 g m^{-3} MB; C = concentration after 12–15-h exposure.

halving time of 6 sec. This is shown in Fig. 1, where final Ct products are plotted as a function of pressure halving times. The figure includes data from de Lima *et al.*, (1994) and from Table 3 adjusted to an initial MB dosage of 58 g m^{-3}. Some of those containers that passed the 10-sec pressure test, monitored at 12–15 h after dosing, had still not achieved the required Ct product but would be expected to have done so at 24 h. On the other hand, of 12 containers that failed the 10-sec pressure test (see Tables 1–3), five failed to achieve the required Ct product of 200 g h m^{-3}.

De Lima *et al.* (1994) suggest that a pressure halving time from 200 to 100 Pa ≥6 sec is sufficient to allow containers loaded with hay to be fumigated with MB without enclosing them under sheets. They recommend adoption of this period as the standard in this specific application. Because AFPL was obliged to work to a 10-sec pressure halving standard, the limited data in this work, obtained in this range of the pressure test, do not substantiate this suggestion. However, de Lima (pers. comm.) has indicated that the recommendation is supported by the results obtained from a series of objective pressure tests.

AFPL's subjective assessment of the pressure tests it has undertaken has indicated that approximately 99% of new plywood-floored containers and 95% of old (3–4 years) plywood-floored containers passed the pressure test. Failure of old containers to pass was usually associated with gross structural damage. Only about 70% of the new plank-

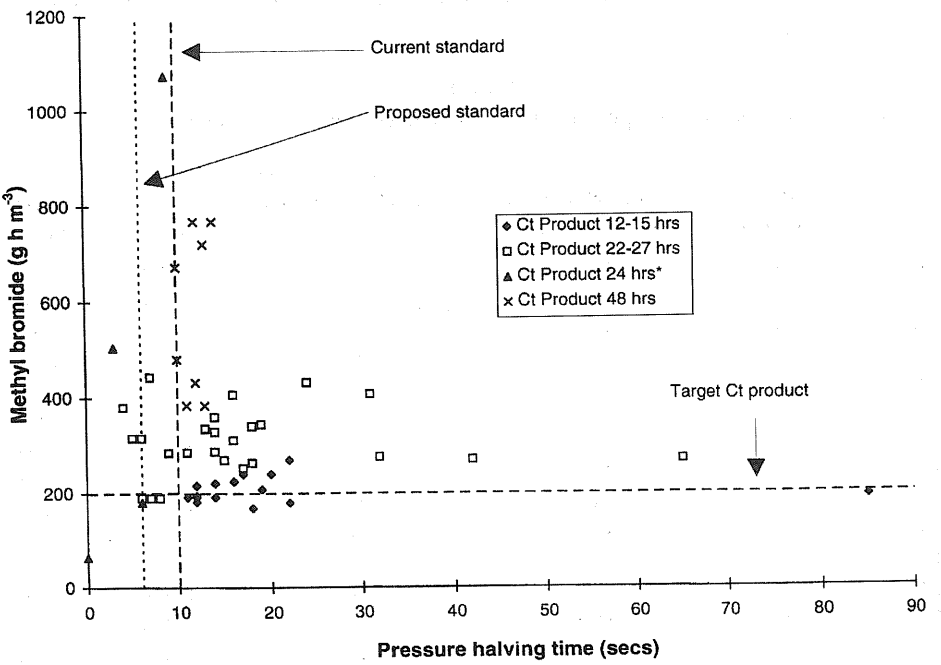


Fig. 1. MB Ct products as a function of pressure decay testing of freight containers loaded with hay.

TABLE 3
Pressure tests of hay-filled plywood-floored freight containers (age unknown) dosed with 44 g m^{-3} MB

Owner	Maximum pressure achieved (Pa)[1]	Pressure halving time (sec)	Concentration after 24-h exposure (g m^{-3})	C/C_0	Ct product (g h m^{-3})	Ct product (g h m^{-3}) corrected[2]
YMLU	120	4	12	0.272	>288	>380
TPHU	150	5	10	0.227	>240	>316
YMLU	150	6	10	0.227	>240	>316
YMLU	150	6	6	0.136	>144	>190
TRIU	200	7	14	0.318	>336	>443
YMLU	180	7	6	0.136	>144	>190
YMLU	180	7	6	0.136	>144	>190
INBU	200	9	9	0.205	>216	>285

[1]Reason for not taking pressure test to 250 Pa unknown. [2]Dosage corrected to 58 g m^{-3}.
C_0 = initial dose 44 g m^{-3} MB; C = concentration after 24-h exposure.

floored containers passed the pressure test; passing depended on the sub-floor treatment. After 1–2 voyages, only <10% of "old" plank-floored containers met the pressure test standard. It should be noted that the undersurface of some new plank-floored containers had been treated with tar (or a similar material) that enhanced the pressure test results on them in comparison to the results on those not treated in this manner. The pressure-enhancing effect is lost after 1–2 voyages because the planks "work" under the weight of the vehicles used to stow cargo in the containers (and subsequently the weight of the cargo itself). This "working" appears to open the seal between the planks. It has also been noticed that gaps open up between the planks when they shrink. The difference we observed between plywood and plank-floored containers in gastightness confirms the observations made by Sharp and Banks (1980) that plank-floored containers can leak extensively through the floor.

Overall, the results of the pressure tests reported here would seem to be favourable, in the long-term, to the adoption of a carbon dioxide technique for in-transit disinfestation of commodities in plywood-floored freight containers (Banks, 1988).

In commercial fumigation practice, use of the CONTESTOR has provided AFPL with savings in labour and time. Using a CONTESTOR, a 2-person fumigation team working at 3–4 different container yards is able to fumigate 25 containers in a 6-h shift, whereas without this instrument the time required for this work could be 8–10 h. In situations where containers are frequently not available until midday, or later, this becomes advantageous. Where large numbers of containers have to be treated, those meeting the pressure test are fumigated immediately while those that fail are sheeted and treated later by a second team.

CONCLUSION

Freight containers loaded with hay can be effectively pressure-tested under commercial conditions to determine their level of gastightness. Those that meet the standard (a pressure halving time from 200–100 Pa ≥10 sec) can be fumigated without being enclosed under sheets and are able to achieve a target Ct product of >200 g h m^{-3} after a 24-h exposure period.

Plywood-floored containers were assessed as more gastight than were plank-floored ones, and they retained their gastightness longer than did those with plank floors.

In commercial fumigation practice, pressure testing has provided savings in terms of both labour and time.

ACKNOWLEDGEMENTS

We thank Peter Annis and Chris Whittle for their advice and comments during the preparation of this paper.

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MANIFOLDING AND SEALING INSTALLATION METHODS FOR MANIFOLDED PHOSPHINE RECIRCULATION SYSTEMS IN MULTIPLE CONCRETE SILOS IN US GRAIN ELEVATORS

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ABSTRACT

The closed loop fumigation (CLF) or phosphine (PH_3) recirculation (PGR) system using a low volume blower and piping system in each tank was patented by James Cook of Houston, TX, in 1980. In 1991, to make CLF more effective, OSU researchers manifolded two large steel tanks (2,000–10,000 t/tank) to one small (0.1 to 1.5 kw) CLF blower. In 1995, manifolded PH_3 recirculation (MPR) system designs for concrete silos were developed. In 1996, 3–18 concrete silos were manifolded to a single blower to operate as 1,500 to 10,000-t storage units at five grain elevators.

At four elevators, all under-roof external wall vents were sealed. Suction pipes connected to only one side or end silo. Gas from all silos flowed through open under-roof internal vents between silos to CLF blower suction pipes. Blower pressure piping manifolds connected to the base of each silo in a variety of plumbing schemes. Silo roof vent and silo base sealing problems required a variety of techniques. In this project, sealing closed openings, gaps and cracks in order to minimize leakage considerably rather than to provide a gastight seal.

There are several benefits to CLF-MPR systems. Smaller work crews are required, thus reducing worker exposure. The response to fumigation is quicker. The cost of fumigant is lower. There are fewer regulations to comply with. The systems achieve better efficacy with less fumigant and less management expertise. Finally, blowers can be moved from site to site, minimizing investment costs.

BACKGROUND

Closed loop fumigation (CLF) was used for methyl bromide fumigation in the early 1920's in the US and other grain producing countries. A closed recirculation process for phosphine (PH_3) fumigation using small, low-power centrifugal blowers and piping systems operating at low pressures was developed in the 1970's and patented by James S. Cook of Houston, TX, in April, 1980. Initial CLF system designs used one blower for each storage tank or silo. This blower was operated throughout the fumigation period.

MANIFOLDED PHOSPHINE RECIRCULATION SYSTEMS ON STEEL TANKS

In the early 1990's, CLF systems in Oklahoma were designed by manifolding two large steel tanks (2,000- and 10,000-t/tank) to operate from a single blower in order to make them more cost effective (Noyes and Kenkel, 1994). Multi-tank installations are called manifolded PH_3 recirculation (MPR) systems.

To further reduce costs, an Oklahoma commercial grain company that owns several country elevators has installed CLF piping systems on steel storage tanks at each location, but they transport their CLF blowers from one elevator site to the next when the storage tanks at each elevator site need to be fumigated.

At 3–4 tank elevator sites at US country elevators, a single CLF blower can recirculate PH_3 on a single set of manifolded tanks for the initial pellet-release period (24–48 h), then be shut off and moved to the next set of tanks. The CLF blower is sized to produce one complete gas-volume exchange in 2–4 h; initial recirculation should be 24–30 h. At 5–10 h/complete gas exchange (2.5–5 gas cycles per day), initial recirculation of 30–40 h is recommended (Noyes and Kenkel, 1994; Noyes *et al.*, 1996a).

If the CLF blower is needed to recirculate gas on another set of tanks, it can be disconnected and the suction and pressure manifold pipes capped off or sealed. It can then be connected to the CLF piping manifold on the next set of tanks and the PH_3 released. Because fumigation of the second set of tanks cannot take place within 1–2 d of fumigating the first set of tanks, the complete facility is fumigated together. Some elevator managers prefer to install a blower for each set of tanks at an elevator site so that fumigations of all tanks are started at the same time.

MANIFOLDED PHOSPHINE RECIRCULATION SYSTEMS ON CONCRETE SILOS

In 1995, the multiple storage MPR system design concept was expanded through a US EPA grant to include concrete silos in Oklahoma and Texas. This project included 17 grain elevators with 12 steel tank systems and 5 concrete silo CLF/MPR model systems (Noyes *et al.*, 1996a, b).

Almost all steel tanks are equipped with aeration, which makes installing MPR easy. To be competitive with steel tanks, concrete silos must use MPR systems with 2–20 concrete silos manifolded to a single blower to operate as one large storage unit. Two of the silo systems in the five elevators that installed MPR on concrete silos, in 1996, included aeration. The smallest MPR concrete system involved three 500-t aerated silos (1,500-t total on the CLF blower), each separated by non-fumigated, non-aerated silos.

Figure 1 illustrates a simplified version of the MPR system design, where the suction pipe is connected to an exterior under-roof vent at the end or side of the elevator. Figure 2 shows the suction pipe connected to a silo roof inspection port cover on one silo. Where possible, the vertical suction pipe should be mounted beside, or connected to the side rail

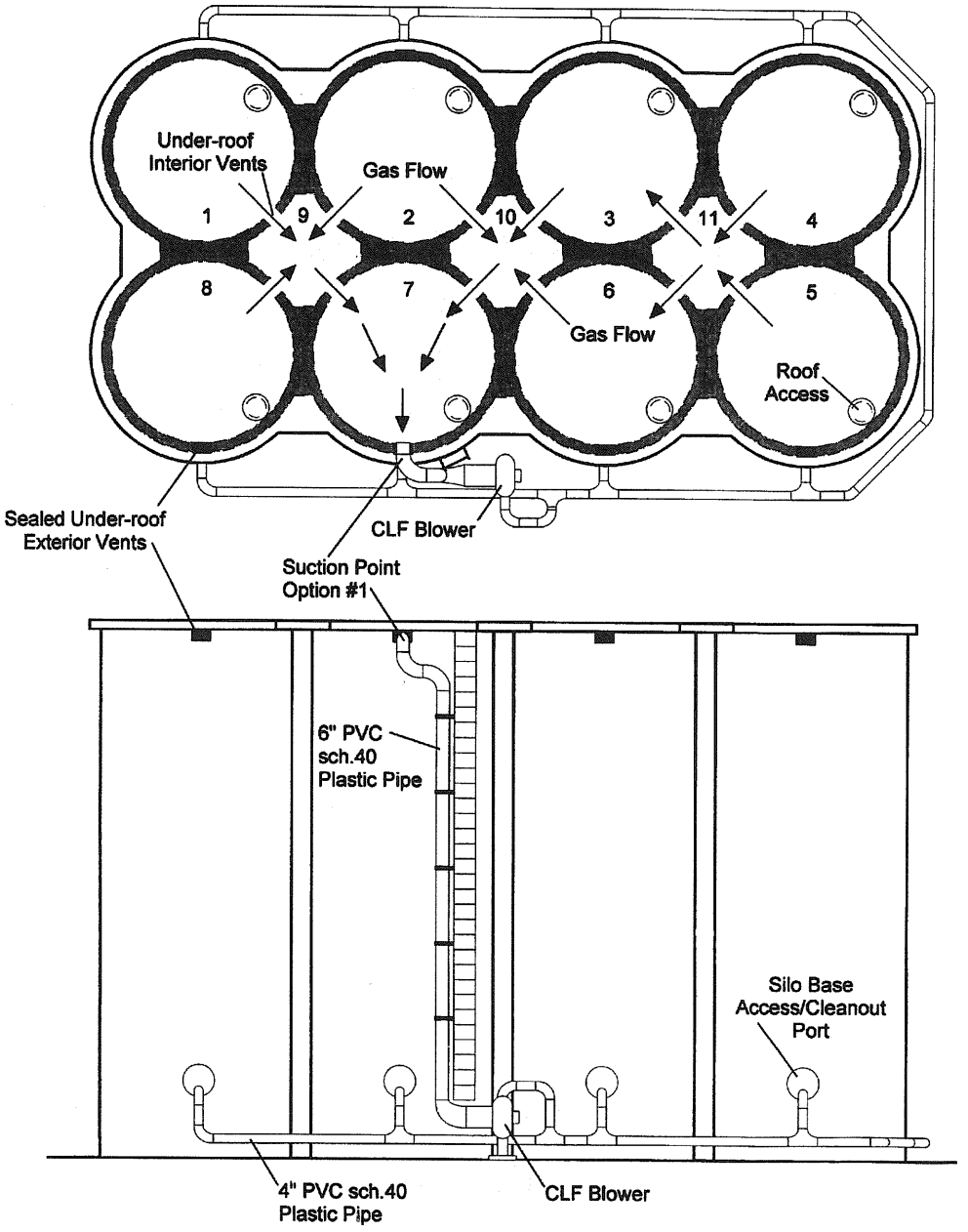


Fig. 1. Manifold phosphine fumigation system pulls gas from the exterior under-roof vent of one concrete silo and recirculates the gas through the base inspection door of each silo.

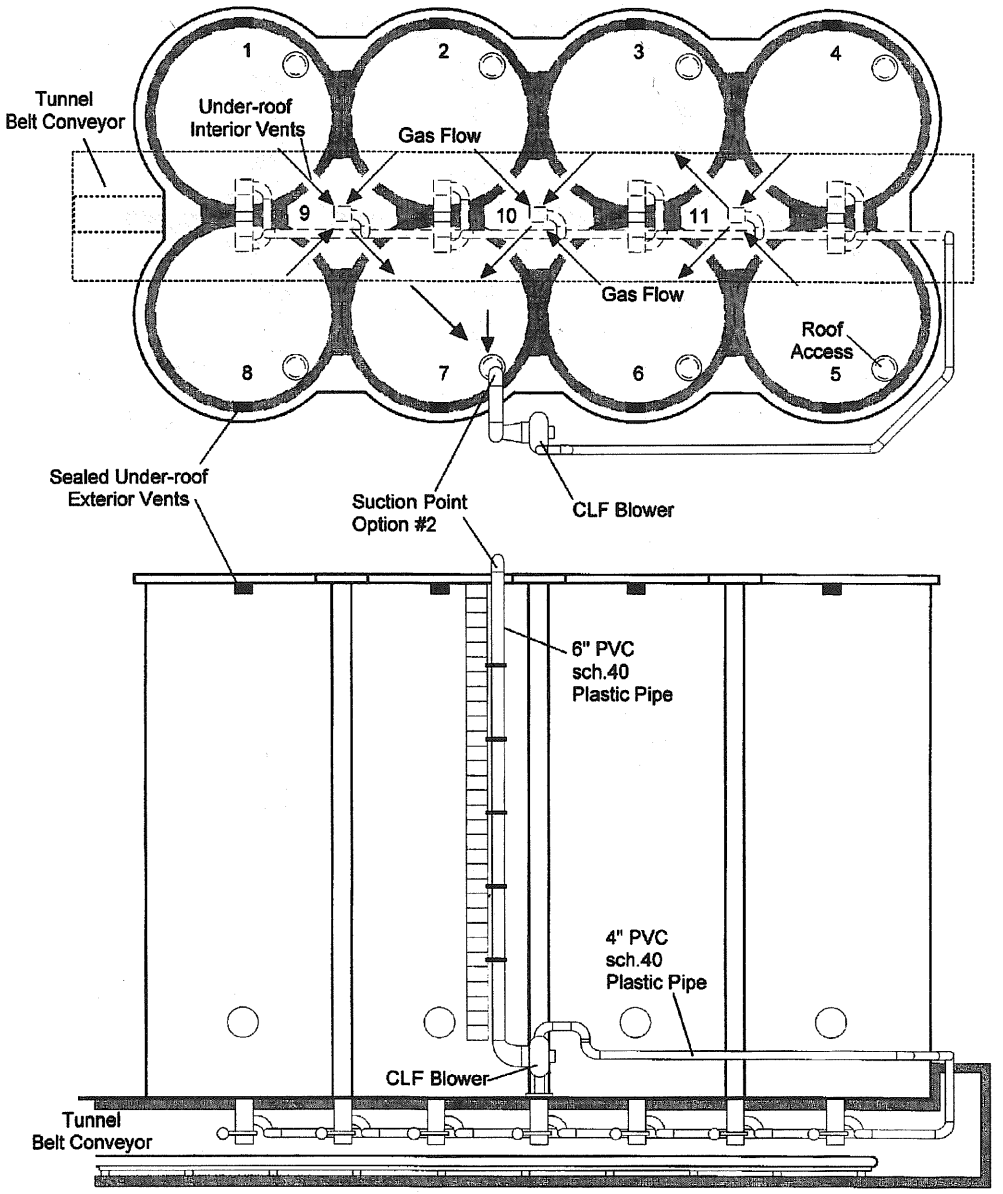


Fig. 2. Manifold phosphine fumigation system pulls gas from the roof hatch of one concrete silo and recirculates the gas through the discharge spouts in the tunnel/basement under each silo.

of, a ladder going down the side of the elevator. The suction pipe can be clamped to the vertical side rail of the ladder using stainless steel hose clamp bands spaced 3–4 m apart. The PVC plastic pipe extends to within 1–2 m of the ground. Flexible tubing connects the suction pipe to the CLF blower inlet. This makes the construction of the suction pipe from roof to base simpler, often saving the cost of a truck with a hydraulic hoist, a crane, or other expensive equipment.

At all five concrete elevators, under-roof external wall vents had to be sealed, as shown in Figs. 1 and 2. The four large concrete elevator silo systems contained 13, 14 (two elevators) and 17 silos connected to form common storage groups from 6,500–8,500 t/elevator. At these four sites, under-roof interconnecting vents between silos were left open to operate as return-flow gas conduits. Thus, gas could flow through the top of each silo into the silo connected to the suction pipe and then through the suction duct to the CLF blower, as shown in top and side views of Figs. 1 and 2. Interior under-roof vents on the 3-silo system had to be sealed in order to isolate the gas in the fumigated silos from adjacent silos.

The CLF blower outlet pipe manifold system had to connect to the base of all silos in each group. One elevator had aeration ducts connected to six exterior and two interior silos. For that elevator, the CLF blower pressure pipe connected directly to the aeration duct leading to the eight silos. Additional pressure manifolds along the aeration duct connected to the six non-aerated silos through discharge spouts and cleanout doors in driveways.

If all silos at an elevator were to be aerated with individual aeration blowers, then a simple MPR piping system could be installed around the elevator with a "tee" pipe connecting to the inlet or outlet of each blower on the silo sidewall. This would look similar to the plumbing-to-base inspection doors (Fig. 1). If all silos in a group were to be served by a central aeration blower through an external duct, the MPR system would be even simpler — the CLF pressure pipe connecting to the aeration duct.

However, since most concrete silos in the US are not equipped with aeration systems, most concrete MPR systems would usually be more complex than steel storage designs (Noyes *et al.*, 1996a, b). The CLF blower outlet pipe would be manifolded to the base of the silos by a variety of plumbing schemes. The pressure manifold for the 3-silo system involved connections to each of the three aeration blower pressure manifolds. In Fig. 1, pressure piping is shown connected to silo base cleanout or inspection doors on all eight silos.

If the concrete silo has a basement and tunnel belt system, it might be easier to connect MPR piping directly to silo discharge spouts between the tunnel ceiling and the discharge slide gate valve (Fig. 2). This would involve cutting a slot opening in the silo discharge spout. Manifold boxes installed over perforated sheet metal (20–40% opening with 1.5–2.5-mm holes) clamped to the duct sidewall would keep grain out of the gas line and allow gas flow into the base of each silo. Sealing of the discharge spouts below the gas manifold is discussed later.

A 10-cm (4-in) diameter main pipe would distribute gas along the tunnel to 5-cm (2-in) ID branch pipes and flexible hoses teeing off from the main pipe and connecting to the manifold box on each square silo discharge spout. An example of the main pipe that runs along the tunnel to the last silo, feeding gas to each silo discharge spout, is shown in Fig. 2. Three concrete elevators in the EPA study used basement tunnels for discharge conveyor belts under the silos.

At one elevator, a gallery covered part of the concrete silo roof deck, protecting a belt conveyor used to fill the silos. The simplest arrangement for a piping system for this elevator was to place the entire MPR system inside the elevator structure. The vertical suction pipe from one of the end silos was banded to the side rail of an unused inside ladder reaching from the silo roof deck floor to the CLF blower mounted on the ground floor. Because the installation was inside the elevator, an explosion-proof 1.3-kw motor on the CLF blower was used to protect against dust explosions.

Concrete elevators with different silo layouts and conveying designs required special MPR plumbing designs. The five concrete elevators in this program presented a variety of unique storage and duct-sealing problems. The alternative sealing options used at these five elevators covered most of the sealing problems that could be expected in US concrete elevator silo systems.

CONCRETE SILO SEALING TECHNIQUES

Because concrete silos are usually constructed of reinforced monolithic concrete sidewalls, sealing them generally involves sealing openings on or at the silo roof and at or near the silo base. In 1994, the Australian government required sealed storage structures to be able to build static pressure up to 25-mm water gauge and to sustain a pressure of no less than 12 mm for 3 min for partially filled or full tanks, or 5 min for empty tanks (Andrews *et al.*, 1994).

"Sealing," as used in this study, does not mean the type of pressure sealing specified or required for controlled atmospheres (CA) by the Australian government or for CA and/or modified atmospheres (MA) by other countries. The sealing techniques used in concrete elevators in this EPA project closed openings, gaps and cracks, greatly minimizing leakage. The objective was not to develop a gastight seal or to maintain pressure in the structure. The goal was to seal the structure so that PH_3 would not drain from the structural walls or base due to gravity, and gas loss through the eave and roof areas was minimal. Several openings requiring sealing were in the silo roof, sidewall and base. They were the silo-roof-deck-to-sidewall-joint gap, under-roof exterior vents, under-roof interior vents when MPR fumigated silos were mixed with non-fumigated silos, the roof entrance and inspection hatches, the roof ventilators and ventilation fans or aeration blowers, the downspouts or conveyor fill spouts through the silo roofs, the entry inspection/cleanout ports near silo bases, the aeration blowers at the silo base, the aeration duct manifolds around the silo exteriors and the basement tunnel or driveway discharge spouts.

SILO ROOF DECK TO SIDEWALL JOINT GAP

Flat concrete roof decks cover most US concrete silos. Often, because the roof deck is poured after the sidewalls, a gap exists between the sidewall and roof cold joint or junction. This gap is usually an irregular opening, too narrow for concrete mortar fill, which can cause a high PH_3 leakage rate.

Suggested filling options are expandable foam insulation or silicone caulking material. The selection of sealant depends on the gap size and elevator preference. If gaps are larger than 0.5 cm, foam insulation is preferable. Gaps less than 0.5 cm can be caulked with silicone. If gaps exceed 1.0 cm, expandable foam or cement mortar paste may be used.

UNDER-ROOF EXTERIOR VENT SEALING

Most concrete silos in the US have flat slab roof decks. Exterior under-roof vents are usually about 15 × 30 cm (6 × 12 in) with 1.25 cm diameter vertical steel reinforcing bars placed vertically about 2.5 cm apart. Roof decks usually extend 15–30 cm beyond the outside silo wall, providing rain protection for the open vents.

Before sealing, the wall vent openings had to be cleaned, using compressed air or brushes, of all dirt and trash. The methods suggested for sealing external under-roof vents were spray foam insulation, plates of plywood or steel and concrete mortar fill.

Expanding foam seal

High-density hole filler expanding spray foam is available from home or industrial supply or hardware stores. A 340-g (12-oz) pressurized spray foam can contains about 9,400 cm^3 (575 in^3). One can should therefore fill either about 1.25 vent openings of 15 × 30 cm, 15 cm thick, or about 1.8 vents 10 cm thick.

To fill the vent opening, one starts by building up layers of foam from the base and works toward the top of the opening. The opening should be filled about 40–50% and the foam allowed to expand upward. One should work back and forth on several vents allowing the foam to expand before adding the final layer to seal off the top of the opening.

The foam should be filled in around each steel bar and should cover at least 0.66% of the width of the concrete wall; the foam barrier, with the steel bars of the center of the plug, should be at least 10 cm thick. The steel bars anchor the expanding foam plug. After the opening is filled, to finish the seal a final continuous foam bead should be run around the opening against the concrete. To provide ultraviolet (UV) protection, the outer foam surface should be painted with exterior-grade paint. Once in place, the expanding closed cell foam barrier should provide excellent long-term sealing against gas leaks through the vents.

The expanding spray foam should be applied at temperatures of 15–38°C (60–100°F). It should be tack-free and not sticky after 30–45 min of curing at 22°C (70°F) and fully cured in 6–8 h. Workers who apply spray foam should wear plastic safety gloves, to keep foam off their skin, and avoid breathing fumes from the spray foam.

Wood or steel plate and silicone caulk seal

When using plywood or steel plating, several vent openings should be measured to ensure that they are all uniform in size. A 0.80–0.95 cm (5/16–3/8 in) thick exterior-grade plywood should be used. If the vent opening size is uniform, one should cut the required number of rectangular plywood plates 0–0.15 cm (0 to 1/16 in) undersized. The rough texture of the concrete around the vent opening will allow plywood to be press-fit or hammered against the vertical steel bars.

For loose-fitting plywood or 0.25–0.50 cm steel plating, one should drill a 0.5–0.6 cm diameter hole near the center of the board or plate and insert a "J" bolt (or formed all-thread rod) that can be hooked around one of the vertical steel bars. The nut on the bolt should be tightened to draw the plate tight against the steel bars, then the bolt threads sealed with silicone or similar caulking to keep the nut locked in place. To finish the seal, one should lay a 0.5–0.8 cm bead of silicone or similar caulking sealer material around the wood or steel plate to concrete a junction. The wood or steel caulking seal should then be painted with a suitable exterior-grade all-weather paint.

Concrete mortar

A third option for sealing exterior or interior vents is to fill the openings with an expandable concrete mortar. This may require that one worker be inside the silo to fill the opening while a second worker in safety harness or on scaffolding holds a flat plate or board against the opening during placement of the mortar to assure uniform filling and a tight seal.

UNDER-ROOF INTERIOR VENT SEALING

If fumigated silos are separated by non-fumigated ones, interior under-roof vents need to be sealed. Sealing these vents will probably require that the silos be filled with grain to within 1.5–2 m of the roof. If interior vents have vertical steel "bird" bars, either press-fitting, using plywood or steel plates with "J" bolts and caulking the edges, or using expandable foam similar to that for the exterior vents should be a suitable means of sealing them.

Wood plug seal

If there are no vertical steel bars on interior vents, expandable foam which does not require painting for UV protection should be a good alternative. Because of fumes from the spray foam, workers must be provided with a suitable fresh air supply or venting. When crews are working inside the silos, all electrical equipment should be locked and tagged out and the workers should wear suitable safety harness with security ropes.

For vents without steel bars, another alternative is to cut solid wood blocks or plugs, about 10–12 cm (3.5–4.6 in) thick and slightly undersized in height and length, to fit the openings. The wooden plug should be pushed into the opening and centered on the wall, after which the wood should be caulked to the concrete junction with a heavy silicone caulking (or similar suitable caulking compound) on both sides of the plug to anchor it and seal it securely in place.

ROOF ENTRANCE/INSPECTION HATCHES

Silo roof entrances or inspection hatches typically have shallow round steel or cast iron pans or lids that are set down over a raised steel lip to provide protection from storm water. These lids can be sealed by wrapping 5–10 cm fumigation-quality sealing tape or industrial-quality duct tape around the metal-to-concrete junction. An alternative is the use of expandable foam to fill the space extending from under the lip of the hatch cover to the steel flange ring and then down to the deck, usually a 2.0–3.0-cm gap. The foam should then be painted with a UV-resistant paint for long-term use. This type of sealing can be left in place for long periods if access or inspection entry is not needed.

ROOF VENTILATORS, VENTILATION FANS OR AERATION BLOWERS

Sealing of roof ventilators, fans or blowers should be done as a temporary measure at or near the time of fumigation if these devices are used during the year. A common method of sealing devices that protrude above silo roofs is to place heavy plastic bags over the equipment and seal the bag around the base of the ventilator, fan or blower using either 5–10 cm fumigation tape or a fumigation spray adhesive that will hold the plastic bag against the steel surface.

On some vents or fans, 4–6-mil plastic sheeting may be suitable, with spray adhesive and fumigation tape used to seal the plastic to the steel surfaces. The primary objective is to seal off the opening, preventing air and gas leakage from the structure.

DOWNSPOUTS OR CONVEYOR FILL SPOUTS THROUGH SILO ROOFS

Downspouts or conveyor discharge spouts in the silo system are often the toughest points to seal because of their inaccessibility. Downspouts are steel pipes or tubes that are often cast in place when the concrete roof is poured. To cut sections of the tubing out and flange them for easy removal for sealing may be desirable, but it is an expensive option.

Spouts that project even a short distance through the roof can be sealed with plastic and tape when the silo is filled with grain. If grain distributors are accessible, down spouts may be plugged or sealed with plastic sheet and tape from above the silos. Since this part of the MPR system is under slight negative pressure, sealing of grain-fill points is important, but these points are not as critical from the standpoint of leakage as are silo discharge spouts. However, from a personnel safety standpoint, fumigant leakage up the downspouts can cause safety problems in other parts of the elevator system.

ENTRY INSPECTION/CLEANOUT PORTS NEAR SILO BASES

These round steel or cast iron openings are usually positioned about 2–3 m above ground level. They are used for entry to the silo base for inspection and cleanout. These plates

usually fit closely into the reinforced opening frame; therefore, use of a silicone caulk bead or fumigation tape at the crack or gap between the plate and frame should provide a suitable seal.

AERATION BLOWERS AT SILO BASE

Centrifugal aeration blowers at the bases of concrete silos may be installed as either pressure or suction systems. On centrifugal blowers that deliver pressure, the inlet is often shielded by a wire guard to keep rodents and birds out. When a flanged frame covers the inlet, heavy flat plastic sheeting can be used with a light spray adhesive around the sides of the frame which allows the plastic to be secured sufficiently to make taping for final sealing easier. If an easily-unbolted frame is attached to the side of the blower housing, a plastic sheet can be tacked to the side of the housing and the frame replaced over the plastic, providing a good seal. In this case, the plastic should extend beyond the flange so it can be taped down to provide a positive seal.

For suction systems, the blower usually has a gravity-shuttered exhaust that provides weather and pest protection when the blower stops running. These shutters often have flanges that can be removed so that plastic sheeting can be placed over the opening and the shutter replaced, sandwiching the plastic sheeting. Again, the plastic should be extended and the edges sealed with fumigation or duct tape. The alternative is to use a plastic bag over the outlet, which is then sealed with tape.

The blower wheel drive shaft clearance opening through the blower housing sidewall is quite often overlooked on aeration blowers. This clearance opening should be carefully tape-sealed to prevent leakage. All seams on blowers, blower transition ducts, flexible connectors between blower and duct should be checked for leaks. On pressures systems, the blower should be operated and all seams and joints checked for leaks using a soap detergent. Flange to flange connections are often inadequately caulked. Tape or silicone can caulk any leakage points.

AERATION DUCT MANIFOLDS AROUND EXTERIORS OF SILOS

Large aeration blowers can be manifolded to a large steel duct serving several concrete silos. Minor duct leaks, that are not a problem for aeration, can cause serious leakage when the ducts are used for MPR fumigation systems. Leakage points to check and seal are the connecting joints, the seams along the pipes and clearance openings for shut-off or control valve shafts.

A detergent soap solution can be used to check all possible leak points on external or interior ducts (where ducts go through the silo walls to inside silos). Flanges, longitudinal seams and connecting joints can be tape sealed with fumigation tape. Control valve shaft clearance openings should be sealed with silicone caulk. If valves are turned and the caulking disturbed or torn between fumigations, the shaft should be recaulked for future fumigations.

BASEMENT TUNNEL OR DRIVEWAY DISCHARGE SPOUTS

Discharge spouts in basement tunnels or driveways present two primary sealing problems: the outlet opening and the slide gate. Discharge spouts that feed tunnel belts often have dust hoods attached that create a major sealing problem. In some cases, the dust-system hood may have to be reconfigured so it can be disconnected from the discharge spout.

The simplest method of sealing rectangular discharge spouts is to plastic-bag the outlet. Heavy plastic bags that are specifically designed for fumigation vent and blower sealing should be used. Double bagging may be necessary to achieve strength adequate to handle the pressure of the CLF blower outlet that is attached just above the discharge slide gate. A light-tack spray adhesive to help secure the plastic bags for taping may be advisable.

The slide gate area should be silicone-caulk sealed above and below the slide as well as around the rack-and-pinion shaft openings. The CLF blower should be operated without fumigants and a soap detergent used to check for pressure leaks on the bag and silicone-caulk seals. It is imperative that basement tunnel and drive discharge spout seals be extremely tight because of their close proximity to the MPR pressure manifold that cycles the gas to the silos above. Any fumigant leaks at this point will drain the system, rapidly diluting the fumigant mixture.

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SEALING EFFICIENCY ASSESSMENT IN MODIFIED ATMOSPHERE STORAGES

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ABSTRACT

The leak area that would permit air infiltration into an experimental 665.7-L silo was assessed with constant pressure tests using 23–1,006-mm^2 orifice cross-sections. The influence of both the area of the orifice cross-section and its depth (wall thickness) on variations of the empirical constants that describe the constant pressure test was also demonstrated. Based on a series of tests, an empirical equation to estimate leak area was proposed.

The experimental silo was filled to 92% of its capacity with ca. 500 kg wheat and tested, using variously sized orifices, for carbon dioxide (CO_2) loss. The measured CO_2 concentrations were compared with the calculated values based on equations that took into consideration initial CO_2 adsorption by the wheat, diffusion of CO_2 through the leak, and variations in temperature and barometric pressure. Under the experimental conditions, close agreement between the measured and calculated values was obtained. The influence of temperature and barometric pressure variation on infiltration of air into the silo was also analyzed. The information obtained from the constant pressure tests and from analysis of the weather conditions provided guidelines concerning how long a certain concentration could be maintained in a CO_2-treated structure. Leak rates for any structure may be assessed by the method developed in these experiments.

INTRODUCTION

A well-sealed storage structure is fundamental for the application of modified atmospheres (MA's). A MA created by the introduction of carbon dioxide (CO_2) can be maintained in such a structure at an efficient level because air ingress is restricted; otherwise it would eventually lead to dilution of the CO_2 concentration. To control stored-product insects, Jay (1971) recommended a concentration of 60% CO_2 in air for 4 d at a temperature of 27°C or above. Gastightness has been a problem for years in the application of fumigants in storage. The consequences of poorly sealed storages under fumigation have been discussed by Banks and Desmarchelier (1979) and Banks (1981), though the requirement that storages be gastight appears to be more critical in using MA's than in applying fumigants (Monro, 1969).

The degree of gastightness that is satisfactory, as well as what gas concentrations can be maintained under given environmental and structural conditions (Hunt, 1980), should be decided prior to the application of a MA. These decisions should be weighed against the investment involved in sealing a leaky structure to prevent excessive loss of the MA.

The present work assesses gas-leak rates from grain bulks treated with CO_2 at different temperatures and atmospheric pressures.

MATERIALS AND METHODS

Equipment

Pressure measurements were carried out using a Pace Wianko pressure transducer kit with a model CD25 indicator and a model KP15 transducer having interchangeable diaphragms. The transducer source was calibrated against a Dwyer model 40023 manometer.

Temperatures were determined with thermistor probes connected to a Yellow Springs Instrument Co. Model 47.1 scanning tele-thermometer. Two Yellow Springs model 80A continuous recorders were connected to the equipment, one to the pressure indicator and the other to the scanning tele-thermometer. These readings were recorded on a 25.4 cm scale, using a Hewlett-Packard model 7128A recorder with a 25.4 cm/h scale.

Barometric pressure changes were recorded on a Belfort Instrument Co. No. 5-800A microbarograph with a weekly chart record. This microbarograph was calibrated and periodically checked against a Fortin-type Fisher Scientific Co. Model 02-380 mercury barometer.

Air flow was measured by using either Gilmont K-3200-00 float-type variable-area flowmeters having several ranges or a Sierra Instruments, Inc. model 715 mass flow-meter. Flowmeters were calibrated against a bubble-type flowmeter. The mass flowmeter was used for air volumes up to 50 L/min, while higher air volumes were measured with an Alnor Thermoanemometer Model 8500.

Atmospheric gas composition was analysed with a Fisher-Hamilton Model 29 dual column gas-chromatograph equipped with a thermal conductivity detector. Peak areas were measured with a Hewlett-Packard integrator model 3390A.

A 665.7-L silo, 259.5 cm high with an internal diameter of 57.15 cm, was used for the experiments (Fig. 1). The silo was constructed by welding together three commercially available metal drums, the wall thickness of which was 0.825 mm.

Variously sized orifices, simulating leaks in the experiments, were prepared by using hypodermic needles having measured diameters for orifices less than 3 mm wide or by drilling holes on removable flanges mounted on the experimental silo.

Constant pressure test for determination of leak area

In two series of tests both small and large leaks were studied. In the series with small leaks, needles with openings of 0.57, 1.14 and 1.42 mm^2 in area, and orifice lengths (representing simulated wall thickness) of 5, 20 and 40 mm, were employed. To minimise

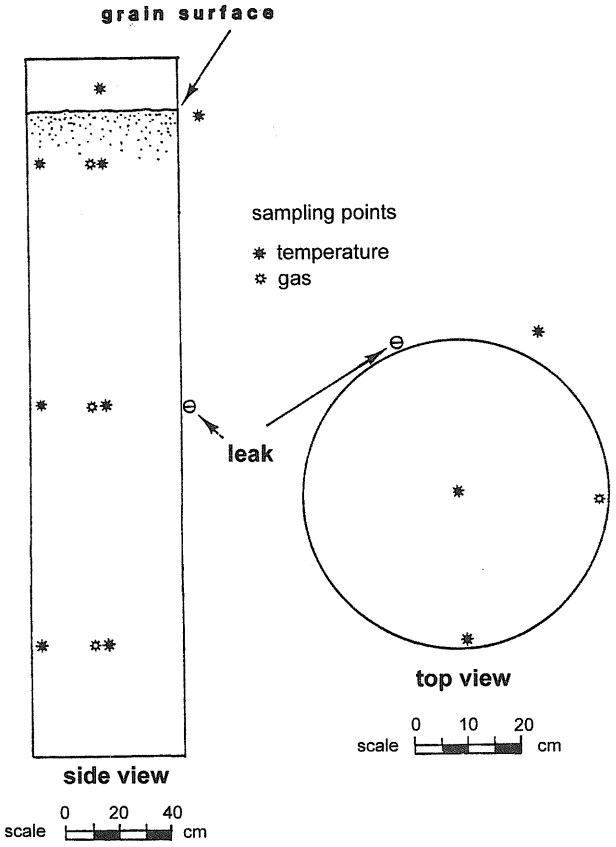


Fig. 1. Experimental silo designed for assessment of gastightness in modified atmosphere studies.

the influence of variations (in both the ambient temperature and barometric pressure) that would affect the results obtained when the small leaks were located on the silo wall, it was necessary to reduce the volume of the silo. Therefore a parallel series of experiments was carried out with needles mounted on a 0.95-L glass container.

Large leaks were tested on a 3.15-mm thick removable sliding side plate. Orifices were 23 to 1,006 mm^2. During a test, air from a pressurized cylinder was introduced into the silo and the ratio of volumetric flow to final equilibrium pressure recorded. The general equation (Anon. 1972; Hill and Kusuda, 1975):

$$Q = KA \cdot \Delta P^n \tag{1}$$

was used to obtain the value for the proportionality constant (KA). Since the airflow into the silo at equilibrium equals the total air leakage out of the silo, the pressure difference (ΔP) could be correlated to K (the empirical constant) and A (the effective leak area). Sharp (1982) and Dickson (1981) found values of n close to 0.5 for sharp-edged orifices and 1 for cracks.

CO_2 decay tests

CO_2 decay tests were carried out with the silo containing 499.4 kg of soft red winter wheat at 12.5% moisture content (m.c.). The wheat bulk occupied 92% of the silo volume.

CO_2 gas was supplied from pressurized cylinders. After a certain CO_2 concentration was reached, 2–3 d were allowed to elapse to enable the gas to attain a uniform concentration. Gas samples were taken periodically using a 10-ml Hamilton model 1010 gastight syringe fitted with a 5-cm size hole model 6-90224 needle. Gas sampling ports were located at 42, 131 and 218 cm from the bottom of the silo (Fig. 1). CO_2 concentrations were analyzed by gas chromatography.

The silo was equipped with eight temperature measuring points. Gas pressure within the silo was measured from the center of the silo through a 5.7-mm diameter tygon tube connected to the transducer. All tests were carried out in a room maintained at 25 ± 1°C.

Calculation of CO_2 loss

The calculation of CO_2 loss was based several assumptions. The effect of wind was assumed to be negligible since the leak site was protected from any direct air circulation which could have been caused by the air-conditioning system in the room where the silos were held. The CO_2 produced by grain respiration was also assumed to be negligible and the grain was assumed to be free from insect infestation. The volume occupied by the grain, with a calculated void space of 288.7 L (including the head space) was assumed to remain constant. The corrected diffusion rate through the leak was assumed to be 16.1 mm^2/sec at 25°C and 101.325 kPa (Roberts, 1963), and the concentration of CO_2 in the silo was assumed to be uniform, while the CO_2 concentration outside the silo was assumed to equal the atmospheric CO_2 concentration. Finally, the representative silo temperature was measured in the center of the empty silo and in the silo containing wheat, and the external temperature was assumed to affect only the headspace volume.

Variations in temperature and barometric pressure and diffusion through the leak all caused air to infiltrate into the void space in the silo. The air infiltrating into the silo caused a change in CO_2 concentration due to a uniform diluting effect. The CO_2 concentration at time t (C_t) was uniform throughout the silo. Appropriate equations were developed to determine the volume of air entering the silo and thus causing changes in the CO_2 concentration.

Based on these assumptions, the change in concentration (dC) is equal to the concentration (C) times the fraction of the volume withdrawn dV (Nelson, 1971):

$$dC = -\frac{C\,dV}{V} \tag{2}$$

This integrates to give a resultant concentration C_t:

$$C_t = C_0 \exp\,(-V_a / V_e) \tag{3}$$

where V_a is the volume of air infiltrating the silo and V_e the void volume of the silo and C_0 is the initial concentration.

The volume of air/CO_2 mixture diffusing out of the silo is equal to the volume of air entering the silo from the diffusion rate equation:

$$V_d = \frac{AD \cdot \Delta C \cdot 3.6t}{L10^8} \tag{4}$$

where V_d is the amount of CO_2 that diffused through the leak in m^3; L is length of the leak in mm; A is the cross-sectional area of the leak in mm^2; t is the time during which a given amount of CO_2 diffused in h; ΔC is the CO_2 concentration difference between the silo atmosphere and ambient atmosphere in %; and D is the diffusion coefficient in mm^2/s.

Fluctuations in gas temperature result from solar radiation, ambient air temperature, wind and precipitation, all of which depend on the thermal properties of the silo's construction material. The total number of temperature changes is directly proportional to the frequency and amplitude of the temperature fluctuations in the silo's void space. Therefore, changes in the silo gas temperature were approximated by the linear functions used by Meiring (1982):

$$T_t = T_0 + R_T \cdot t \tag{5}$$

where T_t is the silo gas temperature in °K at time t; T_0 is the initial silo gas temperature in °K; R_T is the temperature rate of change in °C/h and t is the time in h.

Since air infiltrates into the silo void space as a result of a decrease in gas temperature, the ratio of silo gas temperature to the initial silo gas temperature can be used as a measure of the influence of temperature on the volume of air which infiltrates the silo. In practice, the drop in nighttime temperature affects both the headspace temperature of the silo and that of the wheat to a depth of approximately 0.15 m from the outside wall (Oxley, 1948; Muir, *et al.*, 1980). If the void space portion of the silo under the influence of ambient temperature is V_h, the volume of air entering the silo due to temperature change is:

$$V_t = \frac{V_h \cdot R_T \cdot t}{T_0} \tag{6}$$

Similarly, an increase in barometric pressure causes air to infiltrate the silo. Changes in barometric pressure may be approximated by the linear function:

$$P_t = P_0 + R_P \cdot t \tag{7}$$

where P_t is the silo gas pressure in Pa at time t; P_0 is the initial atmospheric pressure; and R_P is the rate of atmospheric pressure change in Pa/h. Since the atmospheric pressure would affect the effective total void volume (V_e) of the silo, the volume of air entering the silo due to atmospheric pressure change is:

$$V_p = \frac{V_e R_P \cdot t}{P_0} \tag{8}$$

The basic assumption in calculating V_t and V_p is that the volume infiltrating the silo is not restricted by leaks. Obviously in a gastight structure not equipped with a pressure relief valve air infiltration into the silo during sudden changes in temperature or barometric pressure will be independent of the leak characteristic. When this happens, the pressure differential required to cause air infiltration is approximately 50% of the pressure differential value. The time required for this process is important. For a given time t_p:

$$\Delta P = \frac{V_t + V_p}{2t_p V_e} \tag{9}$$

by substituting (9) into (1)

$$Q = KA \left[\frac{V_t + V_p}{2t_p V_e} \right]^n \tag{10}$$

so that

$$\log Q = \log KA + n \log \left[\frac{V_t + V_p}{2t_p V_e} \right] \tag{11}$$

If A and t_p are sufficiently small values, then Q will be:

$$Q < \frac{V_t + V_p}{t_p} \tag{12}$$

A quick volume change would result in a pressure change in the system and the value Q should be combined into (3) as:

$$C_t = C_0 \exp\left(-(V_d + Q)/V_e\right) \tag{13}$$

But if A and t_p are large values, then Q is:

$$Q > \frac{V_t + V_p}{t_p} \tag{14}$$

and equation (3) can be transformed to:

$$C_t = C_0 \exp\left(-V_a/V_e\right) \tag{15}$$

where $V_a = V_d + V_t + V_p$.

The validity of the above assumptions was investigated using the 665.7-L experimental silo containing wheat. When a silo is purged with CO_2, the initial change in concentration due to sorption also needs to be considered. If the sorption rate is known, the expected drop in CO_2 concentration can be estimated. The volume of CO_2 sorbed by wheat will

cause negative pressure in the system, and air infiltrating the silo will further lower the CO_2 concentration. This will cause a different rate of sorption than that obtained at a constant CO_2 concentration. Since the proportion of grain volume to the total capacity of the bin may vary, the resulting sorption rate would also vary in relation to the changing CO_2 concentration.

RESULTS AND DISCUSSION

Constant pressure tests

Volumetric flow in small leaks or cracks is strongly dependent on orifice length (*L*). In a grain bin or silo, this orifice length may constitute the thickness of the wall. This relationship was measured for a 1.42-mm^2 cross-section with various orifice lengths. The longer the orifice was, the higher the value of the empirical parameter *n* was. Kreith and Eisenstadt (1957) found that the expression of length-to-diameter (*L/D*) was a function of *n* when determining flow characteristics of short capillary tubes. Using a similar approach, the results obtained for different *n* values were plotted against orifice length/diameter (*L/D*) ratios for leaks of various lengths (Fig. 2).

This relation would clearly indicate that where cracks exist in the structure, the flow characteristics necessary to determine *KA* (Equation 1) will be strongly dependent on the orifice length which affects the pressure-drop expressed by the exponent *n*.

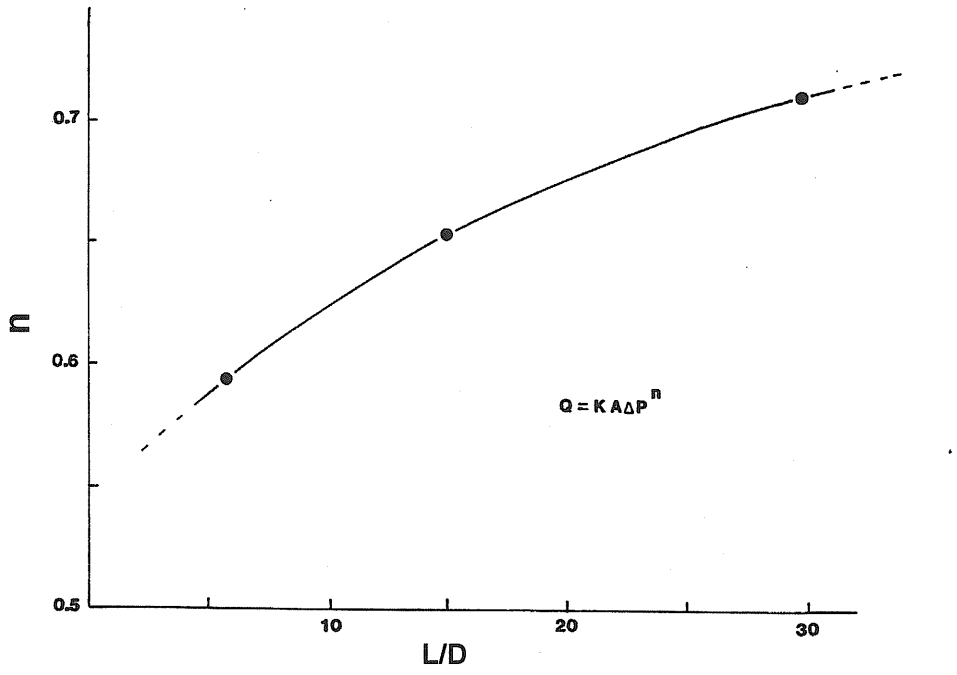


Fig. 2. Relationship between orifice ratio of length to diameter (*L/D*) and pressure difference exponent (*n*).

These results indicated that unless the orifice length is maintained constant, *KA* can not be generalized in relation to exponent *n*. Additional research is needed to quantify the relationship of these constants.

Various orifice sizes 3.15 mm long were studied to determine the relationship of *KA* and *n*. From a practical view, determination of the leak area of a given silo where exponent $n = 0.55105$ is applicable, would require only a single constant pressure test. The empirical values obtained for different areas in cross section were tested for values up to 60 Pa (Fig. 3). Since the current values were studied on round orifices having a fixed length, more experimental values are needed to determine the relation of *KA* and *n* for different leak configurations.

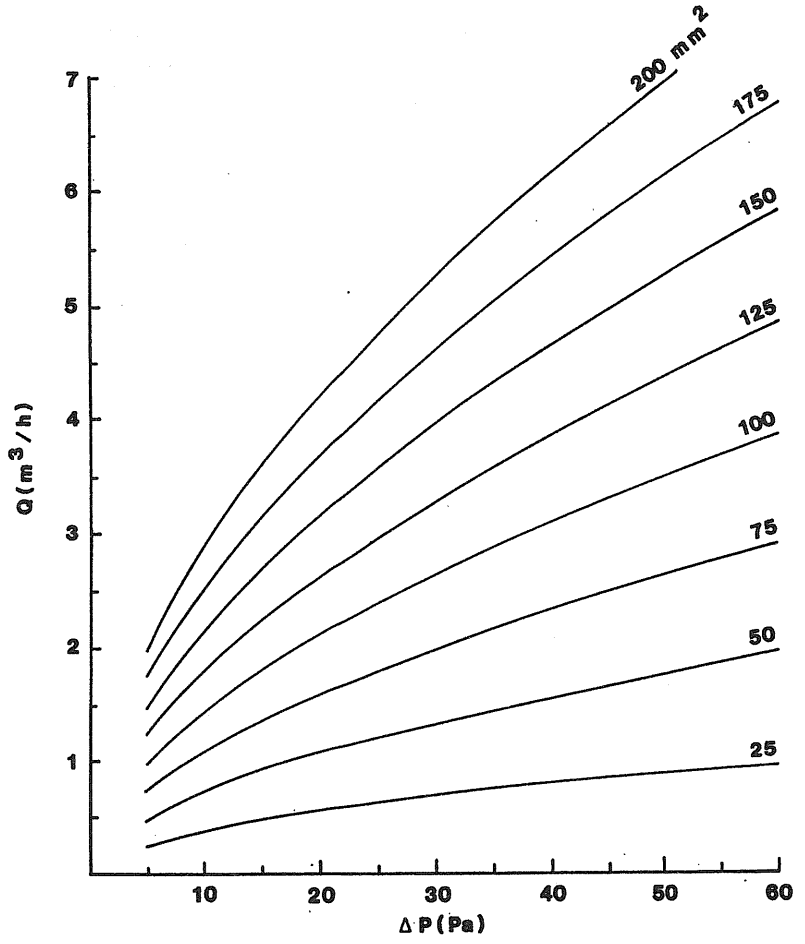


Fig. 3. Calculated family of curves for leak cross-section areas varying between 25 and 200 mm^2, based on constant pressure tests using air at 25°C, where the empirical parameters were $K = 4.0516 \times 10^{-3}$ and $n = 0.55105$.

Influence of temperature

Although the experimental silos were maintained in a controlled temperature room with a thermostat setting of 25°C, the changes in temperature outside the room influenced the frequency of thermostat activation due to heat loss from the room to the outside air. This loss caused the thermostat to activate the heating unit for intermittent periods, especially when the ambient temperature outside the room was in the 0–10°C range. The periodic operation of the heater to maintain the room at 25°C was accompanied by an increase in pressure in the silo, and these periodic changes in internal silo pressure caused a pumping effect which resulted in either blowing out the CO_2-air mixture or sucking ambient air into the silo.

Temperature changes which affected the silo's internal pressure could best be detected when the silo had a leak with a cross-sectional area of less than 1.478 mm^2. Typical silo pressure changes recorded during the observations with leak cross-section areas of 0.586 and 0.068 mm^2 were recorded (Fig. 4). The smaller the leak was, the greater the influence of temperature on the build-up of internal pressure was also. This may be especially important in horizontal silos having a large head space volume. To prevent damage to tightly sealed silos during critical pressure increases, use of a pressure relief valve is recommended by Banks and Annis (1977).

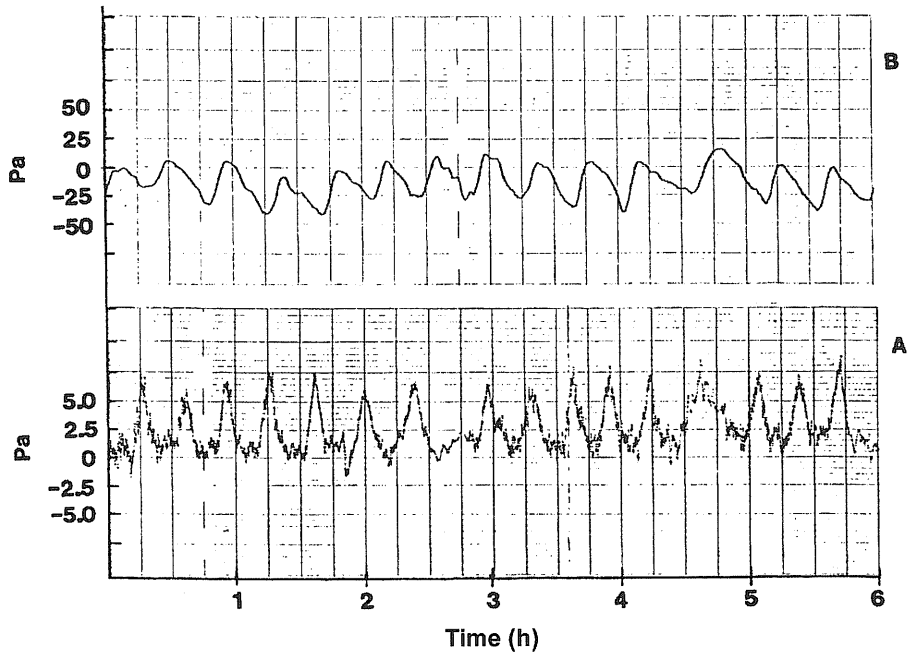


Fig. 4. Typical silo pressure changes recorded under the influence of temperature fluctuations in the experimental silo containing ca. 500 kg of wheat and with 0.586 mm^2 (A), and 0.068 mm^2 (B) leak cross-section areas.

The typical ambient temperature changes recorded in these observations do not represent field conditions where temperature changes may be much greater and last for extended periods of time. However, R_T values (Equation 5) calculated in these experiments ranged from +0.13°C/h to +3.56°C/h. These values may be compared with temperature changes under maritime weather conditions, which may vary between +3°C/h and +10°C/h (Meiering and Wenner, 1970).

Meiering (1982), working with gastight silos, found that when temperature rises 3°C/h, there is sufficient time for gas flow to create pressure equilibrium with the atmosphere at a specific permeability of 0.2 mm^2/m^3 (ratio of leak cross-section area to void volume of silo). Our experiments were carried out with specific permeabilities of 0.23, 2.03, 5.12 and 76.67 mm^2/m^3 and, accordingly, sufficient time existed for gas flow to create pressure equilibrium with the atmosphere.

Influence of barometric pressure

Changes in barometric pressure are important in infiltration of air into silos which are under MA treatment. The dominant cause of air infiltration was the change in barometric pressure when control of room temperature was discontinued. Variations in barometric pressure ranged between 11 and 17 Pa/h in the test room. Under maritime weather conditions, Meiering and Wenner (1970) observed typical atmospheric pressure changes of +25 Pa/h. Their data and results in Fig. 5 demonstrate the importance of local weather observations, when a MA is to be applied, in order to enable the CO_2 loss from the storage structure to be assessed.

Air infiltration into the experimental silo

To control stored-product insects Jay (1971) recommended a MA mixture of 60% CO_2 in air for 4 d at a temperature at or above 27°C. An initial concentration of 60% CO_2 will gradually decrease due to sorption of the gas by the grain (Banks *et al.*, 1980). In our experiments the CO_2 purge was carried out from a single port at the bottom of the silo; this created an initial CO_2 gradient between the different sampling ports. This gradient progressively changed (due to diffusion) until a uniform CO_2 concentration was obtained. The initial sorption of CO_2 by the grain has a significant effect on reducing the initial CO_2 concentration attained. Therefore, in the application of CO_2 to maintain a concentration effective to control insects, addition of CO_2 in the maintenance phase should be balanced against the expected mass of CO_2 to be adsorbed by the commodity.

Four experiments with leak areas of 0.068, 0.586, 1.478 and 22.134 mm^2 were carried out while temperature, barometric pressure and gas composition of the experimental silo were monitored. Results of CO_2 loss and calculated CO_2 concentrations based on Equation 15 are shown in Fig. 6.

Characteristic changes in barometric pressure and ambient temperature were considered in calculating CO_2 loss, and they were plotted against each period of time (20 h, 10 h or less).

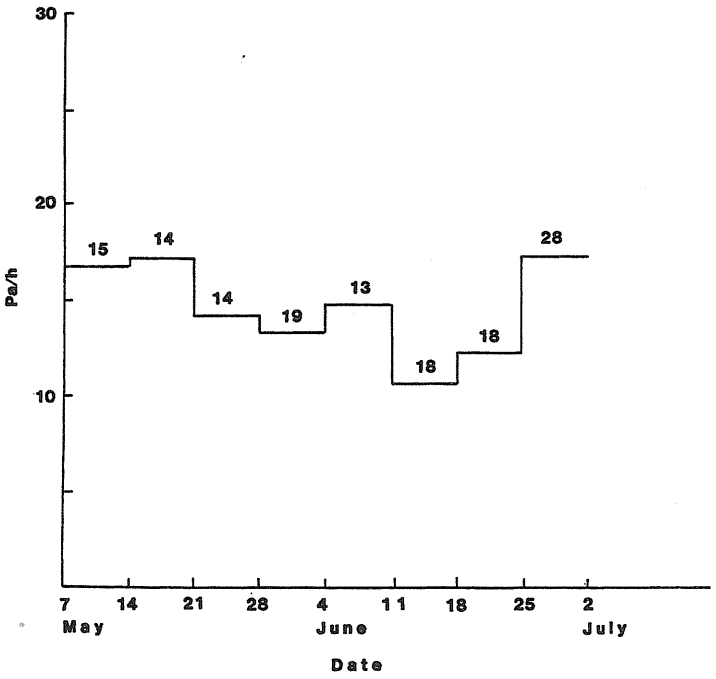


Fig. 5. Typical weekly barometric pressure changes recorded during the tests. Number of changes in barometric pressure are given in figures shown for each weekly period.

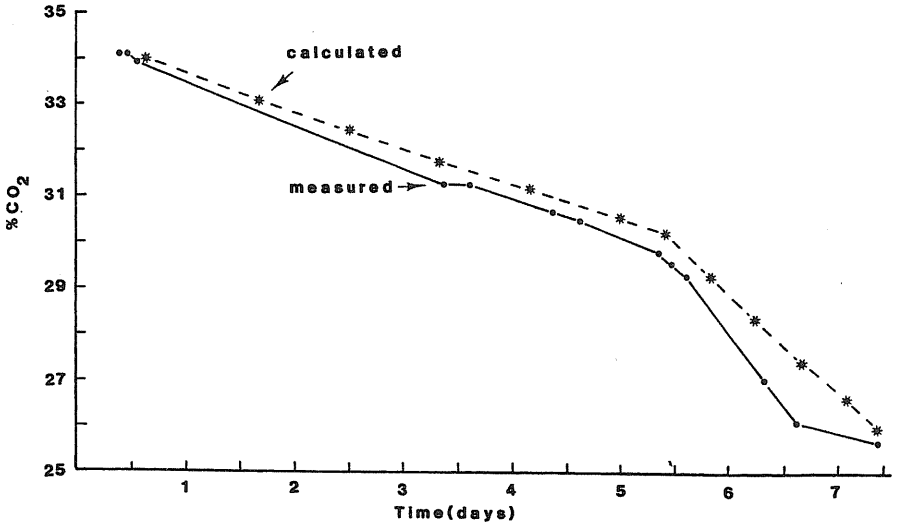


Fig. 6. Measured and calculated CO_2 concentrations obtained in the experimental silo containing ca. 500 kg of wheat and equipped with a leak cross-section area of 22.134 mm^2.

Other parameters affecting CO_2 loss, such as wind and chimney effects, were not considered in these studies. Under extreme weather conditions, the effects of temperature gradients and gas density differences would clearly have additional effects on the CO_2 loss from treated structures (Banks *et al.*, 1975). These weather parameters would require complicated computations using simulation models. Empirical models to measure air infiltration into structures have been proposed by Sherman *et al.* (1980), but application of this information to grain storage facilities requires additional research. If the leak-rate estimates given in the present study indicate that a silo is unsatisfactory for the application of CO_2 (or other MA's), efforts should be made to render the storage structure more gastight. The expected CO_2 loss from a silo containing grain can be assessed by estimating the total leak area and gathering information on expected changes in local temperature and atmospheric pressure. This assessment is essential for evaluating the cost of maintaining an adequate CO_2 concentration during the purge and maintenance phases of treatment. It is also very important in enabling comparison of added treatment costs with the capital investment necessary to render the storage structure gastight.

ACKNOWLEDGEMENT

The author wishes to thank Drs E.G. Jay and J. Leesch, USDA, ARS, Stored-Product Insects Research and Development Laboratory, Savannah, GA, for their support, and Mr G.C. Pearman Jr. of the same laboratory, for his technical assistance during these experiments. Thanks are also due to Dr Ruth Marcus, ARO, Bet Dagan, Israel, for help during the preparation of the manuscript. This research was supported in part by Grant No. I-303-80 from BARD, the United States–Israel Binational Agricultural Research and Development Fund.

LIST OF SYMBOLS USED

A = orifice cross-section area, mm^2.
D = diffusion coefficient, mm^2/s.
L = initial length, mm.
C_0 = initial CO_2 concentration, %.
C_t = CO_2 concentration at time t.
ΔC = CO_2 concentration difference between the silo and ambient.
M = mass of grain, kg.
t = time, h.
t_p = time to cause pressure differential, h.
P_a = atmospheric pressure, Pa (Pascals).
P_t = silo gas pressure, Pa (Pascals), at time t.
P_0 = initial atmospheric pressure, Pa.
ΔP = pressure difference, Pa.
R_P = change of atmospheric pressure, Pa/h.
Q = volumetric flow rate, m^3/sec or m^3/h.

T_t = silo gas temperature, °K, at time t.
T_0 = initial silo gas temperature, °K.
R_T = change of temperature, °C/h.
V_e = silo gas volume, m^3.
V_h = silo headspace gas volume, m^3.
V_a = volume of air infiltrating the silo, m^3.
V_d = volume of air infiltrating the silo due to diffusion through the orifice, m^3.
V_t = volume of air infiltrating the silo, due to temperature change, m^3.
V_p = volume of air-infiltrating the silo, due to atmospheric pressure change, m^3.
n = characteristic pressure difference exponent, empirical parameter varying between 0.5 and 1.0.
K = empirical parameter, multiplier of the orifice cross-section area (A) in constant pressure test.

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SEALING OUTDOOR STORAGE AND FUMIGATION FACILITIES USING PLASTIC SHEETING

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ABSTRACT

Plastic sheeting used for modified atmosphere facilities for storing grain, dried fruits and bee-hives were discussed. Several aspects in the production of plastic membranes and in ensuring sufficiently sealed facilities were reviewed. There are prerequisites for sealed outdoor facilities. The plastic membrane of the structure should have adequate physical strength, a low gas-permeability rate and good UV resistance. The structure should be sufficiently gastight either to allow metabolic processes to create the desired atmospheric composition or to enable modification of the atmosphere by the addition of gas. Manufacturers should perform leak tests before the units leave the production plant.

On-the-spot sealing is employed only for very large units (such as bunkers for grain storage of over 10,000 t or sealed storage of dried fruits containing tens of tonnes) since sealing techniques demand high professional skills, including the use of hot air guns and adhesives. Where grain is stored in units of up to 1,000 t, sealing of liner sections with easily locked plastic tongue-and-groove zippers is convenient.

Prefabricated units have the advantages of a lower price, due to a standard manufacturing process, and ease in operation. On-the-spot-installations are less cost-effective, and testing is rather complicated. In addition, each time a fresh commodity is stored a new sealing process is required.

Tests used for determining airtightness include physically controlling seams and weldings, inflation and audio control, inflation and measurement of drop in pressure, inflation with colored smoke and ultrasound.

INTRODUCTION

In the seventies PVC sheeting was developed for the storage of military equipment in the open, a method frequently termed "dry storage". It was necessary that military emergency equipment be stored in a way that ensured minimal maintenance and maximum preservation of the stored equipment. Tanks, heavy artillery and planes are stored in plastic envelopes which prevent water vapor (and dust) from penetrating through the sheeting into the storage space. At the same time, because it is designed to be used outdoors, the sheeting has to be extremely robust, resistant to degradation by UV light and resistant to rodent penetration.

When the scientists of the Agricultural Research Organization (ARO) requested us to manufacture a plastic membrane for the hermetic storage of grain, it was clear that the properties of the sheeting described above also made it suitable for this civilian use. However the requirements for hermetic storage are different from those for military dry storage.

In the case of dry storage, the prevention of any movement of water vapor across the liner is the main issue. For hermetic storage and storage under modified atmospheres, the sheeting should prevent (or reduce to a minimum) the penetration of oxygen (O_2) from outside into the O_2-deficient atmosphere created within the liner, and it should at the same time prevent the carbon dioxide (CO_2) produced within the grain bulk from permeating outwards into the atmosphere. Our tests and experience have shown that the thickness of the PVC liner is crucial both in terms of gastightness and UV resistance. To put it differently, the thinner the PVC, the poorer will be both the gastightness and the UV resistance.

PVC sheeting is made flexible by the addition of plasticizers to the PVC resins. These oily substances tend to migrate from the resins under the influence of sunshine, thus causing the sheeting to become stiff and brittle with time. Only experienced producers of PVC lining can fabricate a membrane in which this migration process is sufficiently retarded to enable the material to have a reasonable life span. Under Israeli conditions, in certain areas there are 305 d of sunshine a year. We have, however, been able to develop a liner with a life span under constant exposure to solar radiation of over 10 years. Recent advances in the chemistry of plasticizers have resulted in the development of dry plasticizers which enable the life span of the sheeting to be doubled. However, these dry plasticizers are not very widely employed due to their high price.

MATERIALS AND METHODS

Sheeting requirements

Plastic sheeting used for sealed outdoor facilities should meet several conditions: the sheeting should be adequately gastight (impermeable to water and water vapor and with low permeability to CO_2 and O_2); the technique used for connecting the sheets together should provide a sufficiently gastight seal; the sheeting must be UV resistant; the sheeting should be rodent repellent or rodent proof; the price of the material should be reasonable; the sheeting should be flexible and easy to handle; and the sheeting should be easily welded by high frequency (HF) sealing methods.

Structures for storage

To provide a suitable and user-friendly storage solution that incorporates the PVC-based sheeting, a good design for the storage structure is required. Such a structure should meet several conditions. In the case of grain storage, it should be tailored to fit either bag-stacks or bulk. In the case of fumigation or artificial gasing, it should be manufactured to fit the size of the packed commodity. It should be simple to erect and to dismantle. Final sealing should be easy. The storage unit should be rodent proof. The price of the structure should make it cost-effective. The structure should be easy to repair.

Finally, the stack or the bulk load should be easy to move into the structure, given local usage, labor costs and transport facilities (O'Dowd *et al.*, 1987).

The best sealing results are obtained by hot-air welding or HF sealing. HF sealing is preferable since this technique does not affect the molecular substance of the PVC and is performed in workshops where final testing is easier than in the field. The structure should be sealed in such a way that the natural modification of the atmosphere, caused by the aerobic respiration of the grain, stored-product insects and microflora, will take place within a few days after sealing. The level of hermetic seal is best evaluated by a pressure test. This is done by creating a negative pressure within the liner using a vacuum cleaner and measuring the rate of pressure change (a negative pressure of 5 cm water gauge to fall to 2.5 cm in 15 min or more is usually taken as acceptable).

Three types of structure employing PVC liner technology are at present in use for grain storage (Navarro *et al.*, 1990). They are Volcani cubes for 5–150 t grain in bags, 250-t, 500-t and 1,000-t mobile silos and bunkers for large bulk storage (up to 10,000–15,000 t).

Although cereal grains are without any doubt the most abundant commodity suitable for hermetic storage, such additional applications (using artificially modified atmospheres) as storage tents for fumigation and storage of honey combs under CO_2 and storage of dried fruit under CO_2 have been developed. These latter two structures will be reported elsewhere.

RESULTS

Frameless flexible envelopes (Volcani cubes)

These consist essentially of the same PVC liner as that used for the larger structures. They are intended for bag storage of small quantities (approximately 10, 20 and 50 t) of cereal grains. Since no rigid frame is required, the liner is made of an upper and a lower section which can be zipped together to form a gastight seal. The envelopes, also termed "cubes", are easy to erect and dismantle. They are particularly suitable for on-farm storage and storage by farmer cooperatives. For trucking operations they can be transported with the grain load, and the sacks can be off-loaded directly into the cubes at the point of destination (Donahaye *et al.*, 1991).

The storage cubes consist of a lower floor-wall section of heat-welded flexible 0.83-mm-thick PVC formulated sheeting, laid on the ground, onto which the sacks are stacked. When fully loaded, the upper roof-wall section is drawn over the stack and the two sections are hermetically sealed using the gas-proof zipper. The zipper is covered by a protective over-flap. The "10-t" liner is $336 \times 298 \times 150$ cm ($l \times w \times h$) (Fig. 1), giving a maximum storage volume of 15 m^3. It weighs 43 kg when empty. The "20-t" liner is $445 \times 336 \times 200$ cm ($l \times w \times h$) with a maximum storage volume of 30 m^3. It weighs 76 kg when empty.

Weld-mesh walled silos (mobile silos)

These are suitable as medium-sized silos with a capacity of up to 1,000 t. A circular bag contained within a vertical wall of galvanized weld-mesh is used. After the ground is

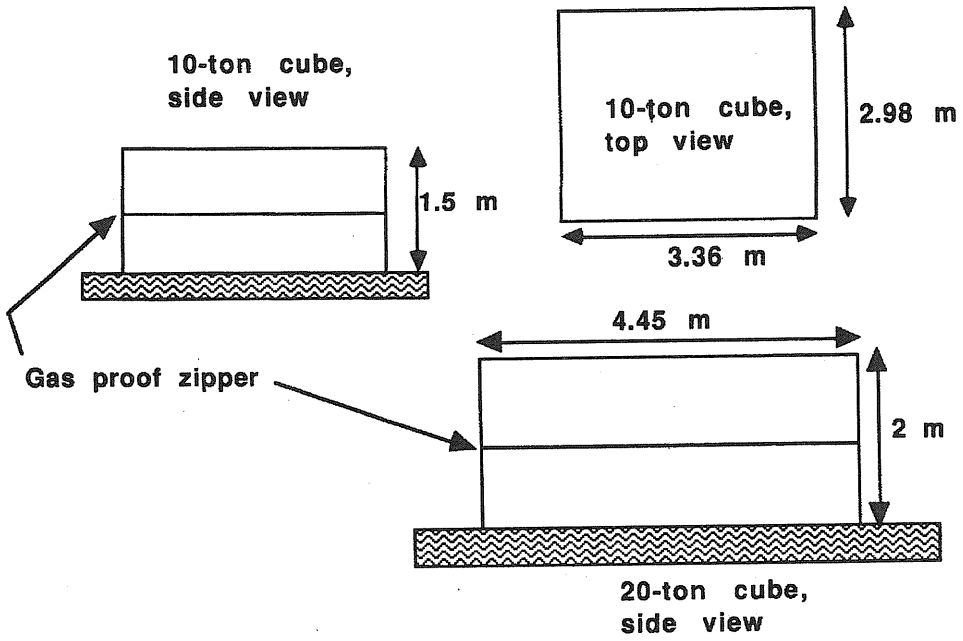


Fig. 1. Plan of 10- and 20-t capacity Volcani cubes for bag-storage of grain.

leveled and cleared of stones, weld-mesh sections are bolted into place to form a circle leaving the floor-wall package within the perimeter. The package is then opened and the walls of the liner are tied to the weld-mesh. These silos can be equipped with aeration systems, in which case the aeration ducts should be placed in position before loading the grain (Fig. 2). Care must be taken to load the silo exactly from the center point. The roof section is then placed over the grain, using a pre-attached rope to pull and unfold the PVC liner. After the roof section is evenly spread over the grain, it is zipped to the wall to obtain a gastight seal (Fig. 3). This method is highly suitable for mechanized grain handling and the integration of aeration systems for bag or bulk storage. The suitability of these silos for buffer storage of emergency stocks in arid regions has been evaluated (Calderon *et al.*, 1989).

Bunker-type storage

This is proposed for capacities larger than 1,000 t. It consists of a bunker bordered on three sides by ramps of earth, excavated from both inside and outside the site, which form the structural wall of the silo. The following is merely a description of the conditions under which the first storage trial in Israel was carried out. Detailed results of this trial have been published elsewhere (Navarro *et al.*, 1984). This bunker was 150 m long and 50 m wide. The earthen floor was bordered on three sides by earth ramps upturned from the floor during leveling and grading, and the fourth end was left open for grain loading. The ramps were 2 m

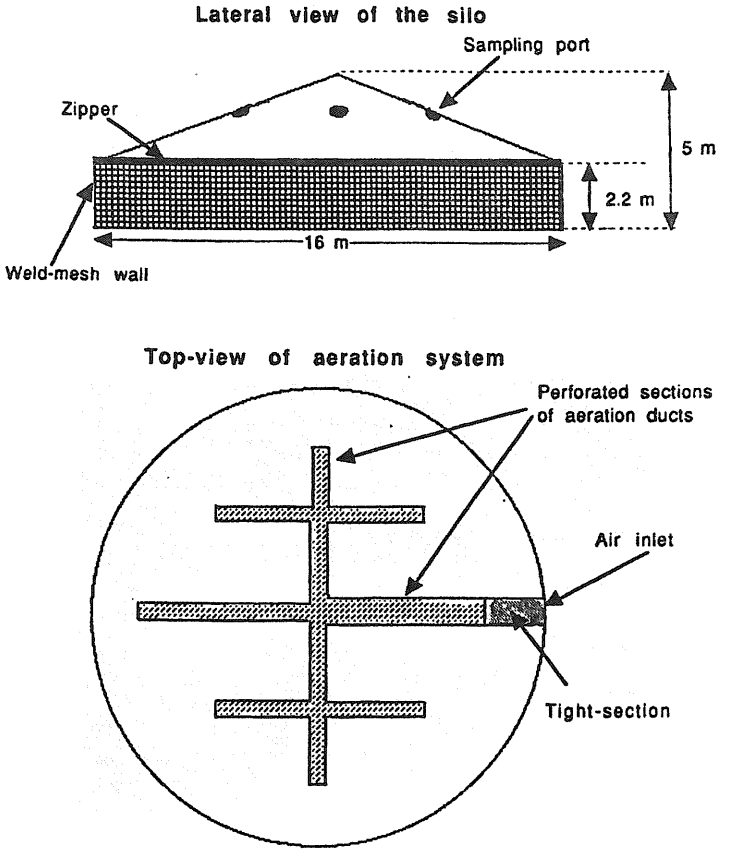


Fig. 2. Schematic view of a 500-t capacity weld-mesh silo including lay-out of aeration ducts.

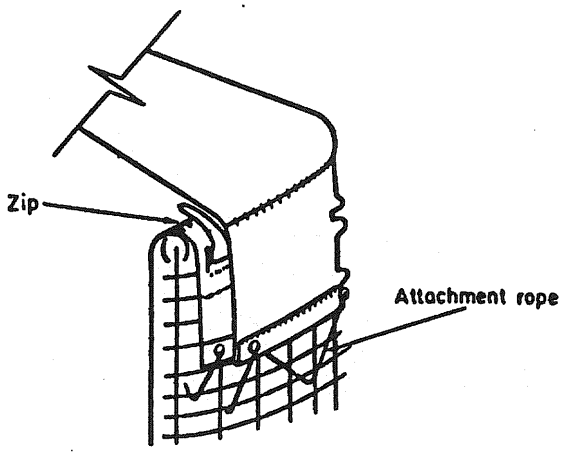


Fig. 3. Method of securing roof-cone to floor-wall unit and weld-mesh walls in silos.

high and 8 m wide at the base, and they were leveled to permit drainage of rain water away from the outer sides (Fig. 4). Before loading, the floor and ramps were lined with overlapping strips of 0.25-mm-thick polyethylene sheeting laid transversely to form a continuous underliner. The overliner was 0.83-mm-thick PVC formulated sheeting, factory-welded into strips 4 m wide, that were laid over the grain surface and then welded together *in situ.* The two liners were joined at the top of the ramps, where they were overlapped, folded and buried in a 60-cm-deep trench to form a hermetic seal (Fig. 5). The ramps were then further protected against erosion by rain-water run-off in winter by a 4-m-wide extension welded to the base of the overliner. Since completion of the first bunker trial, this method of storing grain reserves has become routine practice in Israel.

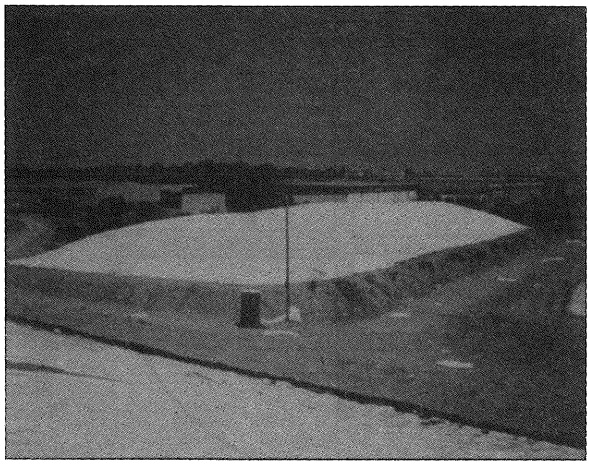


Fig. 4. General view of a bunker containing 15,000 t of wheat.

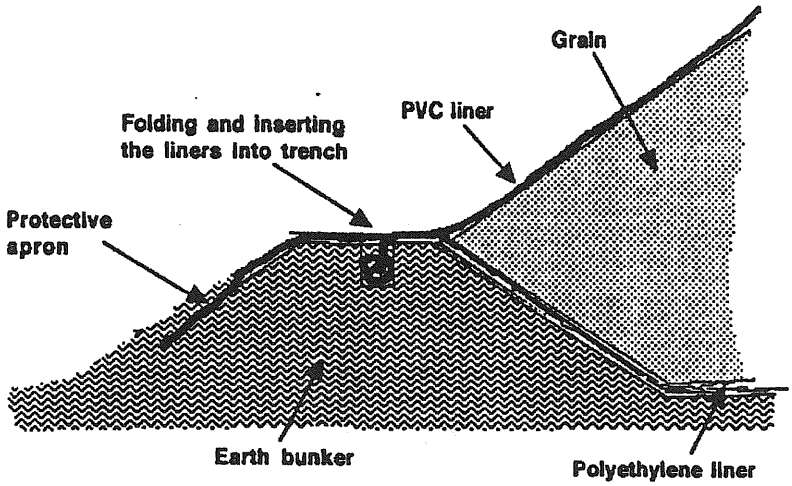


Fig. 5. Section of earth bank to show folded liners in trench and the protective apron.

DISCUSSION

The structures here described are recommended only for dry-grain storage (Navarro and Donahaye, 1976; Kenneford and O'Dowd, 1981). Although hermetic storage of moist feed-grain has been investigated in temperate climates (Hyde and Oxley, 1960), its use in the tropics has not so far led to encouraging results (Hyde, 1969). Loading and unloading cannot be carried out during rainfall unless the structures are under cover. Otherwise, loading and emptying can be carried out only during dry spells. All three types of structures described should be filled to their rated capacity and should not be left partly full (Navarro and Donahaye, 1985).

Rodents can gnaw through the plastic liners. However, the construction system and the layout of the material over the grain, which keeps the plastic under tension, provides a slippery surface which makes it extremely difficult for rodents to make an incision in the material with their teeth. This has been confirmed in trials carried out in our laboratory with liners kept under tension and exposed to both roof rats and house mice captured in the field. Field trials at heavily infested sites have revealed only isolated cases of rodent damage in full silos and bunkers. However, empty silos and envelopes are much more susceptible and should be folded up and stored above ground level to guard against rodent attack.

The durability of the plastic material and its resistance to adverse climatic conditions of solar UV irradiation and high temperatures both offer distinct advantages. The systems can be sealed to a degree of gastightness sufficient to control insects (Fig. 6), based on the principle of hermetic storage (Hyde *et al.*, 1973). Should the necessity arise for chemical control, the hermetic seal ensures effective retention of fumigant concentration. For larger bulks, mechanization of grain handling, temperature monitoring equipment and, for weld-mesh silos, aeration systems can all be easily incorporated.

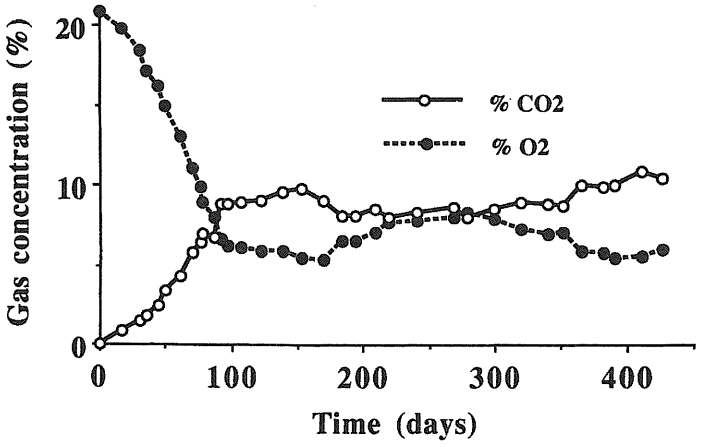


Fig. 6. Average CO_2 and O_2 concentrations (%) in the bunker-type silo containing 15,567 t of wheat bulk during the storage period (Navarro *et al.*, 1984).

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THE RESPONSE OF THE SILO MANUFACTURING INDUSTRY IN AUSTRALIA TO THE SEALING OF TRANSPORTABLE GRAIN SILOS

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ABSTRACT

Research in Australia in the 1970's suggested it was possible to seal farm-grain storages to create an environment in which fumigation would be effective. The information was communicated to all government advisory agencies across Australia for dissemination to manufacturers and users of grain silos. In 1982 in Western Australia this information was transferred by the Agriculture Protection Board through its extensive network of officers. Sealing standards were recommended to silo manufacturers and advice on the best fumigation practice was communicated to farmers. In the eastern states of Australia the extension network, although comprehensive, relied on individual efforts by advisers to create transfer opportunities.

In Western Australia sealing techniques were adopted by all manufacturers. The economics of manufacturing and the requirements of purchasers influenced refinements in design. In the eastern states of Australia, advice was acted on by only some manufacturers as there was low demand for sealed silos from growers. Later campaigns to stimulate interest in the production and use of on-farm sealed grain storages created interest in better fumigation techniques. The stimuli for a change to sealed silos and the developments in silo design are discussed.

INTRODUCTION

The silo manufacturing industry in Australia has undergone gradual change as the technology for sealing grain storages (Banks and Annis, 1980) has been adopted. Andrews *et al.* (1994), summarising the barriers to adoption of on-farm sealed silos, cited, among other reasons, the availability of contact insecticides in most Australian states, the lack of extension material and resistance to change.

Dean (1994) provided an overview of the Western Australian (WA) approach to integrated grain protection in which these barriers were overcome by restricting the availability of contact chemicals and providing good extension. Silo manufacturers were encouraged to experiment with modifications to existing manufacturing methods to create

a sealed silo. Because in 1982 the extension campaign exerted influence on growers to request sealed storage from manufacturers, demand created supply.

In the eastern states of Australia (New South Wales, Queensland, Victoria and South Australia) the technology to create sealed structures was promoted, but it was not widely adopted by manufacturers. Early extension work was characterised more by individual effort than by a co-ordinated statewide approach. The initial publicity encouraging growers to switch their insect-control techniques from contact chemicals to fumigants did cause some demand for sealed silos, and subsequent extension work relied on generalised references to the use of sealed storages, newspaper articles, pamphlets (B.E. Wallbank, 1995, personal communication) and the manufacturers' own advertising. Some opportunities were created by individual officers in state departments of agriculture. Through the media of grower groups, field days and articles, the use of sealed silos was promoted among a wide range of grain management techniques (P. Botta, 1995, personal communication).

Later campaigns by the Grains Research and Development Corporation (GRDC) encouraged manufacturers to switch to the production of sealed silos by 1996. Commitment was achieved when manufacturers agreed to offer sealed silos as part of their range although not as their entire line. The grower-targeted campaign GRAINSAFE was outlined by Andrews *et al.* (1994); it had a significant impact on the adoption and understanding of the sealed-silo option. Despite widespread endorsement by growers, silo manufacturers and extension workers, due to lack of commitment in terms of funding and staff resources (A.S. Andrews, 1996, personal communication), the momentum of the project was not maintained.

In the eastern states, both the commercial availability of contact chemicals and anecdotal evidence circulating in the farming community about moisture problems in sealed silos remain a barrier to change. A much wider use of aeration and perceived problems in maintaining sealed storages in working condition have reduced the demand for fully sealed silos. Manufacturers, who are not committed to the production of sealed silos, suggest that the phosphine metering system, SIROFLO®, is the solution to grain insect control in silos.

SILO MANUFACTURERS SURVEYED

A telephone survey of 30 manufacturers across Australia, all members of the Australian Silo Manufacturers and Grain Storage Association Inc., revealed the different approaches to extension taken by the various states.

In WA, where the use of sealed silos was strongly promoted, manufacturers reported that sealed silo production comprised 80 to 100% of their total silo production, including speciality silos for fertiliser and finely divided grain products. For on-farm storage of whole grain, sealed silos now comprise 100% of production. Problems arising from the use of sealed silos are usually answered promptly or passed on to Agriculture Western Australia.

In the eastern states, sealed-silo production as a percentage of total production varied among manufacturers from 1 to 100%. Many manufacturers expressed doubt about their ability to answer growers' queries concerning grain management or fumigation in sealed storage and said that they did not have ready access to a source of expert advice.

The survey asked questions, discussed in greater detail below, about silo design and construction.

THE CHANGE TO SEALED SILOS

Some manufacturers effected change quickly. These manufacturers were usually those already producing a silo close to a sealed standard such as silos that were fully welded or had sealed eaves. Some manufacturers took 12 months to modify the design so it would accept a sealant to produce a durable seal. Modifications continue to be made in response to both buyers' requests and the economics of the construction process. Enthusiasm for the concept of sealed storages among the manufacturers surveyed varied, some endorsing and some rejecting the idea. The attitude of each manufacturer was usually related to the design of his original silo. If the silo was too difficult to re-design, it was decided not to change it so long as sales remained steady. In WA this was the decision of one manufacturer. The extensive publicity campaign in that state, however, caused consumers to switch to sealed silos, and that particular manufacturer's sales plummeted; silo production is now a minor part of that enterprise.

Wall construction

Most manufacturers of transportable sealed silos in Australia use either fully-welded or riveted-wall construction methods.

The majority of the walls of transportable sealed silos are riveted. They are made from 1–1.2 mm galvanised steel sheeting or ZINCALUME® (aluminium/zinc coated steel sheeting). The steel, purchased in coils, is passed through a series of rollers to create a circle (Fig. 1). During this process, indentations (such as ribs for rigidity or a flange to lock consecutive wall sections together) are pressed into the sheet. The ends of the sheet are overlapped by approximately 150–200 mm to form a ring and then riveted. Fully welded silos use a thicker, 1.6–2 mm, steel and the formed ring is butt-welded. Transportable sealed silos range up to 190 m^3, with an average farm silo being about 64 m^3.

A silo is usually constructed by joining a completed ring to a finished roof section; the joined sections are then lifted and the next wall ring moved underneath for fixing. One manufacturer, however, constructs the silo barrel from narrow coils of sheet steel in a continuous lock seam, starting from the completed roof and forming the walls in a spiral.

Silos that are manufactured for on-site erection use short curved wall sections which are punched, curved and ribbed prior to delivery and then bolted into a circle before being joined to the completed roof.

In WA 40% of manufacturers use the flange/cap system of wall joining. In the rolling process both edges of the sheet are turned down at an angle of 120–90°. When joining two

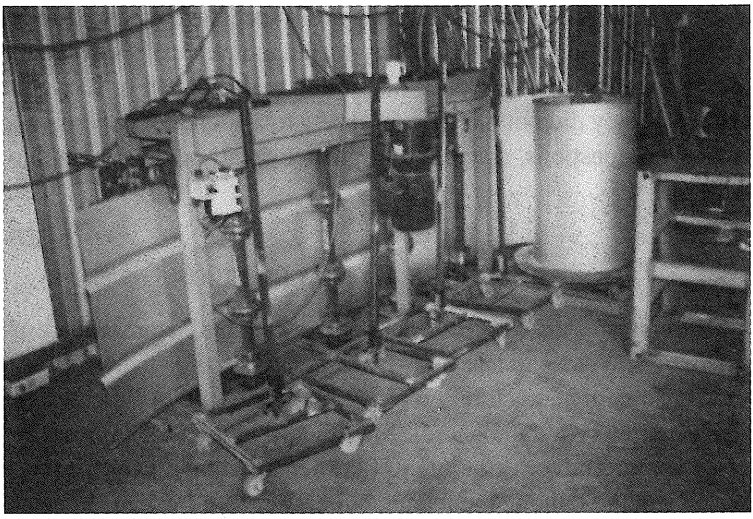


Fig. 1. Rolling machine used to form the silo sections.

consecutive rings, rivets are punched through the flange and cap on the outside of the wall and sealant pressed into the natural vee that is formed on the inside (Fig. 2). The alternative wall-jointing technique is to overlap consecutive sheets about 50 mm and rivet through. The sealant may be applied either between the sheets or over the top of the completed join (Fig. 3). Some manufacturers use high-tensile, sealed, lock-stem rivets, while others use a two-piece swaged-rivet fastening system that requires two people to

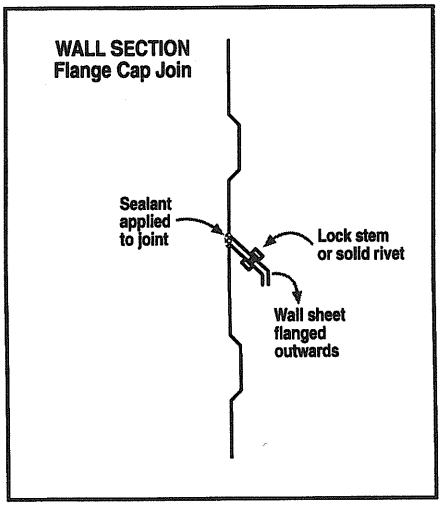


Fig. 2. Wall section, flange cap join.

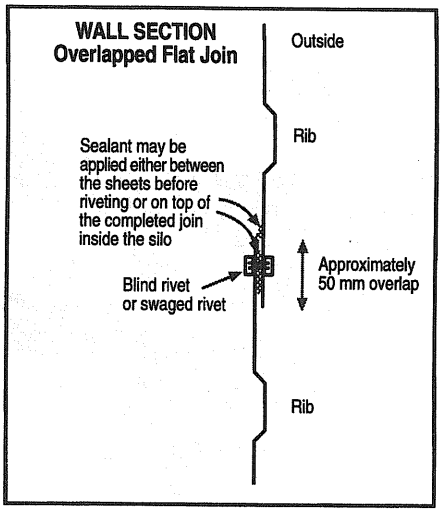


Fig. 3. Wall section, overlapped flat join.

install, one to place the locking nut on the inside before the bolt is pulled through into place and the other on the outside.

Flat floor silos, ranging up to 1,280 m^3 for on-farm use, are constructed by assembling the roof section and then raising the structure on jacks before bolting on the lower sections. A sealing kit, offered as an optional extra, can be fitted during construction. A more expensive alternative is to seal the silo externally or internally with sprayed-on membrane-type paint.

Elevated on-farm silos constructed on site usually have a capacity of 128–833 m^3. The cone base is transported to the site in sections and either welded or bolted together. Because ZINCALUME® is not available in thicknesses over 1.2 mm, the silo walls are typically constructed from 1,200 × 2,400 mm plates of flat, galvanised 1.2–2 mm sheet steel. The silo is again constructed roof first, but it is then raised by a mobile crane to enable the fixing of the cone base to the last ring (Fig. 4). Silicone is applied between the joints and the bolts are sealed with a neoprene washer.

Roof construction

Silo roofs are either segmented, lock-seamed or fully welded. The traditional segmented style is commonly found on vertical silos in Australia. It is a strong roof and does not require bracing except over a very wide area, but a higher labour input is needed to seal the individual segments. A development of this style is the fully lock-seamed roof that has fewer joins to seal but requires additional support for strength (Fig. 5). The choice of roof appears to depend largely on the construction preferences of the particular manufacturer. The factories that have adopted a lock seamed style claim the roof is simpler,

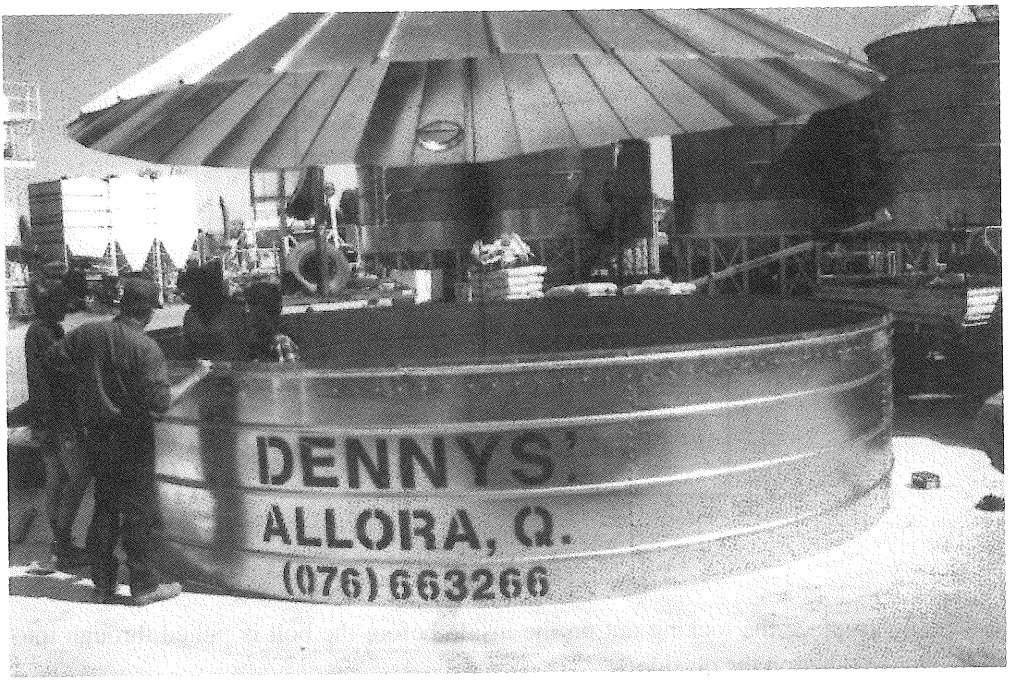


Fig. 4. Assembling silo section on site before joining to the roof.

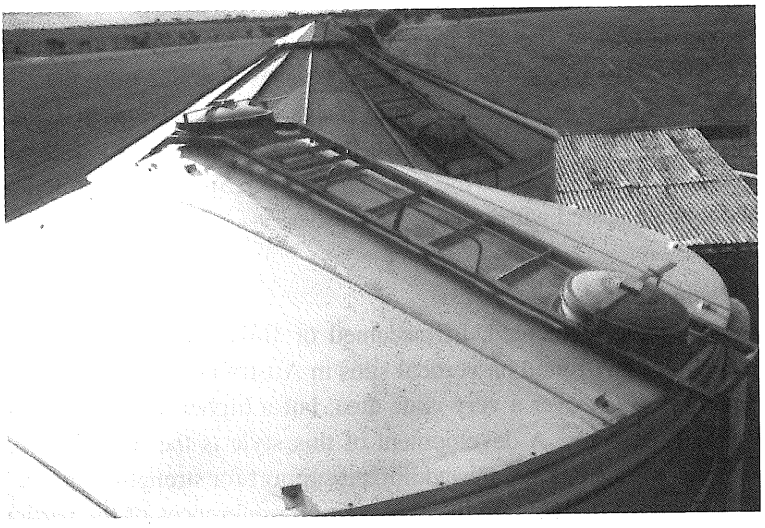


Fig. 5. Lock-seamed roof-front. Segmented roof-track.

more economic to construct and easier to seal. The manufacturers that construct a segmented roof contend that it is more convenient to their operation and that sealing presents no problems.

Cone-base construction

Almost all transportable elevated steel silos made in Australia have a fully welded 1.2–2 mm sheet steel base. One manufacturer in WA is producing a lock-seamed cone base with some welds for support. The majority of silo manufacturers use sheets of hot rolled steel; the rest use galvanised sheet steel. Some manufacturers in the eastern states have developed automatic devices to weld the seams of a galvanised cone base. The support frames vary considerably in design and materials. A large proportion of manufacturers use SILO TUBE® as the upper support ring. This is a modified rolled hollow section (RHS) tube, made from black or galvanised steel, designed for use in silo construction. One edge is angled to allow better sealing of the cone to the wall sections. Sealant is applied on the inside in the angle formed by the joint (Fig. 6). The base support ring may be made of rectangular RHS. Some support frames use galvanised pipe for the upper and lower rings. The legs, of the same material, are crimped at each end for ease of welding to the pipe curve. A few silo base frames are made with curved angle irons for the upper support ring, and some have a bar of flat steel curved around the silo to which the legs can be welded.

Heat reduction

Hill *et al.* (1989) investigated steel silo-wall materials to find out which reflected the most solar radiation, thus reducing the internal temperature. They found that a white coated sheet-steel silo provided the most reflection and that a weathered galvanised silo

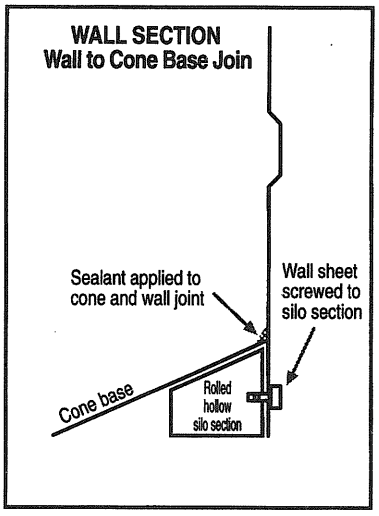


Fig. 6. Wall section, wall to cone base join.

was most absorptive of solar radiation. ZINCALUME® was rated close behind white steel sheeting.

Most manufacturers of silos in WA have changed their silo wall material from galvanised to ZINCALUME® steel sheeting. A white silo is not offered as part of the range for sale because, manufacturers point out, they are more expensive, and in addition the colour is often damaged in transit which results in the delivery crew's being delayed while the paint is repaired. Despite the research and extension to outline the advantages of a white silo, few farmers in WA have painted their silos either because the paint has to be maintained or because ZINCALUME® retains its reflective ability for many years. Silos are usually painted white only when existing farm silos are sealed.

In the eastern states of Australia, ZINCALUME® is used by a smaller number of manufacturers but still represents a significant portion of the market. A few manufacturers build a fully welded silo from hot rolled steel which is then painted white. Others construct riveted, galvanised silos and offer a painted white silo as part of their range. One manufacturer offers part of its range of silos in white COLORBOND®. Overall demand for white silos is, however, low.

Inlets and outlets

Most manufacturers of sealable silos use inlet ports of a similar design. The lid is spun from a single sheet of galvanised steel. The ring or band on which the lid sits may be a single spinning or a rolled band with a welded seam. Rubber stripping is affixed to either component to create a seal (Fig. 7).

The design of the outlets is influenced by the users of the silo and varies considerably. In WA some manufacturers produce a fully sealed rotating boot for the lower outlet; others construct a simpler fixed, sealed design (Fig. 8). The outlet boots are sealed with rubber stripping when the door or lid is closed. A recent trend in WA has been the production of an optional removable hopper, but in eastern Australia it is more common for silos to have a separate auger hopper.

The grain control is most commonly a slide gate, but some silos are fitted with a butterfly valve operated by a remote lever system (Fig. 9). Where a boot is fitted, the control is located inside it and may be operated externally or internally.

All elevated steel silos are now fitted with a safety access hatch in the cone base facilitating cleaning when the silo is empty. In New South Wales this is a mandatory requirement, and all other states have followed voluntarily. The hatch is fitted inside the cone base and may only be opened when the silo is empty, thus preventing accidental discharge. The silo can be cleaned from this base hatch, partially obviating the need for an internal ladder. Some manufactures are now removing internal ladders entirely and fixing a permanent guard in the upper hatches to prevent personnel entry.

Seals and sealants

Strips of rubber are used to seal the inlet ports. The type and shape of the rubber used varies considerably among manufacturers. The most common is the rectangular



Fig. 7. Spun lids with rolled welded band.

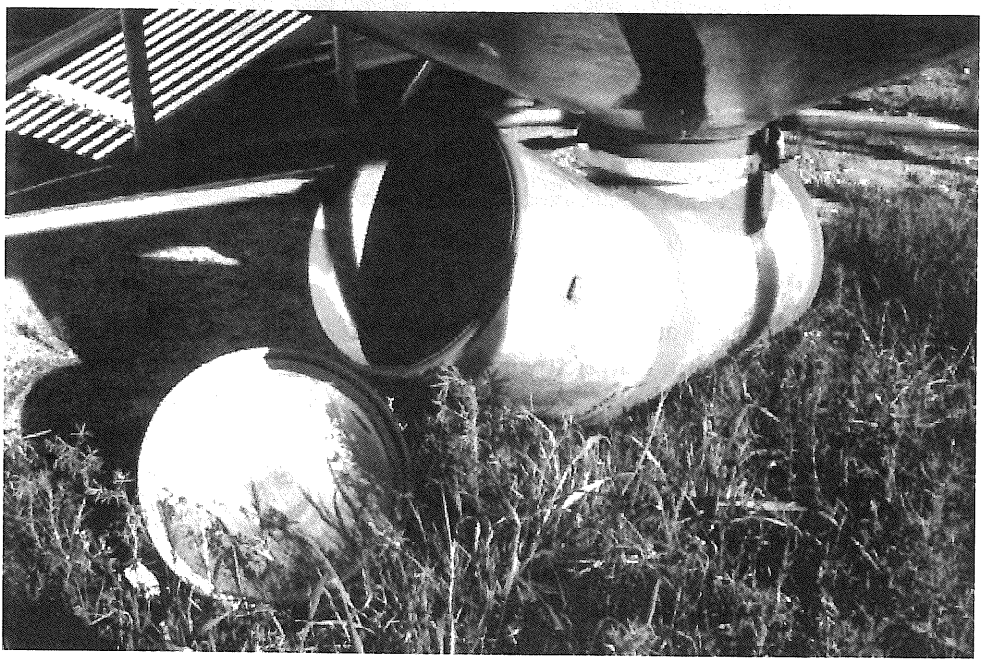


Fig. 8. Sealed rotating boot with internal grain control.

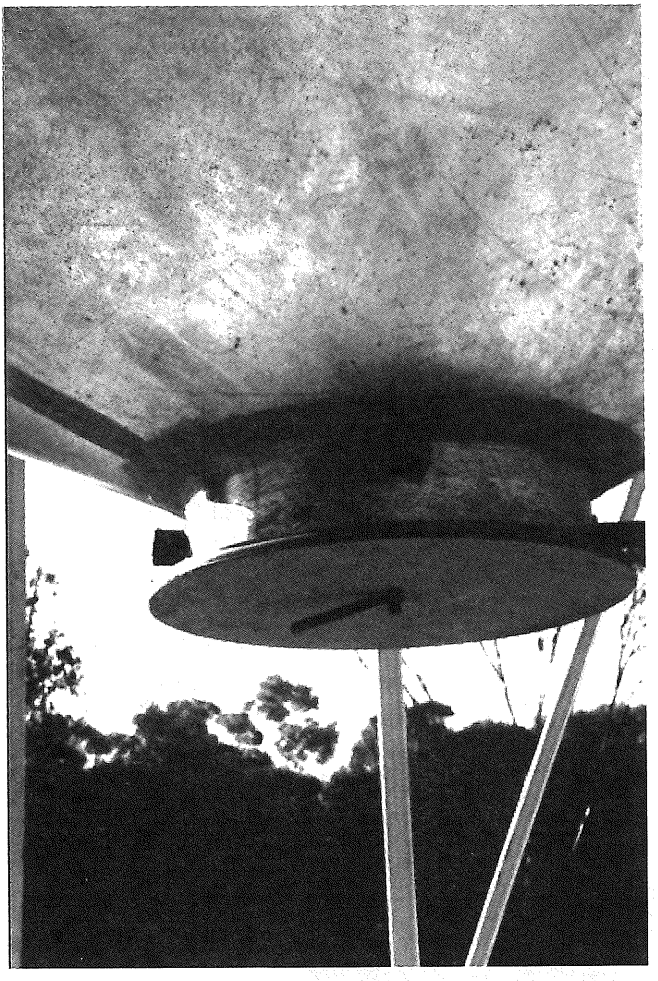


Fig. 9. Sealing plate for a butterfly grain control.

section. Others are round, and some manufacturers use a split section to fit over the ring or band. Some silos are delivered with a polyester foam rubber strip which needs to be replaced at least annually. More durable is the EPDM (Ethylene Propylene Diene Mixture) type of foam rubber that is being used by an increasing number of manufacturers. The outlet ports are fitted with a variety of rubber profiles to create a seal. Flat sheet rubber is used on grain control slides equipped with a levering or bolting device to create pressure on the silo. Most manufacturers seal their silos with a silicone-based neutral cure-caulking compound. Other commonly used sealants are of the polyester adhesive/sealant type. A few manufacturers use spray-on flexible membrane paints, but these are primarily confined to use in later sealing of existing on-farm silos.

Pressure relief valve types and position

The positioning of the pressure relief valve has a bearing on the speed at which air can be drawn into the silo under vacuum conditions which may occur in a thunderstorm on a hot day or during outloading. A valve fitted into the upper wall of the silo, giving the most rapid compensation, also allows faster dilution and loss of fumigant gasses. In this position it is also difficult to see the oil level in order to check the half-life pressure-decay test.

A valve fitted into the lower wall of the silo below grain-load level gives slower air compensation on demand but reduces the dilution of fumigant gasses to a minimum. It has also been suggested that when the silo is under fumigation the lower valve provides a haven for insects due to some admixture with outside air (P.C. Annis, personal communication, 1995).

A compromise between the valve-placement options is a low-set valve with a tube leading into the headspace. This position, due to its remote location, reduces admixture of the headspace gasses. The valve is also more accessible and easier for the operator to read, particularly when he is conducting the annual check of the silo to determine its gastightness.

Of the silos purchased as sealed which were tested by Newman (1994), only 27% passed a standard gastightness pressure test (a half life of 3 min for a full silo and 5 min for an empty silo from a 25-mm head of water gauge). The silos that passed this test were found to be less than 3 years old (Newman, 1989). It was estimated that less than 1% of silo owners test their sealed silos annually for gastightness.

Most manufacturers place the valve where it is convenient to their manufacturing process or where it will be protected from damage when installed.

The most commonly used pressure-relief valve is the box type with a double interconnecting chamber (Fig. 10), made and supplied by plastics manufacturers in various designs. Valves constructed by silo manufacturers tend to be of the PVC tube-and-fittings type (Fig. 11).

During outloading, the pressure relief valve alone is not large enough to allow air inflow without risking the collapse of the silo roof. Several roof collapses have been reported in WA. Operators must open the loading hatch or else fit a vacuum-relief valve large enough to allow sufficient air into the silo when outloading.

Moisture in sealed silos

Most manufacturers report a few complaints from growers about moisture in a sealed silo. Investigation of the problem usually reveals that grain with a higher than acceptable moisture content (m.c.) was stored or that, because no fumigation was conducted at inloading, insect activity has increased. If it is instead found to be a manufacturing fault, the silo is either repaired on site or replaced.

The author investigated a typical complaint from a grower that high moisture occurred in a sealed silo several months after inloading. Two silos were tested for grain m.c. which was found to be just below 12%. Phosphine (PH_3) tablets were added at the recommended rate of 1.5 g/m^3 and the silos monitored over 6 months. The m.c. remained almost constant

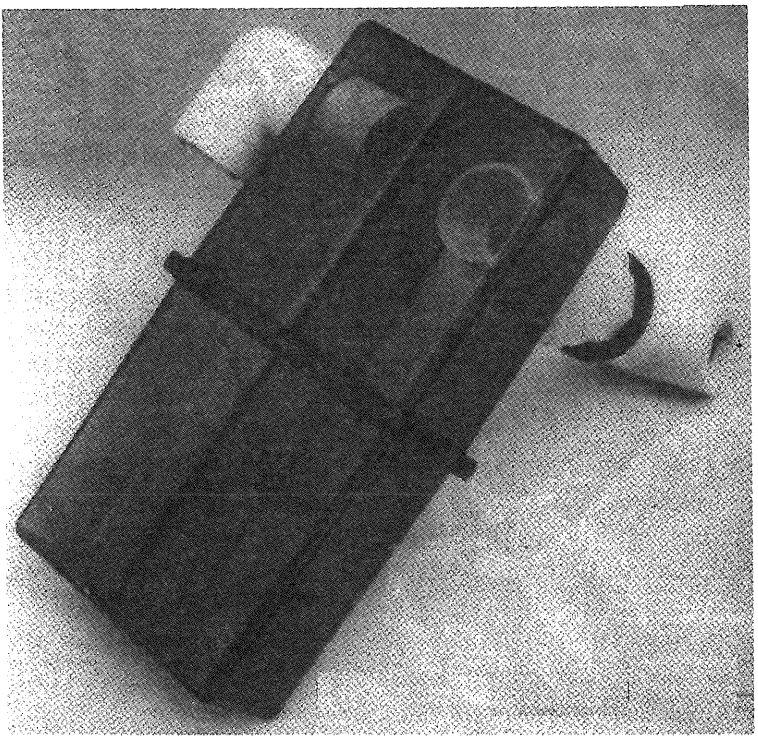


Fig. 10. Box-type pressure relief valve.

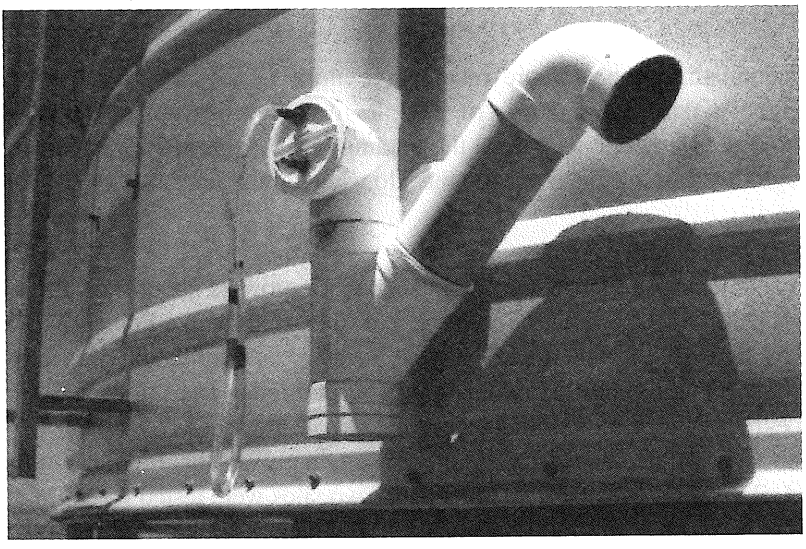


Fig. 11. Example of manufacturer-constructed PVC relief valve connected to the headspace.

in both silos with only minor fluctuations in humidity occurring in the headspace. Prior to this simple test, the grower had been applying PH_3 at the rate of a few tablets per truckload (7 t grain), and he did not possess a moisture meter.

Unless growers can guarantee that grain will be harvested at or below the recommended m.c., silo manufacturers now suggest the addition of a sealable aeration unit to the silo in order to equalise grain temperature and moisture levels and prevent moisture migration.

EXTENSION LITERATURE

Since the inception of sealed silos, government departments across Australia have produced a variety of pamphlets to advise farmers on their correct use and on the management of the stored grain. Manufacturers have produced information on the features of their own silos with additional limited information on grain management and sealed-silo maintenance.

In 1991 WorkCover Authority of New South Wales produced a Code of Practice for the safe design, manufacture, installation and operation of on-farm silos. It is anticipated that a 'code' similar to this will eventually be adopted by statutory Health and Safety authorities around Australia. Under this 'code' manufacturers must provide instructions for the safe operation of their silos. Newman (1995), in consultation with manufacturers across the country, compiled a manual on fumigation and safe operation for the on-farm silo. The GRDC provided the funding to produce this booklet and distribute it to all silo manufacturers in Australia.

Since 1987, the Agriculture Protection Board of WA has produced adhesive signs detailing the correct use and maintenance of a sealed silo. These are supplied to all silo manufacturers in WA to be affixed to the sealed silos leaving the factory. In 1995 this sign was reprinted and offered for sale to all silo manufacturers in other states in Australia.

CONCLUSION

The need to reduce dependence on chemicals for controlling stored-grain insects has driven the campaign to encourage the use of sealed silos. Actual adoption of sealed silos has been slower where there is continued access to chemical insecticides.

High profile extension campaigns led by advisory authorities have significant short-term impact. To maintain the momentum, long-term extension programs must be planned. Problems discovered by users when they adopt a new system can usually not be fully addressed by the manufacturer of the product.

Pressure from grain consumers for a residue-free product will eventually force growers to consider insecticide-free forms of stored-grain insect control. They will consider such alternatives as fumigation or controlled atmospheres in small sealed silos and SIROFLO® in large silos, as well as aeration to cool moist grain to a safe level before fumigation in a sealable silo.

In Australia, the silo manufacturing industry will respond to these challenges and change silo design to accommodate changing control techniques. To transfer this technology successfully, there must be continued input at both the manufacturing and user level, by advisory authorities across the country.

ACKNOWLEDGEMENT

The assistance of A.S. Andrews and B.L. Uren in the preparation of this paper is gratefully acknowledged.

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SESSION 7

INTEGRATED COMMODITY MANAGEMENT METHODS WITH CONTROLLED ATMOSPHERES

Chairpersons:
P.W. Flinn, *USA*
C. Haines, *UK*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 409–422

ETHYL FORMATE AS A FUMIGANT OF SULTANAS: SORPTION AND EFFICACY AGAINST SIX PEST SPECIES

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ABSTRACT

Ethyl formate is used in the Australian dried fruit industry as a fumigant against insect infestation. The rate of sorption of ethyl formate on sultanas was found to be independent of concentration but greatly increased with increased filling ratio and moisture content. There was a slight temperature effect. Sorption, initially rapid with 13% of gas remaining in the headspace at 12 h in containers 95% full, was followed by a more gradual reaction phase. Rate constants per full container for the reaction ranged from 0.077 h^{-1} to 0.038 h^{-1}. At typical commercial dosage rates, concentration × time (Ct) products of 1,493 g h m^{-3} at 8 h and 3,876 g h m^{-3} at 24 h were obtained at 25°C and 60% r.h. in sealed containers. These were shown to be greater than required to control dried fruit pests.

Fumigations at 25°C of mixed-aged cultures of six pests showed that 8-h exposures were more effective than 24-h exposures with the same Ct product. In 24-h exposures, there was 100% mortality of *Oryzaephilus surinamensis*, *O. mercator*, *Plodia interpunctella* and *Carpophilus hemipterus* at 765 g h m^{-3} and of *Tribolium confusum* at 1,158 g h m^{-3}; there was 94% mortality of *T. castaneum* at 1,158 g h m^{-3}. All of these pests were controlled in 8-h fumigations at 541 g h m^{-3} but not at 496 g h m^{-3}. Ethyl formate, where not limited by sorption behaviour, appears to have excellent potential as a replacement for methyl bromide in the treatment of durable commodities.

INTRODUCTION

Australia is the world's fourth largest producer of dried fruit, with a forecast production in 1995–96 of 59 kt of sultanas and raisins (ABARE, 1994, 1995). After harvesting and drying, fruit may be stored for up to 9 months in open crates before being processed. The fruit, generally untreated with fumigants during this period, can be subjected to heavy infestation pressure. Ethyl formate (EF) is applied to the processed fruit as it is packaged into export boxes in order to control any insects surviving the processing treatment.

EF is a versatile organic compound that can be used for a variety of purposes. It has had a long world-wide history as a fumigant used since 1927 on packaged dried fruit

(Simmons and Fisher, 1945) as well as, formerly, on wheat (Neifert *et al.*, 1925; Wilson and Mills, 1946) and other commodities (Pruthi and Singh, 1945; CFTRI, 1979; Muthu *et al.*, 1984). It has been used as a fumigant to disinfest clothing (Busvine and Vasuvat, 1966; David, 1943) and to control pests on fresh fruit, vegetables and flowers (Aharoni and Stewart, 1980; Stewart and Aharoni, 1983; Stewart and Mon, 1984; Wang, 1982) without affecting the quality or flavour of the commodity. It has also been used as a successful fungicide in cereals (Raghunathan *et al.*, 1974; Deo and Gupta, 1986) without affecting their viability or germination. Other uses are in the manufacture of artificial rum, as a flavour for lemonade and essences, as an organic solvent and as a fungicide (Merck Index, 1989).

The insect pests infesting dried fruit are generally the same as those found in cereal commodities. A recent 2-year survey of packing sheds in the Sunraysia district, Victoria (Tarr and Hilton, unpublished data) found that the major pests of dried fruit in Australia were the saw-toothed grain beetle (*Oryzaephilus surinamensis* (L.)), the Indian meal moth (*Plodia interpunctella* (Hübner)) and the raisin moth and related species (*Ephestia* spp.). Less important pests were the merchant grain beetle (*O. mercator* (Fauvel)), flour beetles (*Tribolium* spp.), the dried fruit beetle (*Carpophilus hemipterus* (L.)) and the hairy fungus beetle (*Typhaea stercorea* (L.)). While *O. surinamensis*, *P. interpunctella* and *Ephestia* spp. were the most economically important pests overall, individual outbreaks of all species occurred, depending on local conditions, in different sheds at different times. These findings duplicated those of a survey of the same sheds completed in 1928 (Myers, 1928).

Relatively few modern studies specifically related to the disinfestation of dried fruit have been made on the effect of EF. Vincent and Lindgren (1972) tested examples of four dried fruit pests and found *C. hemipterus* to be the most tolerant to EF, followed by *O. surinamensis*, *P. interpunctella* and *Cadra* (= *Ephestia*) *figulilella* (Gregson). The pupa was the most tolerant stage of all species and the adult the least. Muthu *et al.* (1984) also found that the pupa of *T. castaneum* (Herbst) was the most tolerant stage to EF. All these studies were carried out on insects alone without dried fruit's being present.

This study was undertaken to confirm the effectiveness of the current treatment regime and provide a basis for improvements. It involves both a study of the sorption kinetics of EF on dried fruit under a variety of conditions and toxicological studies on six of the more common dried fruit pests.

MATERIALS AND METHODS

Sorption studies

Samples of unprocessed 'five crown' grade and commercially processed (i.e. washed, freed of sticks and debris and oiled with DURKEX 500®) 'three crown' grade Thompson seedless sultanas were obtained from the Sunraysia District, Victoria. The three series of experiments detailed below investigated the effects of processing, dosage rate, filling

ratio, temperature and moisture content (m.c.) on the sorption of EF. Each set of conditions was measured at least twice.

In the first experiment, unprocessed and processed fruit at 25°C, 60% r.h. and 0.25, 0.50 and 0.95 filling ratio was dosed with 112, 336 and 1,120 g m^{-3} EF. In the second experiment, unprocessed and processed fruit at 15, 25 and 35°C, 60% r.h. and 0.50 filling ratio was dosed with 1,120 g m^{-3} EF. In the last experiment, unprocessed and processed fruit at 25°C, 50, 60 and 70% r.h. and 0.50 filling ratio was dosed with 1,120 g m^{-3} EF.

The amount of water to be added in order to obtain a given humidity was calculated from the sorption isotherm of Pixton and Warburton (1973) together with the observed initial m.c. The fruit was allowed to equilibrate for at least 1 week before being fumigated. Relative humidities were determined by a dew point meter (MBW Model 3-D), and m.c.'s were determined by using a conductance moisture meter ('Type A', DFA of California) with standard minced samples.

Samples were dosed in 120-ml glass vials fitted with Mininert Teflon valves. EF was added by injecting measured quantities of liquid into the headspace using a gastight syringe. Samples were dosed at the stated concentration of EF calculated after making allowance for the exclusion volume of sultanas. The concentration ranges used in this study were based on the current rate used in the Australian dried fruit industry where 6 ml of EF is applied to a 15-kg box of fruit. This gives a maximum concentration of 1,120 g m^{-3} per box. Vials were filled to either 0.25, 0.50 or 0.95 of full capacity, assuming that the fruit weighed 81.00 g when the vial was 100% full. Exclusion volumes were calculated using the true density of sultanas, taken to be 1.43 kg L^{-1}.

EF concentrations were measured using a gas chromatograph (Shimadzu GC-4CM, 6-AM series) fitted with a flame ionisation detector and a 2 m × 0.4 mm glass column packed with 5% AT-1000 on Chromasorb HP. Sample peak areas were compared to peaks of a known standard EF concentration of 50 g m^{-3} to obtain actual concentrations. The vials were shaken prior to sampling, and a sample of the headspace gas was taken using a gastight syringe.

Sorption model

The uptake of EF on sultanas was assumed to occur in two phases: an initial, rapid, reversible phase in which the fumigant was physically sorbed onto the fruitand then a second, slower, irreversible phase in which the fumigant was assumed to react with constituents in the fruit. The rate of reaction of the EF with the fruit is the slope of the linear part of the sorption curve, cast in semilogarithmic form with time after the initial rapid physical sorption phase. This rapid physical sorption phase was typically complete after about 8-h exposure. Extrapolation of the subsequent reaction phase curve back to zero time gave a concentration value (c_i) less than that originally applied (c_0). The difference between the two values, converted into mass units, was taken to be that quantity physically sorbed and thus available for desorption or further reaction.

The curves at greater than 8 h were described mathematically by:

$$c/c_i = e^{-kt} \quad (1)$$

or in logarithmic form by:

$$\ln c = \ln c_i - kt \quad (2)$$

where c is the concentration at time t, c_i is the extrapolated concentration ($t = t_0 = 0$) and k is the apparent first-order rate constant for the system.

The observed value of k under a particular filling ratio, f, was standardised using the filling ratio to give a value, k_f, for the rate constant in a full container, where:

$$k_f = k/f \quad (3)$$

A constant, K, the partition constant, was derived in which the calculated fraction of gas initially sorbed ($c_i - c_0$) at $t = 0$ was corrected for concentration, mass applied and filling ratio. This gives a dimensionless measure of the tendency of a particular sample of fruit to take up EF by physical sorption. The partition constant was defined as:

$$K = \frac{(c_i - c_0) \cdot V_g}{c_i \cdot V_f} \quad (4)$$

where V_g is the gas volume and V_f is the volume occupied by the fruit. Regression analysis on the data was carried out using Genstat 5 and GLIM statistical packages and general data analyses using Excel 5.

Insect mortality studies

Cultures containing all life stages of six dried fruit pests, *O. surinamensis*, *O. mercator*, *C. hemipterus*, *P. interpunctella*, *T. castaneum* and *T. confusum* (Jacquelin du Val) were dosed in a fumigation chamber at 25°C with EF for 8 and 24 h. Cultures were produced by adding 50 adults (beetle species) or 25 eggs (moth species) to 200 g unprocessed sultanas at 27°C and 60% r.h. weekly for 5 weeks (7 weeks for *Tribolium* spp.). Before fumigation, cultures were divided in half to give control and test samples. These were placed in muslin bags and the controls returned to the incubation room. All test cultures were exposed simultaneously, with the total chamber load approximately 1.2 kg of cultures in 0.868 m^{-3}. Test samples were placed in the fumigation chamber, the chamber sealed and the relative humidity measured using a dew point meter (MBW Model 3-D). The humidity was adjusted to 60% r.h. by addition of water if required. EF was injected into the chamber to give a concentration of 37, 73, 95 and 143 g m^{-3} for 8-h exposures and 14, 27, 35 and 52 g m^{-3} for 24-h exposures. Concentrations were measured hourly during the first 8 h and at 24 h (for 24-h fumigations), and the concentration × time (Ct) products for each fumigation calculated. Each fumigation series was replicated twice.

At the end of the exposure period, the chamber was aired for 1 h and the samples moved to a fumehood to be further aired during assessment. Test and control samples were removed from the muslin bags and the insects recorded by life stage as alive or dead

before being returned to the incubation room in glass culture jars. Samples were reassessed for mortality weekly for 3 weeks.

RESULTS

Sorption studies

Sorption of EF on dried vine fruit follows a trend similar to that seen with other fumigants on wheat and other commodities (Banks, 1986, 1990); there is an initial rapid uptake over the first 8 h followed by a more gradual subsequent sorption (Figs. 1 a, b). The curves of sorption at various concentrations at the same filling ratio for log plot of concentration over initial concentration (c/c_0) fall on the same line. This indicates that at a particular filling ratio, temperature and humidity, sorption is independent of concentration (Figs. 1 c, d). Although EF is initially taken up faster by processed fruit, at longer exposures unprocessed fruit sorbs more fumigant because the rate of reaction with unprocessed fruit is higher than that with processed fruit.

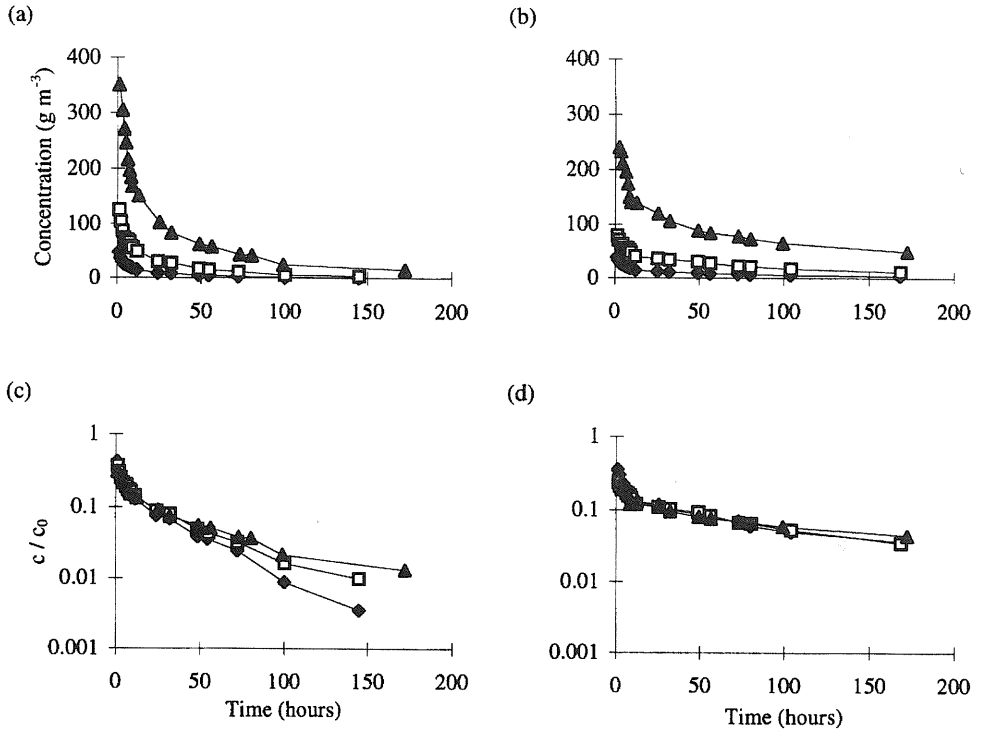


Fig. 1. Sorption curves for ethyl formate on unprocessed (a, c) and processed (b, d) sultanas at a fixed r.h. (60%), temperature (25°C) and filling ratio (0.95) illustrating the influence of concentration, with (a) and (b) showing uncorrected data as a function with time and (c) and (d) corrected on the basis of initial concentration as a logarithmic function with time. ♦ = 112 g m^{-3}, □ = 336 g m^{-3} and ▲ = 1120 g m^{-3} ethyl formate.

Fruit dosed at the same concentration, temperature and humidity but different filling ratios show very different rates of sorption (Figs. 2 a, b). EF is sorbed more rapidly at the higher filling ratios. The log plot of concentration over initial concentration curves against time do not fall on a single line, showing dependence on filling ratio (Figs. 2 c, d).

Adjusting the rate constant according to Equation (3) should remove the effect of filling ratio. An average rate of sorption at specified conditions can then be found. Table 1 shows average k_f values for unprocessed and processed fruit at 25°C and 60% r.h. with the overall average for both fruit types. The sorption rate constant for unprocessed fruit is more than twice that for processed fruit, with k_f values of 0.0262 h^{-1} and 0.0122 h^{-1}, respectively. A trend of decreasing value of k_f with increasing filling ratio at a given concentration is apparent.

The partition constant values (K), calculated using Equation 4, are shown in Table 2 for both fruit types at 25°C and 60% r.h. Samples showed little variation with change in concentration and filling ratio. The average partition constant value for processed fruit (7.78) was slightly greater than for unprocessed fruit (5.92), with a suggestion that at higher concentrations with low filling ratios the partition constant also was greater.

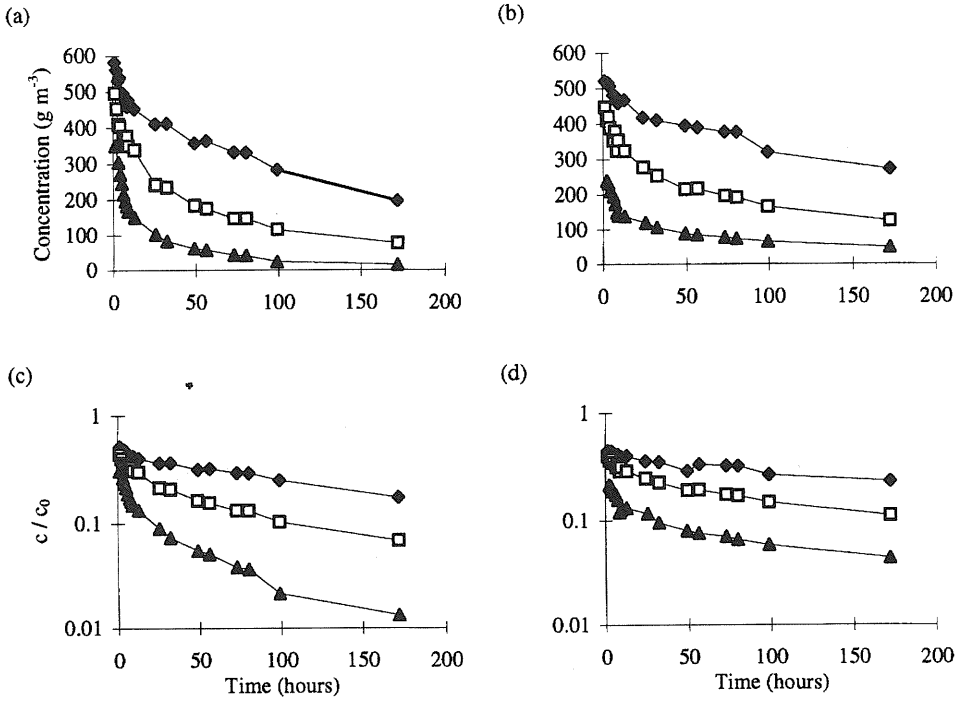


Fig. 2. Sorption curves for ethyl formate on unprocessed (a, c) and processed (b, d) sultanas at a fixed r.h. (60%), temperature (25°C) and concentration (1120 g m^{-3}) illustrating the influence of filling ratio, with (a) and (b) showing uncorrected data as a function with time and (c) and (d) corrected on the basis of initial concentration as a logarithmic function with time. ♦ = 0.25, □ = 0.50 and ▲ = 0.95 filling ratio.

TABLE 1
Average rate constants for sorption per hour for full containers (k_f) for unprocessed (U) and processed (P) fruit at 25°C and 60% r.h., showing effect of filling ratio and concentration

EF dosage ($g\ m^{-3}$)	Fruit type	Filling ratio 0.25	0.50	0.95
112	U	0.0383	0.0328	0.0274
336	U	0.0336	0.0309	0.0225
1120	U	0.0183	0.0183	0.0138
		Mean ± standard deviation = 0.0262 ± 0.00836		
112	P	0.0189	0.0155	0.0091
336	P	0.0106	0.0123	0.0097
1120	P	0.0140	0.0122	0.0077
		Mean ± standard deviation = 0.0122 ± 0.00349		

TABLE 2
Average partition constant values (K) for unprocessed (U) and processed (P) fruit at 25°C and 60% r.h., showing effect of filling ratio and concentration

EF dosage ($g\ m^{-3}$)	Fruit type	Filling ratio 0.25	0.50	0.95
112	U	5.22	5.08	5.95
336	U	6.03	5.14	5.69
1120	U	9.34	7.29	6.99
		Mean ± standard deviation = 5.92 ± 0.835		
112	P	6.38	5.84	7.26
336	P	13.07	7.97	6.70
1120	P	10.19	7.14	7.89
		Mean ± standard deviation = 7.78 ± 2.260		

The effect of temperature and r.h. on sorption on sultanas at 0.50 filling ratio and 1,120 g m^{-3} is shown graphically in Figs. 3 a, b and 4 a, b, respectively. Table 3 shows the rate constant for sorption in a full container (k_f) and the partition constant (K) for fruit at the different temperatures and humidities tested. Unprocessed fruit had a higher rate of reaction than processed fruit at all temperatures and humidities. The partition constant showed a slight decrease with increased temperature in both fruit types, indicating that higher temperatures increase the rate of reaction but slightly decrease the amount of fumigant sorbed. Relative humidity has a much stronger effect on sorption than temperature. At higher humidities, there was a slight increase in the rate of reaction but a dramatic increase in the amount of fumigant sorbed by the fruit which is indicated by the partition constant.

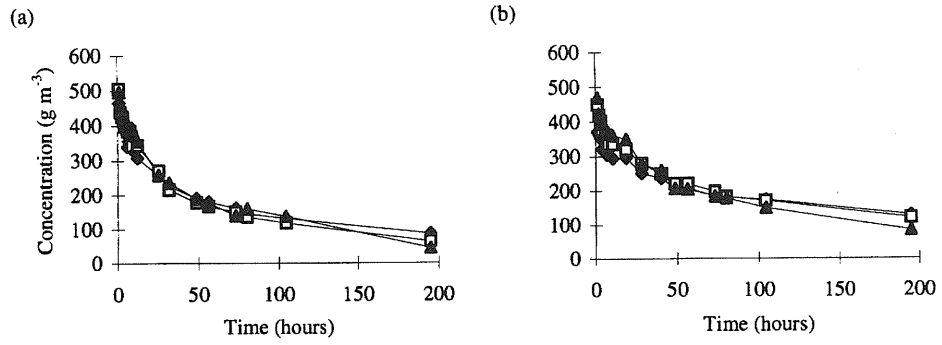


Fig. 3. Sorption curves for ethyl formate on unprocessed (a) and processed (b) sultanas at a fixed r.h. (60%), filling ratio (0.50) and concentration (1120 g m^{-3}) illustrating the influence of temperature. Both graphs show uncorrected data as a function with time. ♦ = 15°C, □ = 25°C and ▲ = 35°C.

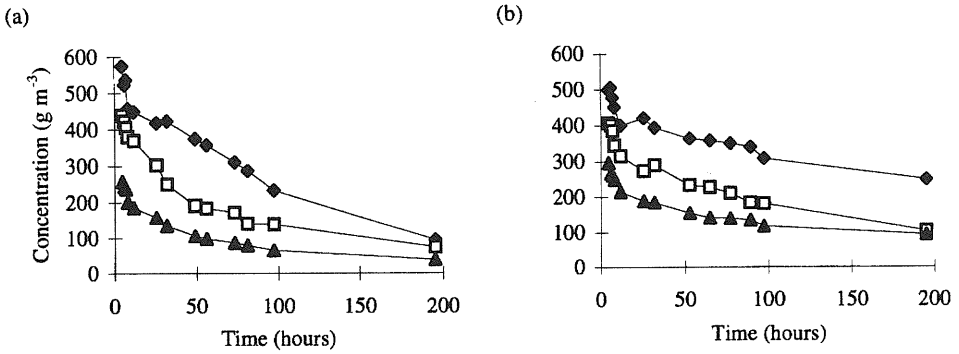


Fig. 4. Sorption curves for ethyl formate on unprocessed (a) and processed (b) sultanas at a fixed temperature (25°C), filling ratio (0.50) and concentration (1120 g m^{-3}) illustrating the influence of humidity. Both graphs show uncorrected data as a function with time. ♦ = 50%, □ = 60% and ▲ = 70% r.h.

TABLE 3

Average rate constants for sorption per h for full containers (k_f) and partition constants (K) for unprocessed and processed fruit at 1120 g m^{-3} EF and 0.50 filling ratio showing effect of temperature and r.h.

Temperature (°C)	r. h. (%)	Unprocessed		Processed	
		k_f	K	k_f	K
15	60	0.0141	8.20	0.0081	9.60
25	60	0.0183	7.29	0.0121	7.14
35	60	0.0224	6.05	0.0153	7.27
25	50	0.0100	4.28	0.0067	4.51
25	60	0.0183	7.29	0.0122	7.18
25	70	0.0179	15.66	0.0100	13.44

Insect mortality studies

Fumigation of six dried fruit pests showed that 8-h exposures with higher initial concentration were more effective at controlling all pest species than with 24-h exposures at the same Ct product (Tables 4 and 5). There was 100% mortality of all species at 541 g h m^{-3} in the 8-h exposures, corresponding to an initial concentration of 73 g m^{-3} (Table 4). The duplicate fumigation with a lower Ct product of 496 g h m^{-3} had a 99.8% mortality of both *O. surinamensis* and *O. mercator* and complete kill of *P. interpunctella* and *C. hemipterus*, indicating that this Ct product is only just achieving control. Eggs and pupae were the only stages surviving any 8 h treatment, with pupae more tolerant than eggs. *Tribolium* spp. showed no survival at any Ct products tested in 8-h exposures.

In 24-h treatments, as compared to 8-h exposures, a higher Ct product was required to control all species. There was complete mortality of all stages of *O. surinamensis*, *O. mercator*, *P. interpunctella* and *C. hemipterus* at 765 g h m^{-3}, corresponding to an initial concentration of 27 g m^{-3} (Table 5). In contrast to their relative susceptibility in 8-h exposures, *Tribolium* spp. were the most tolerant species in 24-h exposures. Larvae and pupae of *T. castaneum* survived at the maximum Ct product tested (1,158 g h m^{-3}). As with the 8-h exposures, eggs and pupae of *O. surinamensis*, *O. mercator*, *P. interpunctella* and *C. hemipterus* were the most tolerant life stages to the fumigant. Larvae and pupae were the most tolerant stages of *Tribolium* spp.

DISCUSSION

Understanding the sorption kinetics of a fumigant on a commodity is important, as the rate of sorption affects the insecticidal efficacy of the fumigant. Sorption onto dried fruit, independent of the initial concentration, was dependent on the filling ratio of the container. There was a greater uptake of EF at 0.25 filling ratio with increasing concentrations (close to point of saturation), probably due to condensation of the fumigant on the fruit. An increase of 10°C increased the rate of reaction by a factor of 1.5 but slightly decreased the amount of fumigant sorbed. Higher humidities have a stronger effect on sorption than does temperature. An increase of 20% in r.h. resulted in a slight increase in the rate of reaction and a fourfold increase in the amount of fumigant sorbed. The very high solubility of EF in water (118.0 g L^{-1}) may explain this trend.

Processed fruit had a reaction rate almost half that of unprocessed fruit over the range of conditions tested. Despite an initial high level of sorption, at long exposures processed fruit sorbed less fumigant than did unprocessed fruit. The dressing oil used to coat sultanas after processing may have caused the increase in the initial amount of fumigant sorbed. Results not reported here indicate that different varieties of unprocessed fruit, ranging in size from small currants (0.20-cm berry) to natural muscatels (1.07-cm berry), showed no significant differences in the rate of reaction or amount of sorption from those of the unprocessed sultanas studied here. The results thus appear to be a general indication of sorption and reactivity of dried vine fruit rather than being specific to the fruit tested.

TABLE 4

Initial concentration, Ct product, mortality (M) and stage surviving fumigation[1] of the six species fumigated with EF for 8 h

Concentration ($g\ m^{-3}$)	Ct product ($g\ h\ m^{-3}$)	*O. surinamensis*		*O. mercator*		*P. interpunctella*		*C. hemipterus*		*T. castaneum*		*T. confusum*	
		M (%)	Stage	M (%)	Stage	M (%)	Stage	M (%)	Stage	M (%)	Stage	M (%)	Stage
37	248	91.1	E, P	99.8	E	100	*	94.1	E	–	–	–	–
37	279	99.8	P	100	*	100	*	78.1	P	–	–	–	–
73	469	–	–	–	–	–	–	–	–	100	*	100	*
73	472	–	–	–	–	–	–	–	–	100	*	100	*
73	496	99.8	P	99.8	P	100	*	100	*	–	–	–	–
73	541	100	*	100	*	100	*	100	*	–	–	–	–
95	566	100	*	100	*	100	*	–	–	–	–	–	–
95	586	100	*	100	*	100	*	–	–	–	–	–	–
142	901	–	–	–	–	–	–	–	–	100	*	100	*
142	920	–	–	–	–	–	–	–	–	100	*	100	*

[1]E = egg, L = larva, P = pupa, A = adult, * = no stage surviving.

TABLE 5

Initial concentration, Ct product, mortality (M) and stage surviving fumigation[1] of the six species fumigated with EF for 24 h

Concentration ($g\ m^{-3}$)	Ct product ($g\ h\ m^{-3}$)	*O. surinamensis*		*O. mercator*		*P. interpunctella*		*C. hemipterus*		*T. castaneum*		*T. confusum*	
		M (%)	Stage	M (%)	Stage	M (%)	Stage	M (%)	Stage	M (%)	Stage	M (%)	Stage
14	321	45.3	all	59.3	all	93.3	L, P	87.5	A, P	–	–	–	–
14	321	68.8	all	61.7	all	95.7	L, P	94.1	P	–	–	–	–
27	572	–	–	–	–	–	–	–	–	46.9	all	71.9	E, L, P
27	583	–	–	–	–	–	–	–	–	56.5	all	77.1	E, L, P
27	592	97.7	E, P	99.1	P	100	*	100	*	–	–	–	–
27	628	98.9	P	99.8	P	98.8	E	100	*	–	–	–	–
35	765	100	*	100	*	100	*	–	–	–	–	–	–
35	783	100	*	100	*	100	*	–	–	–	–	–	–
52	911	–	–	–	–	–	–	–	–	92.9	L	99.7	L
52	1158	–	–	–	–	–	–	–	–	94.4	L, P	100	*

[1]E = egg, L = larva, P = pupa, A = adult, * = no stage surviving.

Short exposure periods with a higher initial concentration of EF were found to be the most effective way of controlling the six insect species tested. In 8-h fumigations, *C. hemipterus* was controlled at 73 g m^{-3} (Ct product of 496 g h m^{-3}), which is consistent with the results of 95% mortality of the pupae at 72 g m^{-3} in 6-h fumigations (estimated Ct product of 432 g h m^{-3}) reported by Vincent and Lindgren (1972). Also in agreement were the results with *P. interpunctella*. Our results showed no survival at any concentrations tested (lowest 37 g m^{-3}), compared to the 95% mortality of pupae at 36 g m^{-3} observed by Vincent and Lindgren (1972). The pupa was the most tolerant stage for both *O. surinamensis* and *O. mercator*, with a 99.8% mortality at 73 g m^{-3} (Ct product of 496 g h m^{-3}), but 100% mortality in the duplicate fumigation (Ct product of 541 g h m^{-3}) indicated that this is the borderline for complete control. Vincent and Lindgren (1972) found 95% mortality of *O. surinamensis* pupae at 48 g m^{-3} in 6-h exposures (estimated Ct product of 288 g h m^{-3}). No *Tribolium* spp. survived at any concentration (lowest 73 g m^{-3}) in 8-h exposures. Shepard *et al.* (1937) found 50% mortality of *T. confusum* at 24.5 g m^{-3} in 5-h fumigations (estimated Ct product of 122.5 g h m^{-3}). In the present work, a Ct product of 541 g h m^{-3} gave complete control in 8-h fumigations of all species tested.

Complete control of *O. surinamensis*, *O. mercator*, *P. interpunctella* and *C. hemipterus* was achieved with an initial concentration of 35 g m^{-3} (Ct product of 765 g h m^{-3}) in 24-h exposures. Pupae of the beetle species and eggs of *P. interpunctella* were the most tolerant. No comparable results for 24-h exposures were found in the literature. Larvae and pupae of *Tribolium* spp. were the most resistant in 24-h fumigations, with 100% mortality of *T. confusum* and 94.4% mortality of *T. castaneum* at the highest concentration tested (52 g m^{-3}, Ct product of 1,158 g h m^{-3}). Neifert *et al.* (1925) achieved 80% mortality of *T. confusum* adults in one experiment at 36.4 g m^{-3} for 24 h (estimated Ct product of 874 g h m^{-3}), which is consistent with our findings. Muthu *et al.* (1984) found pupae of *T. castaneum* to be the most resistant stage, requiring a concentration of 27.9 g m^{-3} (estimated Ct product of 669 g h m^{-3}) for 24 h to obtain a 95% mortality, a slightly lower concentration than in our results. In the present work, a Ct product of 765 g h m^{-3} gave complete control of the more common dried fruit pests tested in 24-h fumigations and 1,158 g h m^{-3} were needed to control *Tribolium* spp. In a gastight fumigation, the commercial dose of 6 ml per 15-kg box of fruit was found to give a Ct product of 1,493 g h m^{-3} at 8 h and a Ct product of 3,876 g h m^{-3} at 24 h. This is well above the Ct product expected to kill all dried fruit pests.

As demonstrated in this study, EF appears to be an effective fumigant of dried fruit (the use to which it is currently put in the Australian industry). It is effective against dried fruit pests, leaves no 'off' odours or tastes, is easy to handle and has a low mammalian toxicity. It is grouped under 'formates' as a food additive for human consumption (FDA, 1979) and is considered to be only mildly toxic by skin contact and inhalation (Sax and Lewis, 1989). Though receiving little attention recently in stored-product literature, EF appears to be well-suited as a fumigant for a variety of dried foodstuffs unless it is too highly sorbed to give insecticidal gas concentrations. Further sorption studies will need to be undertaken to address this point. With the current need for replacements for methyl bromide, EF

appears worthy of reassessment. It seems to have good potential as a 'rediscovered', rapidly-acting fumigant for some durable commodities.

ACKNOWLEDGEMENTS

The authors would like to thank the Dried Fruit Research and Development Council, for supporting this project financially, and Ms Caroline Tarr, CSIRO Division of Horticulture, for assistance in insect collecting and obtaining the fruit samples used in this study.

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CARBON DIOXIDE FUMIGATION OF ORGANIC GRAIN FOLLOWED BY 'REFRIG-AERATION'

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ABSTRACT
(For full paper see "Late-comer" section at end of volume)

Organic grain was fumigated on arrival in storage with carbon dioxide (CO_2). Concentrations were held above 30% for 15 d by continuously topping up with additional CO_2 through a recirculation system. Subsequent aeration with refrigerated air enabled long-term storage without the need for re-fumigation. This integrated commodity management strategy provided the means to outload 60-t batches of insect-free grain over a total storage period of 24 months. The results of insect trapping in the storage area are discussed, and the equipment and operational costs of implementing this strategy are described.

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 425–434

DEVELOPMENT OF A DECISION SUPPORT SYSTEM FOR THE FUMIGATION OF MILLED-RICE BAG-STACKS IN THE TROPICS

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ABSTRACT

A Fumigation Decision Support System (FDSS) is described that enables pest-control operatives in milled-rice bag-stores both to predict when future fumigations will be required and to determine whether previous fumigations were successful. The FDSS consists of an insect monitoring technique, an insect growth model and a pragmatic pest-control threshold. The last two elements are contained in a computer program. The growth model, which is central to the system, is specific to *Tribolium castaneum*, and it is valid only for use in the humid tropics.

The FDSS was tested in rice stores in Indonesia, and a logistic model of insect population growth was found to perform best. The predicted time to fumigation deviated on average by only about 1 week from the actual time to fumigation. The FDSS was also tested, after suitable training, by unsupervised pest- and quality-control staff, at two godown sites in Java, who found the system easy to use. Their performance was evaluated. The predictions were again reliable. The FDSS is now ready for operational use.

INTRODUCTION

Insect pest infestation of stored foods is a major problem faced by store managers in developing countries. To prevent insect damage's resulting in excessive loss of both food quantity and quality, stores are often fumigated and sprayed with insecticide. Long experience has shown that the application of such control measures is frequently poorly timed. Thus expensive pest-control procedures may be used when they are not necessary or even after the damage has already been done. In either case, losses are incurred. A further problem is that pest-control treatments may not be properly applied, making it necessary to repeat treatment sooner than planned.

Previously, no practical system had been developed to establish the optimal timing of pest-control operations in tropical stores. The research described in this report was undertaken to develop a practical package of measures, called the Fumigation Decision Support

System (FDSS), to enable pest-control operatives in milled-rice bag-stores in Indonesia to predict the ideal timing for the application of fumigation, according to their own criteria, and to determine whether or not the most recent fumigation treatment was successful.

The FDSS consists of a trapping method for monitoring the number of pests in milled-rice stores, a population growth model using the trap data to predict future insect numbers, and a pest-control threshold that, when reached, indicates the need for the application of pest control. The growth model and the pest-control threshold are included in a computer program designed to be used by pest-control staff. The program, written in Turbo Pascal, version 7.0, has screens in both English and Indonesian.

To validate the FDSS, two trials were undertaken. The first, a 2-month long 'experimental trial' in West Javanese stores, observed how well the predictions of the time needed to reach the fumigation threshold, made approximately 6 weeks after fumigation, matched the actual time to threshold. This was followed by an 'operational study' in West and Central Javanese storage sites designed both to investigate whether godown staff were able to use the FDSS and to provide further evidence of the reliability of the population-growth predictions.

DESCRIPTION OF THE FDSS

Insect trapping system

Insect populations in milled-rice stores were monitored using bait-bag traps, consisting of 10 × 20-cm plastic net bags with 19 2-mm apertures per cm^2, filled with 100 g of brown rice (Hodges *et al.*, 1985). These traps can give reliable estimates of the insect population present in bag-stacks of milled rice, and Haines *et al.* (1991) established a calibration curve enabling the catches in the traps to be converted into the more familiar units of spear sampling (numbers/kg). For the operation of the FDSS, only adult *Tribolium castaneum* (Herbst) caught in the traps are used for fumigation predictions. In Indonesia, *T. castaneum* is considered the best indicator of stack infestation because it is both the most common species and, almost invariably, the first to arrive after the initial fumigation of new stock. Other important pest species, such as *Sitophilus* sp. or *Rhyzopertha dominica,* may sometimes be found at the start of storage, but they do not generally become re-established if they are killed at the initial fumigation (Haines and Rees, 1988).

A minimum of 20 bait-bag samples from stacks in the range of 175–300 t is required in order to get a good prediction. The number of bait-bags may be reduced or increased pro-rata according to tonnage (Haines and Rees, 1988). Users of the FDSS are advised not to monitor on either very small stacks or fractions of remaining stacks.

The minimum insect catch needed to predict insect population growth with the FDSS is a mean of 1.0/bait-bag. If the mean value is below this, a further sample should be taken at a later date. Earlier experience indicated that after successful fumigations the population will not rise to this level until more than 9 weeks following the fumigation date. However, to allow for exceptionally rapid reinfestation and possible fumigation failures, a sample should be taken at around 6 weeks. If the insect catch is

sufficiently large, it can be used, together with the period of time since the last fumigation, to predict when the pest-control threshold will be reached. This is the time at which another fumigation is recommended.

Insect growth model

Initially, a simple exponential curve was used to predict growth; however, following tests of the program, better results were found to be obtainable from a logistic growth curve. This was fitted to data obtained from trials in East Java (Hodges *et al.*, 1992) in which the development of *T. castaneum* populations was monitored in a total of 6 stores and 12 bag-stacks, using both bait-bag traps and spear sampling (Fig. 1). The logistic curve took the standard form:

$$N = \frac{im}{[i + (m - i)\, e^{-rt}]} \qquad (1)$$

where N is population at time t, i is the initial population (after fumigation), m is the maximum population the stack can support, e^r is the rate of population expansion and t is time in weeks since fumigation.

The growth model was applied with no attempt to correct for variations in grain temperature, moisture content (m.c.) or degree of rice milling. Conditions for all these variables were considered to be sufficiently constant to make such corrections unnecessary. The inside-store temperature close to the equator shows little annual variation. Both the m.c. and milling-degree ranges are subject to regulation; although variable, they are controlled within relatively narrow limits. Thus the FDSS is only valid for the lowland humid tropics where rice is stored in jute bags and is of quality similar to that used in the study (e.g. m.c. 13.0–14.5%, milling degree 85–90%, broken grains 25–30%).

The growth model also does not take into account insects that migrate into a store after fumigation. During the initial phase of reinfestation, the influx of insects is a major component of population growth. However, experience has shown that the number of insects migrating into the store soon becomes insignificant compared with that generated by a breeding population still existing in the rice.

Pest-control threshold

The pest-control threshold used in the program is pragmatic. It is important that pest control be implemented before the live insects in the stores and before both live and dead insects in the rice itself have had a serious detrimental effect on either the cleanliness of the store or the marketability of the rice. Therefore, it was decided to set a threshold ensuring that fumigation was undertaken well before the rate of population growth reached its maximum. Examination of the model showed that this occurred when there were around 150 insects per bait-bag. At 40 *T. castaneum* per bait-bag, population expansion is approximately half the maximum (Fig. 1). This level of infestation was selected as the most convenient pest-control threshold because it allows a reasonable

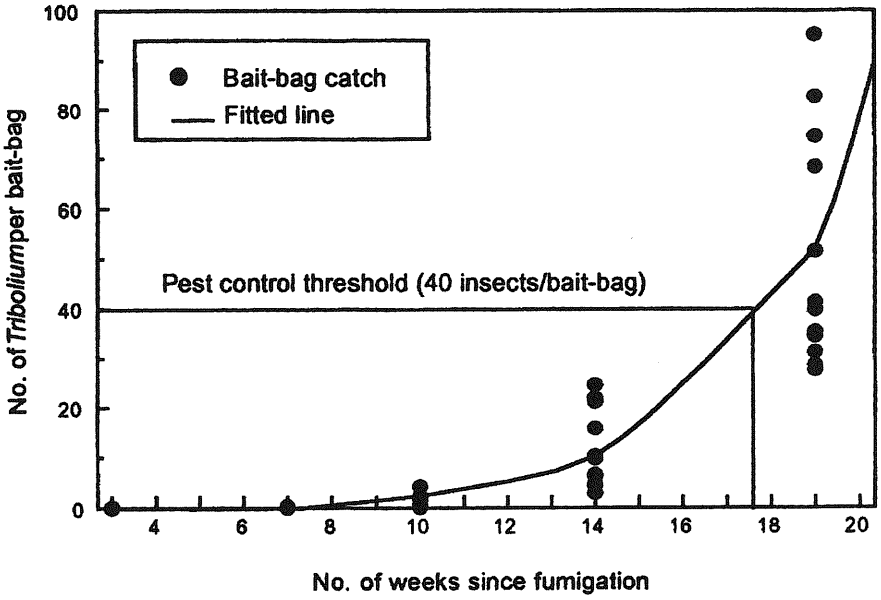


Fig. 1. The logistic growth curve fitted to trial data ($i = 0.03$, $m = 300$, $r = 0.42$).

lead-time to fumigation. The FDSS user can change this threshold to whatever level is convenient and/or appropriate to his system.

Such a threshold has a clear logical basis, but it does not address the issue of such cumulative effects of insect infestation as the contamination and weight loss that occur after successive fumigations. One potentially serious contaminant is quinone secretions from the thoracic and abdominal glands of adult *T. castaneum*. Quinones are mutagenic chemicals, and the possibility of their accumulation in milled rice was investigated; however, no significant build-up could be detected (Hodges *et al.*, 1996). The computer program enables store managers to predict what weight losses might occur in milled rice as a result of insect feeding in the period between the last fumigation and the recommended time of the next fumigation. These predictions are based on weight-loss estimates made by Halid (1988). However, no attempt has been made to compare the financial value of potential rice-weight losses to the cost of fumigation as a means of deciding whether or not pest control should be implemented. This is because high insect numbers will have become unacceptable before there are measurable weight losses. The facility for weight-loss calculation is, however, provided to demonstrate how small weight losses are when compared with the cost of fumigation and how relatively massive is rice contamination with insects and hence quality decline.

The pest-control recommendation

The program displays a table for each stack sampled stating in weeks the time between taking the insect sample and the next necessary fumigation. A recommendation, based on

the stack with the greatest insect population, is then presented for the fumigation of the whole store. A graph can be displayed showing the predicted development of the insect population in each rice stack and the predicted growth for the entire store population, the mean of the sampled stacks.

Success or failure of last fumigation

Fumigation is considered successful if the average bait-bag count for a stack 6 weeks after the last fumigation is estimated to be equal to, or less than, two insects. If the population estimate was made more than 6 weeks after the last fumigation, it is compared with the predicted size of a population that, at 6 weeks, was two insects. This is derived from the recurrence relationship (Equation 2, legends as for Equation 1) based on the bait-bag population each week.

$$N_{t+1} = \frac{1}{\frac{300 - N_t}{300N_t \times 1.525} + \frac{1}{300}} \qquad (2)$$

The choice of an average of two insects/bait-bag is based on the results of trial work in East Java (Hodges *et al.*, 1992) where, after a good fumigation, less than one insect/bait-bag was observed 6 weeks after treatment. The somewhat higher figure of two insects/bait-bag has been adopted as the decision threshold in order to reduce any likelihood of claims that fumigations were failures when they may have been successful. The FDSS user can change the two insects/bait bag default value if this is required.

VALIDATION OF THE FDSS

Experimental trial

The trial was undertaken with 23 bag-stacks of milled rice, distributed among five stores at Cibitung in West Java. The stores had 3000 t capacity; 284–364 t bag stacks were constructed with 100-kg jute bags. The stocks in each store had been fumigated with methyl bromide about 6 weeks prior to the start of the test.

To obtain an initial measure of the insect population, 20 bait-bags were deployed for 1 week on each stack. Seven bags were placed on each long side and three bags on each short side. The bags were placed in suitable crevices in sacks between the third and seventh layer from the floor. To obtain final population estimates, bait-bag monitoring was repeated at a convenient time (around 2–4 weeks after sampling or 7–10 weeks after fumigation). This period was rather shorter than planned because the initial insect infestations were heavier than anticipated.

To test the growth model's ability to predict future fumigation requirements, the time between the initial fumigation and the second insect population assessment was taken as the actual fumigation interval. This was compared with the predicted fumigation interval obtained by entering the first population estimate in the FDSS and setting the pest-control threshold at the value of the second population estimate.

The accuracy of the model's predictions was first tested on the data from all 23 stacks. The stacks were then grouped according to whether their initial bait-bag values were relatively low (13–28 insects/bag) or high (35–57 insects/bag). The program was originally tested using three different exponential growth models and the logistic model mentioned above.

Depending on the growth rate and model, predictions of the fumigation interval had an absolute mean deviation varying from 1.5 to 0.7 weeks (Table 1). When the stacks with low initial bait-bag results are considered separately, there is a similar, but slightly narrower, range (1.1 to 0.8). Where initial bait-bag values are high, the deviation between the predicted and actual fumigation interval was considerably larger. As FDSS predictions will normally be based only on low bait-bag values, the most interesting tests were those carried out on the stacks with low initial values. Here, the exponential ($r = 0.39$) and the logistic growth models, with 33% of the predictions completely accurate, performed better than did the alternative models. However, the absolute mean deviation for the logistic model was smaller (0.8 compared to 1.1 weeks), making it the preferred choice for use in the model; the exponential model ($r = 0.39$) would also be expected to perform well.

This trial was not an ideal test of the program because the insect population in the stacks just 6 weeks after fumigation was much greater than would normally be expected,

TABLE 1
Comparison of actual and predicted fumigation intervals for stacks in the experimental trial

	T. castaneum growth model			
	Exponential			Logistic
Models	$r = 0.48$	$r = 0.40$	$r = 0.39$	$r = 0.42$
Absolute mean deviation (weeks)				
All initial bait-bag values	1.5	1.0	0.9	0.9
Low initial bait-bag values (13–28)	1.0	1.0	1.1	0.8
High initial bait-bag values (35–37)	1.8	1.7	0.7	1.2
Late, early and accurate predictions (%)				
All bait-bag values				
Late	5	17	22	5
Early	87	78	43	69
Accurate	8	5	35	26
Low bait-bag values				
Late	11	44	55	12
Early	67	44	12	55
Accurate	22	12	33	33
High bait-bag values				
Late	0	0	0	0
Early	100	100	64	79
Accurate	0	0	36	21

indicating that the initial fumigations were failures. Consequently, the predictions were based on relatively high bait-bag values, the lowest being 13–28 insects/bag instead of the 1–2 insects/bag required for a good advance warning of the need for fumigation. However, the trial has shown that, at least over short time-spans, the system can predict the rate of insect growth with a useful degree of accuracy.

Operational study

The trial was undertaken at two godown complexes, one in Central Java (Semarang) and the other in West Java (Bandung), using the FDSS with logistic growth model. Both complexes had small-scale laboratory facilities. The trial used four godowns at Semarang and two at Bandung, each containing 284–364 t bag-stacks built with 100-kg bags of milled rice in jute sacks. Four Pest and Quality Control (PQC) staff at each site were given 2 d training in the use of the bait-bag insect monitoring technique, the FDSS and the computer.

Not more than six stacks were monitored in any godown. These were chosen to be as far apart as possible. This is because two or three stacks are confined under the same gastight sheets at the time of fumigation, and such stacks represent a single fumigation that will have either succeeded or failed. Choosing stacks as far apart as possible meant that a greater number of these groups was sampled. Twenty bait-bags were placed on each stack being monitored, seven on the long sides and three on the short sides.

PQC staff began the insect monitoring 6 weeks after the initial fumigation. At Bandung, two population estimates of the same stacks were made, the second 2 or 3 weeks after the first, to confirm the reliability of the initial estimate. Owing to a general shortage of stock, the study was confined to only one fumigation period; thereafter, stocks moved too quickly to warrant pest control. The performance of the pest-control staff was evaluated at the middle and end of the study period.

Staff at both Semarang and Bandung were receptive to training and, by the time the 2-d course had ended, showed competence in using both the computer program and the bait-bag monitoring system. Although initial insect numbers recorded at Semarang were so high, due to earlier fumigation failure, that they could not be counted in full, the staff reported no difficulties in actually using the technique. At Bandung, insect numbers at the first estimate were within the 'normal' post-fumigation range. The second population estimate at Bandung, 2 or 3 weeks after the first, deviated by only 14 and 17% from the values predicted by the first estimate in the two godowns (Table 2); this means that the fumigation interval predicted from the two estimates differed by less than 1 week.

CONCLUSIONS

The FDSS can be used to give reasonably accurate predictions of the optimum timing for future fumigation in milled-rice bag-stores as well as to indicate failures in earlier fumigations. Although the system has the advantage of being simple and effective, it is limited because it is currently configured only for use in stores using jute bags in the humid lowland

TABLE 2

FDSS predictions of *T. castaneum* and population growth and fumigation intervals for two godowns in Bandung

Godown 4	Week 6	Week 8			Predicted fumigation intervals		
Stack no.	Mean no./ bait-bag	Mean no./ bait-bag	Predicted no./ bait-bag*	% deviation actual–predicted no./bait bag	Week 6	Week 8	Difference
1	2.0	8.3	4.7	–43	13	10	–3
2	2.6	6.7	6.0	–10	12	12	0
3	3.0	7.4	7.1	–14	12	12	0
4	2.7	6.7	6.2	–7	12	12	0
5	3.1	7.6	7.1	–6	12	12	0
6	2.5	4.6	5.7	+23	12	13	+1
		Absolute mean deviation 17%			Absolute mean deviation 0.66 weeks		
Godown 3	Week 7	Week 11			Predicted fumigation intervals		
Stack no.	Mean no./ bait-bag	Mean no./ bait-bag	Predicted no./ bait-bag*	% deviation actual–predicted no./bait bag	Week 7	Week 11	Difference
1	4.0	23.5	20.7	–12	12	12	0
2	3.2	15.3	16.3	+6	13	13	0
3	4.8	26.4	24.0	–9	12	12	0
4	3.3	25.6	17.0	–33	12	12	0
5	3.3	16.1	17.0	–6	12	13	+1
6	3.4	14.8	17.5	+18	12	13	+1
		Absolute mean deviation 14%			Absolute mean deviation 0.33 weeks		

*Calculated from first population estimate using growth model.

tropics. In addition, the rice stored must be what is generally regarded as medium grade (m.c. of 13.0–14.5%, 20–35% broken grains and a milling degree of 85–90%). Use of the FDSS under other conditions will require both confirmation of the population growth rate and, if polypropylene bags are used instead of jute bags, confirmation of the relationship between bait-bag catch and the numbers of insects actually present in the rice.

The FDSS uses a simple pragmatic pest-control threshold, easily understood by pest-control operatives, which can be altered to suit local conditions if necessary. Economic aspects are touched on by mentioning actual rice weight losses associated with the predicted insect population, but this is included largely as a matter of information in order to demonstrate that weight losses are small in relation to the costs of pest control, whereas both store and rice insect-contamination is relatively massive. One aspect of the cumulative effects of insect infestation, the accumulation of quinones, has been investigated and found to be insignificant (Hodges *et al.*, 1996). However, a further aspect, the actual accumulation of dead bodies, remains an area for further study. Very few, if any, rice-storage systems in the tropics pay attention to the numbers of dead bodies present in marketed rice even if in theory a threshold does exist. If further versions of the FDSS could be made to take this factor into account, however, it would increase awareness of quality deterioration and, even if the facility was not used immediately for storage operations, it might have an important training function. To take dead-body accumulation into account would require knowing how long a stock would remain in store, the current dead-body contamination rate, the rate at which stocks become reinfested after fumigation and the rate at which dead bodies disintegrate and become, in effect, undetectable. The FDSS could be modified to accept these data and recalculate the pest-control threshold to ensure that dead-body contamination would be below critical level at the stacks' expected discharge time.

The FDSS is one of a very few decision support systems available for use in tropical stores. Another is Ceranyl, developed in West Africa by the German aid agency (GTZ), which assesses the quality of stocks destined for national food security programmes and advises on storage potential. Most other systems are relatively large and complex 'grain store advisors', used principally for training purposes (Wilkin and Mumford, 1994). The FDSS is not currently in operational use although it is being considered in Indonesia. As part of a training 'workbench' developed by the Australian Centre for International Agricultural Research (ACIAR) and the Indonesian National Logistics Agency (BULOG), the system is being prepared in WINDOWS® format with the intention of making it available as both a training tool and an operational system. Copies of the TurboPascal version of the FDSS can be obtained on request from the Food Security Department of the Natural Resources Institute.

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SIMULATION MODEL OF LOW-OXYGEN ATMOSPHERES ON INSECT POPULATION DYNAMICS IN STORED GRAIN

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ABSTRACT

A spatial model of the relationship between *Sitophilus oryzae* (L.) population dynamics and bin temperature was used to predict the efficacy of low-oxygen (O_2) atmospheres in suppressing insect pests in bins of stored wheat. A two-dimensional spatial model was used to predict population growth of *S. oryzae* as a function of grain temperature and moisture. In the model, a cylindrical 82-t steel bin was divided into 12 compartments, allowing the model to predict different insect growth rates based on each compartment's temperature and moisture. Incorporated into the model were equations to predict the effects of low-O_2 atmospheres, grain temperature and insect stage on insect mortality. This model can predict the duration of fumigation required to produce a given mortality using low O_2 levels. It can also be used to predict insect density in grain 1–2 months post-fumigation. Simulations showed that fumigating grain at 29°C for 15 d resulted in 99.9% mortality to all stages of *S. oryzae*. However, fumigating at 17°C for 15 d only resulted in 95.7% mortality of the pupae. Thus, with cool grain near the bin walls longer fumigation intervals are required to kill all development stages of *S. oryzae*, and the pupal and adult stages take longer to kill than the egg and larval stages.

INTRODUCTION

Previous studies have shown that the pupal stage of *Sitophilus oryzae* (L.) is particularly resistant to low-oxygen (O_2) atmospheres (Storey, 1975; Lindgren and Vincent, 1970). At 27°C it requires only 2–3 d to reach 95% mortality for the egg, larval and adult stages. However, the pupal stage requires almost 10 d to reach 95% mortality. Cool grain requires much longer fumigation duration than warm grain. At 21°C, it requires 4–8 d to reach 95% mortality for the egg, larval and adult stages and 20 d for the pupal stage. Grain managers using low-O_2 atmospheres to fumigate grain need to be aware of the additional time required when the grain is cool. Grain temperature is not homogeneous in the grain mass in unaerated bins. In the fall, the periphery of the grain mass cools more quickly than does the center. Insect populations therefore often continue to increase in the centers of the grain masses during the cold winter months.

Computer simulation models have been used to simulate the effects of various control strategies on stored-grain insects. Models have also been used to investigate the effects of grain temperature, moisture, insecticides, aeration and the timing of fumigation (Flinn and Hagstrum, 1990a; Hagstrum and Flinn, 1990). In addition, models have been used to show how insecticide efficacy is influenced by grain cooling (Longstaff, 1988).

A spatial model that simulates changes in temperature and insect population dynamics in a grain bin has been developed and validated (Flinn *et al.*, 1992). By dividing the grain bin into many compartments, the model can simulate different rates of insect growth in each compartment. A spatial model can simulate the effects of non-homogeneous grain temperatures on insect population growth much better than can a model that uses average grain temperatures for the whole bin.

Stored-grain researchers have known for some time that the duration of fumigation required to kill different insect stages using low-O_2 atmospheres varies with temperature. However, there has not been any method for predicting the effects of non-homogeneous temperatures in a grain mass on insect population dynamics. In this paper, we examine the effects of duration of fumigation, grain temperature and insect stage on the efficacy of low-O_2 fumigation for *S. oryzae*. A simulation model of *S. oryzae* population growth, coupled with a two-dimensional bin-temperature model, is used to compare these effects.

MATERIALS AND METHODS

Model

The spatial model used in this study was previously described in Flinn *et al.* (1992). It uses a 2-dimensional representation of the bin, starting at the bin center and proceeding to the bin wall. A cylindrical steel bin with 82-t capacity was divided into 12 regions (Fig. 1). The model predicts *S. oryzae* population dynamics in each of the regions based on the temperatures and moistures predicted by the bin temperature model (Metzgar and Muir, 1983). The insect model uses a distributed delay using 0.10-d intervals to predict insect growth of all stages of *S. oryzae* and includes density-dependent and cold-temperature mortality. The bin temperature model uses hourly weather data for wet and dry bulb temperature, wind speed and cloud opacity to predict changes in grain temperature and moisture. Low-O_2 fumigation mortality equations were estimated using the data from Storey (1975) that predict, based on grain temperature, the number of days required to produce 97% mortality for the egg, larval, pupal and adult stages (Table 1). The composition of the inert atmosphere in Storey's study was <1% O_2 and 8.5–11.5% CO_2, the balance being principally N_2. The regression equations were estimated from data generated by probit equations that were derived from Storey (1975). Regression equations were fitted to data generated by the probit equations for 13 temperatures (15–39°C).

Model simulations

We used 1983 hourly weather data for Topeka, Kansas, and simulations were run from 1 July (harvest) until 1 December. We simulated the effects of fumigation duration, grain

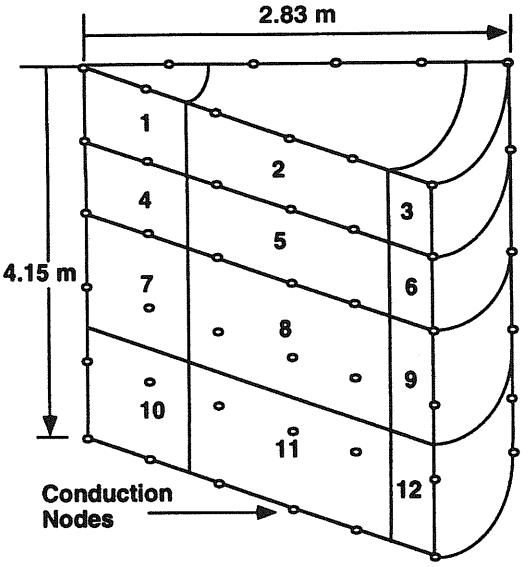


Fig. 1. Diagram showing the 12 bin compartments used in the 2-dimensional model for bin temperature and insect population dynamics in an 82-t cylindrical steel bin.

TABLE 1
Regression parameter estimates for $\log_e$ days until 97% mortality obtained in an atmosphere of <1% O_2 and 10% CO_2 as a function of temperature (°C) for *Sitophilus oryzae*

Life stage	Slope ± SE	Intercept ± SE	R^2	N
Egg	0.01990 ± 0.00112	1.16988 ± 0.03138	0.97	13
Larva	–0.00395 ± 0.00109	1.89115 ± 0.03054	0.54	13
Pupa	–0.11088 ± 0.00224	5.26274 ± 0.06276	0.99	13
Adult	–0.15720 ± 0.00632	5.07174 ± 0.17708	0.98	13

Original data from Storey (1975).

temperature and insect density on fumigation efficacy. We started the simulations using a 32°C grain temperature and 12% moisture content. Although the model predicts the effects of aeration, we simulated a no-aeration condition to maximize the effects of non-homogeneous grain temperature on fumigation efficacy. In the simulations, we assumed that the atmosphere in the bin was homogeneous and had the same composition as in Storey's 1975 study.

Simulation results were compared graphically, and means and standard errors were computed using Systat 5.2 (Wilkinson *et al.*, 1992).

RESULTS AND DISCUSSION

Simulated grain temperatures started at 32°C on 1 July and began decreasing as cooler fall temperatures reduced grain temperature after 1 September (Fig. 2). The model predicted that grain temperatures would fluctuate more in the periphery of the grain mass than in the center. This is because the grain next to the bin wall buffers the grain on the inside of the grain mass from external fluctuations in temperature. Insect population growth rate decreased in the periphery of the grain mass due to the cooler fall temperatures (Fig. 3). In most cases the bin regions that remained warmer longer were predicted to have the highest insect densities. However, the insect density was higher in region 5 than in region 7, even though the temperature remained higher longer in the latter. This was caused by temperatures being higher than optimal in region 7 for part of the storage period.

Insect density at the time of fumigation affected subsequent population recovery (Fig. 4A, B). We simulated the effect of a 10-fold population density by increasing the immigration rate 10-fold. Immigration in the model ceases after 1 October. In Fig. 4B, population density at the time of fumigation was 10 times greater than in Fig. 4A. The resulting population recovery 3 months after fumigation was increased 10-fold in the simulation with the higher immigration rate. These results are similar to those for any insecticide that kills a certain percentage of the population. The larger the population at the time of fumigation, the higher the number of survivors following fumigation. This

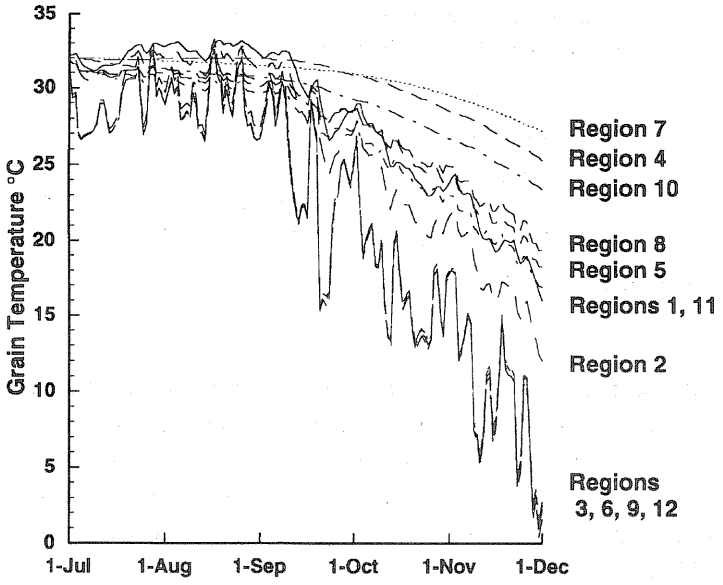


Fig. 2. Simulated grain temperatures in 12 bin compartments for an 82-t capacity cylindrical steel bin located in Topeka, Kansas, using 1983 weather data.

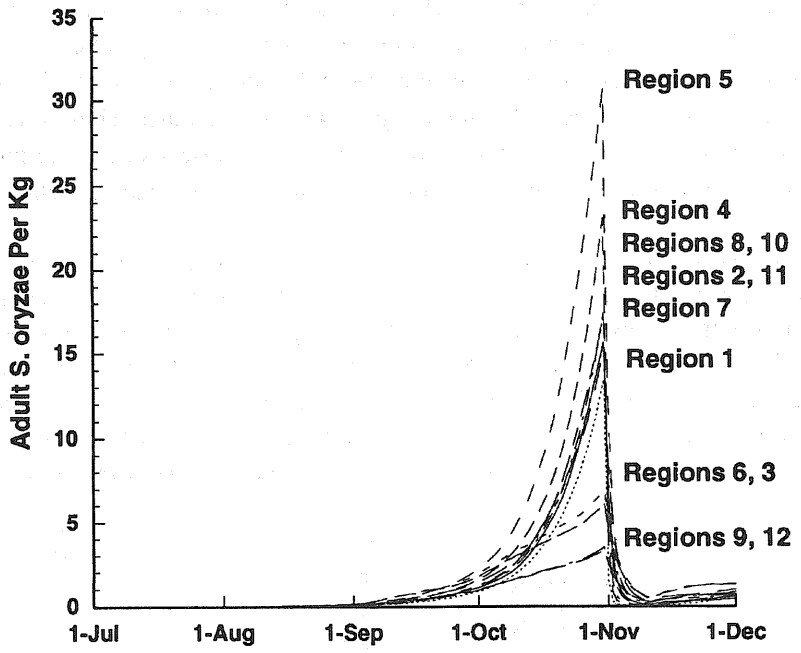


Fig. 3. Simulated *S. oryzae* population density in 12 bin compartments for an 82-t capacity cylindrical steel bin located in Topeka, Kansas, using 1983 weather data. Ten-day fumigation using an exothermic low-O_2 generator was started on 1 November.

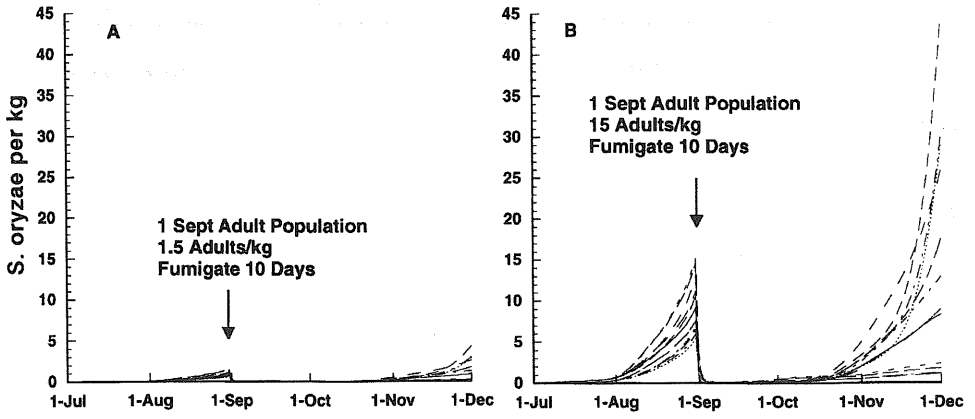


Fig. 4. Simulated population dynamics of *S. oryzae* at a peak density of 1.5 adults/kg (A) and 15 adults/kg (B), fumigated on 1 September.

indicates that population density is an important factor to be considered when making fumigation decisions. A longer fumigation period may be necessary to completely suppress high insect population densities.

The duration of fumigation greatly affected subsequent population recovery (Fig. 5). Our simulated fumigation started 1 September. At this date, the population had reached a density of 16 adult insects per kg. Fumigation for 5, 10, 15 and 20 d resulted in 1,000, 45, 2 and 0 insects per kg, respectively, in bin region 4 by 1 December. Thus, at least a 15-d fumigation period was clearly necessary to kill most of the insect stages and limit recovery. Aeration could of course be used following fumigation to reduce population growth rate.

We examined the effect of temperature on fumigation by simulating the effect of waiting until 1 November to fumigate; by this time, cool autumn temperatures had reduced grain temperatures in the periphery of the grain mass although the center was still warm (Fig. 6). In region 7, at the center of the bin, 15 d of fumigation reduced the egg, larval, pupal and adult stages by over 99.9%. In region 9, at the bin periphery, 15 d of fumigation reduced the egg and larval stages by 99.9% but only reduced the pupal and adult stages by 95.7 and 97.6%, respectively. This is because at low temperatures the pupal and adult stages of

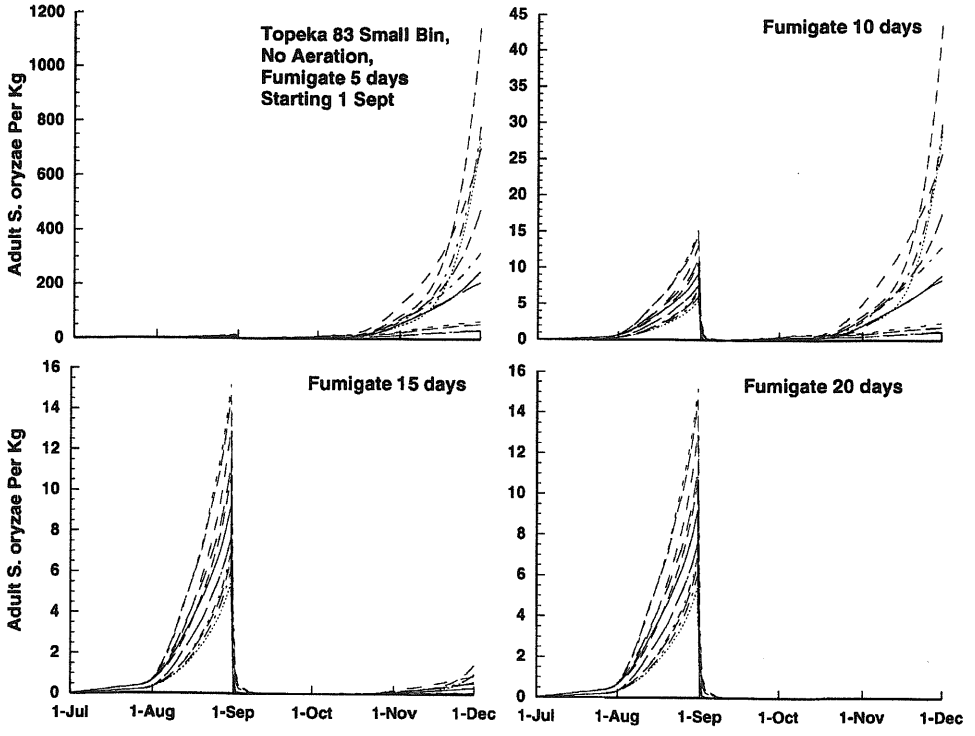


Fig. 5. Simulated population dynamics of *S. oryzae* fumigated for 5, 10, 15 and 20 d starting 1 September. The peak insect density at the time of fumigation was 15 adults/kg.

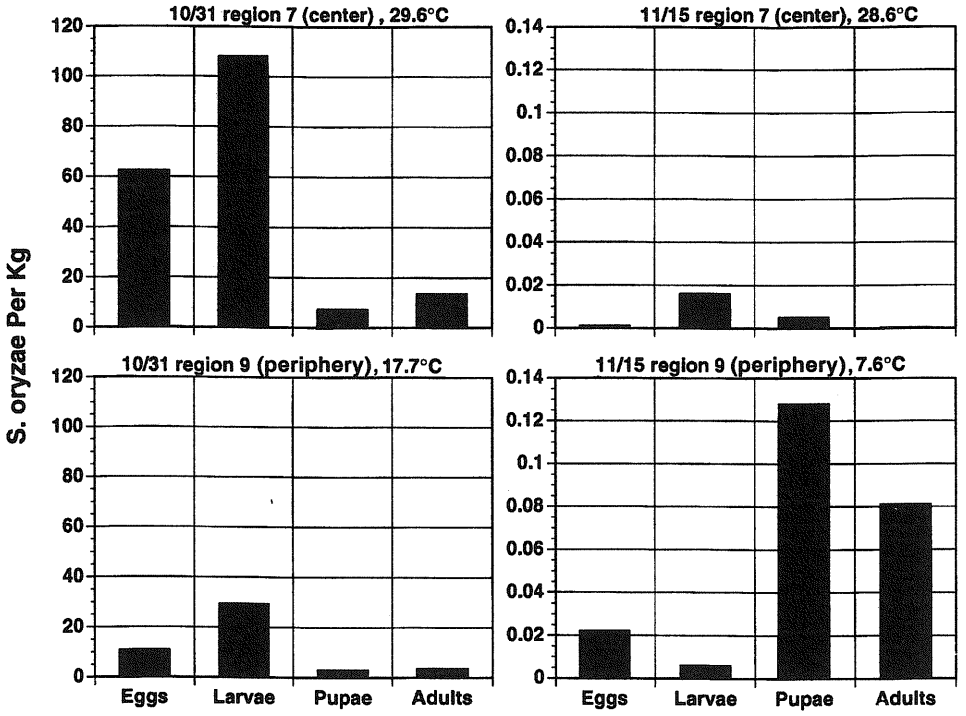


Fig. 6. Effects of grain temperature and low O_2 fumigation on the age structure of *S. oryzae*. The simulated fumigation occurred 1 November and lasted for 15 d.

S. oryzae are more resistant to fumigation than are the other stages. Population density before fumigation was lower in region 9 at the periphery of the grain mass than at region 7 in the center of the grain mass because cooler grain temperature in region 9 suppressed population growth. However, following 15 d of fumigation, the insect density was higher in region 9 than in region 7. It is likely that survivors in region 9 would then migrate into the center of the grain mass. This could cause insect populations to reach high numbers in a few months if the grain center was not cooled by aeration.

We plan to incorporate this model into the Stored Grain Advisor expert system (Flinn and Hagstrum, 1990b) so that it can be used to make low-O_2 fumigation predictions and recommendations for grain managers.

ACKNOWLEDGEMENTS

We wish to thank F. Arthur (USDA, ARS, GMPRC), G. Cuperus (Dept. of Entomology, Oklahoma State Univ.) and E. Soderstrom (USDA, ARS, Hort. Crops Res. Lab.) for reviewing earlier versions of this manuscript.

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SESSION 8

POTENTIAL THREATS TO CONVENTIONAL CONTROLLED ATMOSPHERES AND/OR FUMIGATION (REGULATORY, INSECT RESISTANCE)

Chairpersons:
E.J. Donahaye, *Israel*
J.L. Zettler, *USA*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 445–454

INFLUENCE OF RESISTANCE ON FUTURE FUMIGATION TECHNOLOGY

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ABSTRACT

Although global dependence on fumigation continues to increase, the many constraints on this technology could lead to its extinction. One of these constraints is resistance. Even though phosphine resistance continues to increase in frequency and intensity among pest populations, control failures rarely occur. When they do, however, they can be managed by using other existing, alternative methods or technologies. In spite of our ability to control these resistant populations, future pest-control efforts will become more complex and challenging. Newly developing technologies like genetic engineering could threaten our pest-control security and undermine our efforts to manage resistance. The presence of transgenic food products will increase in the marketplace and have an impact on pest-management technologies in the future.

INTRODUCTION

Fumigation represents our last line of defense against insect infestation and remains the major pest-disinfestation control method worldwide. Dependence on this methodology is increasing for a variety of reasons.

Only two fumigants, methyl bromide (MB) and phosphine (PH_3), remain in widespread use globally. Each of these chemicals possesses certain characteristics that make it suitable for a particular use. MB, the quick action of which requires relatively short exposure periods, is important in fumigation of perishable products; PH_3, with its relatively long exposure period, is better suited for use with durable commodities. Many countries require that imported commodities be fumigated to meet phytosanitary requirements. Many countries during the last decade have (either voluntarily or in obedience to the law) reduced pesticide usage, particularly usage of insecticidal protectants (Matteson, 1995), thereby making fumigation more critical. Australia, for example, has enforced residue-free standards on its grain for many years. The US Environmental Protection Agency has announced a partnership among the EPA, the USDA, the US Food and Drug Administration and volunteer grower groups and utility companies to promote environmental

stewardship in pesticide use. In 1984 the USDA announced a separate initiative to help farmers implement Integrated Pest Management (IPM) on 75% of total crop acreage by the year 2000. Several state agencies in the US no longer recommend insecticidal protectants on grain; instead, they are relying on other, nonchemical methods of maintaining commodity quality. Some large food processing companies in the US have committed themselves to moving towards a less chemical-intensive pest-management program. This is due to litigation about illegal residues, as well as to public opinion (Hegele, 1994). All of this has contributed to more frequent insect infestations in grain stores, and this trend is likely to continue pending implementation of suitable IPM strategies.

Thus, at a time when great dependence is being placed on conventional fumigants, the technology is being attacked on many fronts. MB, because of its ozone-depletion potential, will be banned in the US in 2001 (EPA, 1993). There is also great concern about PH_3 causing chromosomal aberrations and being carcinogenic (Garry *et al.*, 1989; Alavanja *et al.*, 1990). More stringent regulations on PH_3 emissions have raised costs and at times are difficult to implement (Keever, 1990; Keever and Hamm, 1996). Resistance to PH_3 is also increasing both in frequency and intensity. It is fair to say that fumigation as we know it is becoming an endangered technology (Banks, 1994).

Of all the potential threats outlined above, resistance is probably the least important. Nonetheless, because it is the only one over which we have some control, its importance is considerable.

FUMIGANT RESISTANCE

Table 1 shows the results of PH_3-resistance surveys. Several recent reports indicate that fumigant resistance globally is continuing to develop in intensity and frequency (Irshad *et al.*, 1992; Emery, 1994; Rajendran and Narasimhan, 1994; Suliaman *et al.*, 1994; Bell and Wilson, 1995). One of these reports showed that in Indian tobacco stores, control failure with PH_3 was the result of PH_3-resistance in the cigarette beetle (Rajendran and Narasimhan, 1994). Despite increased resistance in these beetles, control failures, as in earlier instances, were overcome by increasing both dosage and exposure time.

COMBATING RESISTANCE

No cases of MB control failures due to resistance have been reported. This fumigant seems to remain effective although its regulatory future is uncertain. PH_3-resistance, on the other hand, has caused control failures. Often these resistant populations have been dealt with by MB fumigation or increasing the PH_3 dose by lengthening the exposure period or increasing the concentration, or both. Lengthening the exposure period is generally most effective (Winks, 1980). These methods are, of course, used only after ensuring that the affected commodity can be sufficiently sealed to contain the gas for the required increase in the Ct (concentration × time) product.

TABLE 1
Incidences of phosphine resistance by region and species*

Region	Number of strains tested (left) and number of strains resistant (right) for each species																					
	Tribolium castaneum		*Rhyzopertha dominica*		*Sitophilus* spp.		*Oryzaephilus* spp.		*Tribolium confusum*		*Cryptolestes* spp.		*Lasioderma serricorne*		*Plodia interpunctella*		*Cadra cautella*		*Trogoderma granarium*	
N Africa	3	1	1	1	4	2	2	0	1	0										
Sub-Sahara[9]	23	7	20	17	7	1	2	2			2	2							1	1
S Asia	17	11	10	8	3	1	2	1												
SE Asia	12	0	16	9	3	0	1	0			2	1								
S America[1]	30	24	34	31	22	21	1	1			14	14								
Australia[2]	1,330	224	551	125	210	58	726	105	27	3										
US[3]	59	22	21	8					17	3			20	8	7	4	18	3		
UK[4]	14	5	2	0	39	17	54	3	4	1	30	4								
Malaysia[5]	22	5	11	11	17	2														
Pakistan[6]	33	5	25	11																
India[7]	5	5	4	4	3	1	2	1			1	0	2	2						
France[8]	1	1																		

*After Taylor (1989) and Zettler (1992).

[1]Sartori *et al.* (1991); [2]Attia and Greening (1981), Herron (1990) and White and Lambkin (1990); [3]Zettler (1991); [4]Mills (1986); [5]Suliaman *et al.* (1994); [6]Irshad *et al.* (1992); [7]Rajendran (1989), Rajendran and Narasimhan (1994); [8]Becton *et al.* (1988); [9]Bell and Wilson (1995).

INCREASING EXPOSURE TIME

Following the first reported case of control failure with PH_3, an analysis of resistance levels in the affected insects showed that an effective PH_3 dose for the resistant insects would be 150 mg L/h for a minimum of 3 d (Tyler *et al.*, 1983). Treatment schedules for PH_3-resistant insect populations given by Mills (1986) required a minimum exposure time of 6 d at 25°C. The effective dose for PH_3-resistant cigarette beetles reported by Rajendran and Narasimhan (1994) is a Ct of 480 mg L/h for a minimum of 10 d at 30°C.

Thus, while it is possible to control PH_3-resistant populations with PH_3 by increasing the exposure time, clearly at some point in the future there will be a practical limit to the extent of such an increase. Most fumigated structures are not sufficiently gastight to provide adequate fumigation for pesticide-susceptible insects. How long, then, can we expect to be able to simply increase the exposure period in order to deal with resistance? Given the present state of storage facilities in most parts of the world, it is reasonable to expect resistance to continue to increase, forcing us to concomitantly increase exposure times.

OTHER FUMIGATION TECHNIQUES

Dealing with fumigant resistance is not yet an uncontrollable problem. Resistance can be managed by effective pest control. Many suitable technologies and methodologies exist to deal with it. However, most resistance occurs in developing countries where suitable equipment and sanitation practices are generally lacking. Despite these limitations, economic advances have been made in the proper fumigation of sheeted bag-stacks. Both laminated PVC sheets and large sandsnakes (Taylor and Harris, 1994) and laminated wall and floor coverings (Xu and Wang, 1993) can greatly improve fumigation efficiency.

Hermetic storage, because of its simplicity, is ideal for use in developing countries. It is environmentally friendly and has global applications for protection of stored commodities in both developing and advanced countries (Navarro *et al.*, 1994).

Fumigant recirculation technology has been known for more than 50 years and used for HCN and MB fumigations. This technology has been applied to modified atmospheres (MA's) (Wilson *et al.*, 1980) and PH_3 fumigations (Cook, 1984). Recent technological advances include the closed-loop system used in the US (Kenkel *et al.*, 1993; Noyes and Kenkel, 1994), the circumfluent system used in China (Sun *et al.*, 1993; Lu *et al.*, 1994), the PHYTO-EXPLO® system (Chakrabarti *et al.*, 1994; Vacquer *et al.*, 1993a, b) and J-SYSTEM® (Degesch) for fumigation of large, flat bulk storages, silos and holds of export grain ships (Zettler *et al.*, 1984; Leesch *et al.*, 1986). These technologies usually reduce the dose of fumigant required to produce a lethal Ct product in grain pests and thus improve the efficiency of conventional types of PH_3 fumigation.

Flow-through systems like SIRO-FLO® (Winks, 1993) and cylinder-based PH_3/CO_2 mixtures (Bell *et al.*, 1993; Chakrabarti *et al.*, 1994) are efficient because they rely on the increased susceptibility of pest insects to continuous steady-state concentrations of PH_3 rather than on increasing/decreasing the concentrations experienced during normal conventional fumigations. In addition, the concentrations required are lower than normal and

thus more economical and also more environmentally friendly. This allows the extension of exposure periods for control of resistant populations at no additional expense. Finally, these technologies are forgiving in that they can be effective even in leaky storages, although this is certainly not to recommend that they be used in leaky situations in lieu of properly sealing the store.

Combination treatments of PH_3 with carbon dioxide (CO_2) (Carmi *et al.*, 1990, 1994) and with CO_2 and heat (Mueller, 1993) have proved useful under some circumstances for fumigating large silos and flour mills. CO_2 helps the movement of PH_3 through commodities (Leesch, 1990), and the addition of heat lowers the effective dose of PH_3 required for a lethal Ct product.

The list of alternative fumigants is small. Several new compounds being tested or retested were listed by Banks (1994). Carbonyl sulfide (Desmarchelier, 1994) and methylisothiocyanate (Ducom, 1994) have shown promise in stored-grain situations but they are not labeled for use on any commodity.

BIOTECHNOLOGY AND THE FUTURE OF POSTHARVEST PEST CONTROL

Biotechnology has the potential to significantly affect postharvest pest control via genetic engineering. This branch of molecular biology is developing rapidly. The first engineered gene to successfully confer insect resistance on plants was constructed less than 15 years ago (Meussen and Warren, 1989). Today, more than 50 plant species have been genetically manipulated for some specific purpose (Gasser and Fraley, 1989), i.e. resistance to disease, resistance to insect predation and herbicides or an increase in shelf-life of fresh fruits and vegetables.

Three areas of research are presently being actively investigated with the goal of improving stored-product insect pest management.

DNA fingerprinting

Polymerase chain reaction (PCR) is a technique that amplifies or clones DNA fragments which can then be utilized as a fingerprint for that particular DNA. This DNA fingerprint is unique for each individual or population within a species (Beeman, 1994). Recently a variation of the PCR technique called random amplification of polymorphic DNA (RAPD) has been used to fingerprint populations of *Plodia interpunctella*. Comparisons of RAPD fingerprints within regions could provide new insights into population movements and infestation sources which would be potentially useful in tracking the global movement of resistant populations of stored-product pests.

Resistance detection

PCR can be used to fingerprint resistance genes. For example, the lindane resistance gene, prevalent throughout the world, has recently been cloned and its structure determined (ffrench-Constant, 1993). Shortly thereafter, a PCR-based resistance detection assay was developed and tested on *Tribolium castaneum* (Andreev *et al.*, 1994). This

assay system should be useful in detecting other types of resistance once the relevant genes are identified and molecularly characterized.

Insect-resistant seeds

Transgenic corn, incorporating the delta endotoxin of *Bacillus thuringiensis* (Bt), has been approved by the EPA (Anon., 1995). Similarly, the cotton plant has been genetically engineered to incorporate the 'Bollgard gene,' derived from Bt, that is toxic to cotton bollworm, tobacco budworm and pink bollworm (Barton, 1995). A transgenic potato plant has also been engineered to contain a Bt gene which is toxic to the Colorado potato beetle (Wassell and Knorr, 1995).

These insect-resistant crops all contain Bt endotoxin genes which may in actuality be just another chemical insecticide to which insect pests will develop resistance (Oberlander, 1995). The controversy raised by this possibility has led to considerable concern about resistance management (McGaughey, 1990; McGaughey and Whalon, 1992; Tabashnik and McGaughey, 1994).

The insecticidal components of these transgenic plants are manifested in the foliage. However, they can also be manipulated to be manifested in the seeds themselves. Indeed, a genetically engineered garden pea has been developed which contains an amylase inhibitor protein (Shade *et al.*, 1994). The gene for this protein was taken from the common bean that, by virtue of its amylase inhibitor protein, is normally tolerant to the cowpea weevil, *Callosobruchus maculatus*, and the Azuki bean weevil, *C. chinensis*. The potential to insert this gene into seeds of other crop plants exists. Other candidate insect control proteins that might be used to confer insect resistance to seeds of other transgenic plants include Bt toxins, proteinase inhibitors, vitamin binding proteins, venoms and chitinases (Beeman, 1994).

What are the implications of these biotechnological advances for postharvest entomology? Some day our silos and warehouses may be filled with transgenic grains and oilseeds containing a variety of pest-control agents. Based on previous history, one can assume that the resistance problem will not be alleviated but is instead likely to increase. The insecticides or pesticides to which pests will be exposed will likely be more environmentally friendly, and perhaps more selective in their toxicities, than those available today. However, insects are likely to develop resistance to them. This translates into higher pest infestations and more pest-control failures. Consequently, there is likely to be a continued need for conventional and MA fumigation, even in the transgenic arena.

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RESISTANCE — A THREAT TO THE USE OF CONTROLLED ATMOSPHERES FOR STORED-PRODUCT PROTECTION?

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ABSTRACT

According to data in the literature, adult stored-product insects could be selected for up to a ninefold increase in tolerance to controlled atmosphere (CA) treatments over the tolerance of unselected strains. Carbon dioxide (CO_2)-atmospheres with high residual oxygen (O_2) contents produced higher resistance factors than did hypoxic atmospheres. High moisture contents (m.c.) favoured insect survival. Tolerance to various CA's correlated with adult body weight. However, when developmental stages were examined, lethal times of the naturally most tolerant pupal stages could not clearly be prolonged by selection. In a comparison among ten *Sitophilus granarius* strains from three continents, including strains formerly resistant to methyl bromide, phosphine or CO_2, the LT_{99} values of pupal stages in the least susceptible strain were maximally double those of the most susceptible strain. At present we have no proof that resistance threatens the use of CA's for stored-product protection. To minimise the risk of resistance developing, the number of control treatments per commodity should be reduced and emphasis placed on preventive measures and early pest detection within the framework of integrated stored-product protection. All control techniques available, including the use of CA's and toxic fumigants, should be alternated, and incomplete control treatments must be avoided.

INTRODUCTION

Due to the very limited number of stored-product protection agents available today, potential insecticide resistance is a serious threat which increases the need for a sound resistance-management policy. The information available on the mode of action of toxic fumigants and development of resistance to them in stored-product pests was reviewed by Price (1986).

Controlled atmospheres (CA's) low in oxygen (O_2) and/or high in carbon dioxide (CO_2) have been registered in many countries during the last two decades for stored-product protection. On the one hand, users did not readily accept this technology which

takes more time, requires a higher degree of gastightness and is often more costly than is fumigation with toxic substances. On the other hand, use of this technique may be increasing because it does not leave residues in the treated product, minor leaks do not produce health risks to exposed people and there is no unpredictable environmental risk in the use of nitrogen (N_2) or CO_2.

According to several laboratory studies, adults of some stored-product Coleoptera could be selected that were several times more tolerant to a given CA than random individuals. The present paper summarises current knowledge about resistance to CA's.

REVIEW OF CURRENT KNOWLEDGE

What is known about resistance to CA's? It is possible to experimentally select strains with an increased tolerance towards certain CA's (Table 1). Bond and Buckland (1979) exposed adult granary weevils *Sitophilus granarius* (L.) to an atmosphere of 42% CO_2, 46.4% N_2

TABLE 1
Literature data on the selection of stored product pests for tolerance to controlled atmospheres

Source	Species[1]	Temperature (°C)/r.h.(%)	Gas mixture	Selection (no. of generations)	Resistance factor/level
Bond and Buckland (1979)	*S. granarius* (A)	25/100	42% CO_2 46.2% N_2 11.6% O_2	7	3.3 (LT_{99})
Navarro *et al.* (1985)	*S. oryzae* (A)	26/100	75% CO_2 (rest air)	10	3.34 (LT_{95})
Navarro *et al.* (1985)	*S. oryzae* (A)	26/60	75% CO_2 (rest air)	10	2.87 (LT_{95})
Donahaye (1990a)	*T. castaneum* (A)	25/95	65% CO_2 15% N_2 20% O_2	40	9.2 (LT_{95})
Donahaye (1990b)	*T. castaneum* (A)	25/95	99.5% N_2 0.5% O_2	40	5.2 (LT_{95})
Annis (1991)	*S. oryzae* (P)	25/65	40% CO_2 (rest air)	7	–[2]
Annis (1991)	*S. oryzae* (P)	25/65	65% CO_2 (rest air)	7	–
Annis (1991)	*S. oryzae* (P)	25/65	95% CO_2 (rest air)	7	–

[1]In paranthesis, selected development stage: A = adults; P = pupae.
[2]No significant resistance was recorded.

and 11.6% O_2 (by volume) at 25°C and close to 100% r.h., until 60% mortality was achieved. After selecting six subsequent generations in this way, they determined that the tolerance factor was 3.3 at the LT_{99}-level as compared to an unselected population. Navarro *et al.* (1985), by selecting adult rice weevils *S. oryzae* (L.) for ten generations in an atmosphere of 75% CO_2 at 26°C and approximately 100% r.h., achieved a tolerance factor of up to 3.34 at the LT_{95} level. When this tolerant strain was exposed to the same gas mixture at 60% r.h., there was a tolerance factor of 2.87 (LT_{95}). Since O_2 content of up to 21% did not markedly change either the LT's or the tolerance levels, the authors attributed the tolerance to the action of CO_2. These data correspond to those of Adler (1994), suggesting that at least part of the toxic action of CO_2 is due to acidosis blocking the glycolytic pathway and thus inhibiting further metabolism even in the presence of sufficient O_2.

Donahaye (1990a, b) selected adult *Tribolium castaneum* (Herbst) for 40 generations at 25°C and 95% r.h. not only to a CO_2-enriched atmosphere (65% CO_2, 15%N_2 and 20% O_2) but also to a hypoxic atmosphere (99.5% N_2 and 0.5% O_2). While the level of tolerance against the hypoxic atmosphere reached a value of 5.2 after 26 generations and could not be increased thereafter (Donahaye, personal communication), the tolerance level against the hypercarbic gas mixture increased gradually from 6.3 to 9.2 after 40 generations of selection. This may indicate that physiologically it is easier to withstand the toxic effects of CO_2 in the presence of sufficient O_2 than the physiological stress caused by lack of O_2.

All laboratory selections were carried out at extremely high r.h., and Donahaye (1991) noted that such conditions were not to be expected in a practical situation. However, one has to keep in mind that condensation processes, leaky storage structures and heavy infestations in so-called "hot spots" may lead to very high local moisture contents (m.c.'s).

In a comparison of eight laboratory strains (-L) and two field strains (-f) of *S. granarius* from Australia (AUS), Canada (CN), France (F), Great Britain (GB), the United States of America (USA) and Germany (D), Adler (1991, 1993) found that there were differences in their susceptibility to certain atmospheric gas mixtures, i.e. 99% N_2, 1% O_2 (Atmosphere 1); 80% N_2, 1% O_2, 19% CO_2 (Atmosphere 2); and 4% N_2, 1% O_2, 95% CO_2 (Atmosphere 3).

LT_{99}-values among the strains, calculated by Probit analysis according to Finney (1971), showed up to a twofold difference in the pupal age group and up to a fourfold difference in adults (Tables 2–7, Figs. 1–3). However, no single strain was consistently more tolerant through all stages than were others. This may be due in part to differences in the average time needed by each strain for egg-to-adult development. The granary weevil is known for its unsynchronised development. At 25°C and 75% r.h., from eggs oviposited on the same day weevil emergence may be up to 21 d apart. A comparison of untreated cultures reared at 25°C and 75% r.h. showed differences in the average development times of the tested strains (Table 8). Therefore, developmental stage 5 (Table 6), treated 29–32 d after oviposition, may have contained a large number of relatively susceptible late larval stages in a slow-developing strain or some already very susceptible emerging weevils in a fast-developing strain. Both effects would reduce the LT_{99} to be

TABLE 2

Susceptibility of ten *S. granarius* strains (stage 1: eggs, reared at 25°C/75% r.h., exposed 1–4 days after oviposition) to three different CA's at 20°C/75% r.h.

	Atmosphere 1		Atmosphere 2		Atmosphere 3	
Strain	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits
GB-L2	14.8	14.0–15.8	**10.3**	9.7–10.9	14.1	13.2–15.1
D-L	15.6	14.6–16.7	14.7	13.7–15.8	**15.7**	14.5–17.1
CN-L2	12.3	11.7–13.0	13.2	12.5–13.9	**11.9**	11.4–12.5
CN-L1	**11.7**	11.0–12.4	12.4	11.6–13.2	12.1	11.5–12.7
GB-L1	13.0	12.4–13.6	14.6	13.9–15.2	15.0	14.2–15.9
D-f	13.0	12.2–13.9	13.4	12.7–14.1	13.2	12.6–13.9
USA-L	14.7	13.7–15.8	14.4	13.3–15.6	13.6	12.5–14.9
AUS-L	**17.2**	16.5–18.0	14.3	13.8–14.9	14.7	14.0–15.5
F-L	14.9	13.9–16.1	14.3	13.3–15.4	14.3	13.2–15.5
AUS-f	14.5	13.9–15.1	**14.9**	14.3–15.5	15.4	14.6–16.2

Atmosphere 1: 99% N_2, 1% O_2; Atmosphere 2: 80% N_2, 19% CO_2, 1% O_2; Atmosphere 3: 95% CO_2, 4% N_2, 1% O_2. Bold numbers indicate highest and lowest values. -L: laboratory strain; -f: field strain (e.g. CN-L1: laboratory strain from Canada).

TABLE 3

Susceptibility of ten *S. granarius* strains (stage 2: larvae, reared at 25°C/75% r.h., exposed 8–11 days after oviposition) to three different CA's at 20°C/75% r.h.

	Atmosphere 1		Atmosphere 2		Atmosphere 3	
Strain	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits
GB-L2	**10.6**	9.7–11.6	8.5	7.9–9.1	4.0	3.6–4.5
D-L	9.9	9.1–10.8	**8.6**	7.9–9.5	**2.5**	2.0–3.1
CN-L2	8.5	7.9–9.1	7.8	7.2–8.3	4.4	4.0–4.8
CN-L1	8.5	7.9–9.2	7.4	6.9–8.0	3.8	3.4–4.2
GB-L1	6.6	6.0–7.3	6.8	6.3–7.3	2.7	0.7–10.3
D-f	8.2	7.6–8.8	7.8	7.3–8.3	3.3	3.0–3.6
USA-L	**5.1**	4.6–5.8	7.2	6.4–8.1	3.0	2.4–3.7
AUS-L	6.0	5.5–6.6	6.8	6.3–7.4	**4.7**	3.7–5.9
F-L	7.6	6.6–8.7	**5.1**	4.5–5.7	2.6	2.1–3.3
AUS-f	7.8	7.2–8.5	6.9	6.4–7.4	3.2	2.3–4.3

Atmosphere 1: 99% N_2, 1% O_2; Atmosphere 2: 80% N_2, 19% CO_2, 1% O_2; Atmosphere 3: 95% CO_2, 4% N_2, 1% O_2. Bold numbers indicate highest and lowest values.

TABLE 4

Susceptibility of ten *S. granarius* strains (stage 3: larvae, reared at 25°C/75% r.h., exposed 15–18 days after oviposition) to three different CA's at 20°C/75% r.h.

	Atmosphere 1		Atmosphere 2		Atmosphere 3	
Strain	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits
GB-L2	14.1	13.0–15.3	11.6	10.8–12.4	5.0	0.4–59.0
D-L	**17.3**	16.0–18.7	**15.7**	14.4–17.1	5.8	5.2–6.5
CN-L2	12.5	11.8–13.2	10.6	10.0–11.5	**7.4**	6.9–7.8
CN-L1	11.7	11.0–12.5	11.2	10.6–11.8	6.5	6.1–6.9
GB-L1	**7.1**	6.7–7.6	**8.6**	8.4–9.4	5.0	4.4–5.8
D-f	13.2	12.4–13.9	12.9	12.0–13.9	4.7	0.8–26.7
USA-L	10.0	9.2–10.9	10.5	9.7–11.4	**4.3**	3.9–4.9
AUS-L	8.9	8.4–9.4	9.5	8.9–10.1	5.6	4.9–6.4
F-L	11.9	10.9–13.0	11.6	10.7–12.6	4.4	3.8–5.1
AUS-f	12.8	12.0–13.6	10.4	9.9–11.0	5.7	5.0–6.4

Atmosphere 1: 99% N_2, 1% O_2; Atmosphere 2: 80% N_2, 19% CO_2, 1% O_2; Atmosphere 3: 95% CO_2, 4% N_2, 1% O_2. Bold numbers indicate highest and lowest values.

TABLE 5

Susceptibility of ten *S. granarius* strains (stage 4: larvae, reared at 25°C/75% r.h., exposed 22–25 days after oviposition) to three different CA's at 20°C/75% r.h.

	Atmosphere 1		Atmosphere 2		Atmosphere 3	
Strain	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits
GB-L2	18.6	16.2–21.3	20.2	19.2–21.2	15.0	9.3–24.2
D-L	23.8	22.8–24.9	22.0	21.0–23.1	**19.9**	18.6–16.7
CN-L2	21.5	20.9–22.2	22.6	21.7–23.6	15.6	14.8–16.4
CN-L1	22.5	21.8–23.3	23.0	22.1–23.8	17.9	17.1–18.8
GB-L1	**14.7**	13.6–15.9	**15.2**	14.5–16.0	**9.2**	5.5–15.3
D-f	**28.4**	27.6–29.2	**26.3**	25.0–27.7	18.6	17.6–19.6
USA-L	18.6	17.6–19.7	20.9	19.9–22.0	14.0	12.9–15.3
AUS-L	17.2	16.3–18.2	17.9	17.0–18.8	13.0	11.7–14.5
F-L	19.1	18.3–20.0	20.4	19.4–21.5	15.5	14.5–16.7
AUS-f	22.5	21.5–23.5	25.8	24.3–27.3	15.0	14.0–16.2

Atmosphere 1: 99% N_2, 1% O_2; Atmosphere 2: 80% N_2, 19% CO_2, 1% O_2; Atmosphere 3: 95% CO_2, 4% N_2, 1% O_2. Bold numbers indicate highest and lowest values.

TABLE 6

Susceptibility of ten *S. granarius* strains (stage 5: mainly pupae, reared at 25°C/75% r.h., exposed 29–32 d after oviposition) to three different CA's at 20°C/75% r.h.

	Atmosphere 1		Atmosphere 2		Atmosphere 3	
Strain	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits
GB-L2	**19.5**	17.5–21.8	22.1	21.3–23.0	18.3	17.3–19.4
D-L	22.5	21.5–23.5	21.2	20.1–22.3	18.5	17.5–19.6
CN-L2	24.1	23.5–24.7	24.6	23.7–25.4	19.5	18.7–20.2
CN-L1	**28.8**	28.0–29.3	26.7	25.8–27.6	22.5	21.7–23.4
GB-L1	20.5	19.9–21.2	22.2	21.5–22.9	**11.3**	6.2–20.8
D-f	26.6	25.9–27.4	22.4	21.6–23.2	19.7	18.8–20.7
USA-L	22.2	21.2–23.3	23.3	22.4–24.4	20.2	19.0–21.5
AUS-L	21.8	21.0–22.5	20.7	20.0–21.5	19.3	18.3–20.4
F-L	20.4	19.3–21.5	**20.2**	19.1–21.4	15.5	14.5–16.5
AUS-f	28.4	27.3–29.5	**29.7**	28.1–31.4	**23.2**	22.1–24.2

Atmosphere 1: 99% N_2, 1% O_2; Atmosphere 2: 80% N_2, 19% CO_2, 1% O_2; Atmosphere 3: 95% CO_2, 4% N_2, 1% O_2. Bold numbers indicate highest and lowest values.

TABLE 7

Susceptibility of ten *S. granarius* strains (adults, reared at 25°C/75% r.h., exposed 1–3 weeks after emergence) to three different CA's at 20°C/75% r.h.

	Atmosphere 1		Atmosphere 2		Atmosphere 3	
Strain	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits	LT_{99} (d)	Fiducial limits
GB-L2	8.0	7.5–8.6	8.1	7.5–8.6	**8.1**	7.6–8.7
D-L	7.3	4.8–11.2	5.6	3.9–7.8	4.5	3.9–5.3
CN-L2	7.6	6.5–9.0	6.8	6.1–7.6	5.8	5.0–6.6
CN-L1	6.3	5.5–7.2	7.4	6.6–8.4	5.5	4.8–6.2
GB-L1	4.3	4.0–4.6	**2.5**	0.1–64.5	**3.4**	3.0–3.7
D-f	5.5	5.1–5.8	**10.3**	8.3–12.7	5.5	4.8–6.2
USA-L	6.6	4.5–9.6	5.2	3.8–7.0	4.4	3.8–5.1
AUS-L	**4.2**	3.9–4.4	3.2	2.9–3.5	4.0	3.6–4.4
F-L	**9.0**	1.1–23.5	6.6	1.3–32.9	4.4	3.8–5.1
AUS-f	4.3	4.0–4.6	2.9	0.7–11.4	3.8	3.4–4.2

Atmosphere 1: 99% N_2, 1% O_2; Atmosphere 2: 80% N_2, 19% CO_2, 1% O_2; Atmosphere 3: 95% CO_2, 4% N_2, 1% O_2. Bold numbers indicate highest and lowest values.

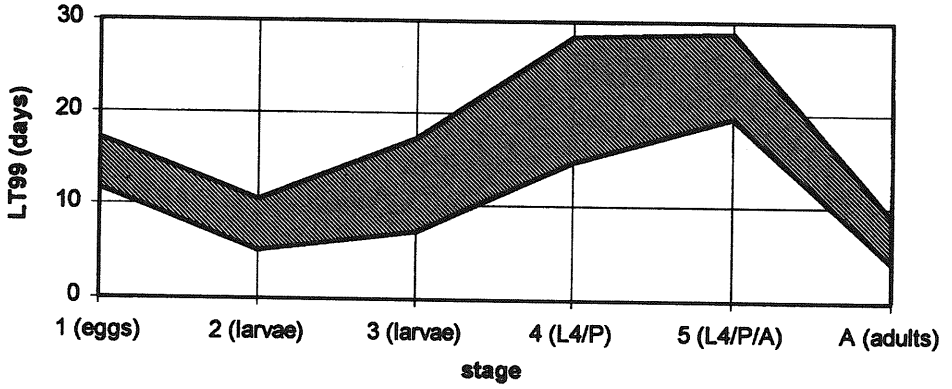


Fig. 1. Variations in the LT_{99} of ten *S. granarius* strains produced by an atmosphere composed of 99% N_2, 1% O_2.

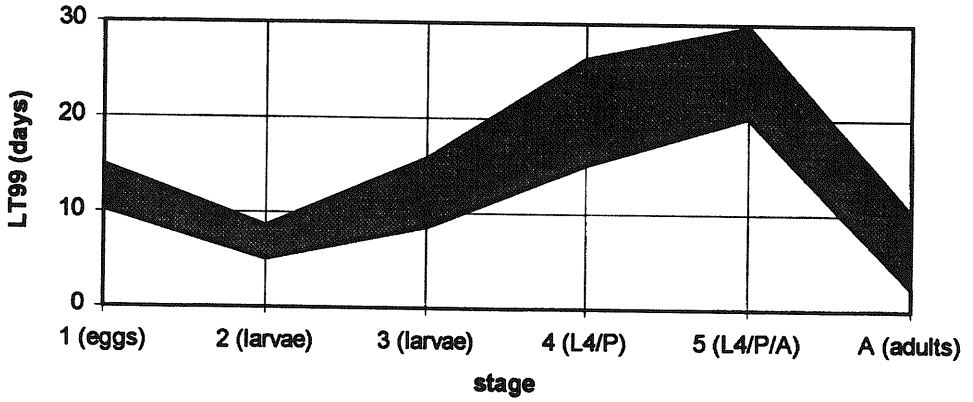


Fig. 2. Variations in the LT_{99} of 10 *S. granarius* strains produced by 80% N_2, 19% CO_2, 1% O_2.

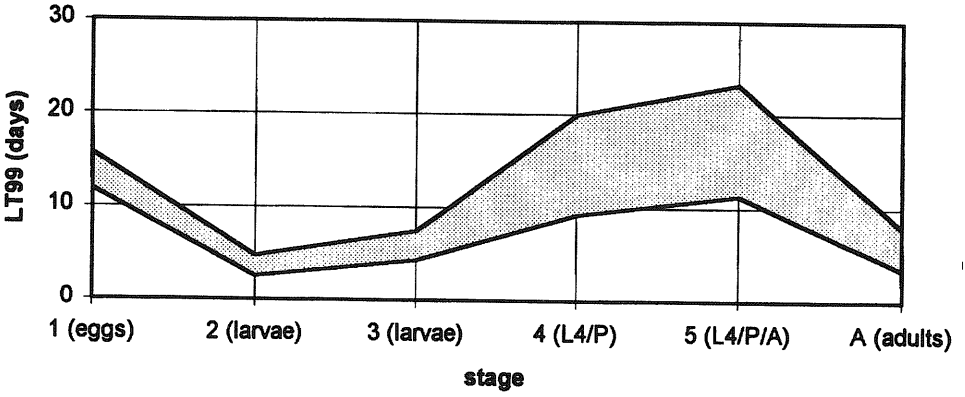


Fig. 3. Variations in the LT_{99} of 10 *S. granarius* strains produced by 95% CO_2, 4% N_2, 1% O_2.

TABLE 8
Mean development times of ten *S. granarius* strains

Strain	Mean development time (d)
GB-L2	43
D-L	38.5
CN-L2	41
CN-L1	38.5
GB-L1	47
D-f	38.5
USA-L	45
AUS-L	49
F-L	40
AUS-f	42

Time (days) from oviposition to adult emergence at 25 ± 1°C/75 ± 5% r.h., estimated by weekly examination of untreated samples.

expected from treatment of only young pupal stages. This complicates the comparison of mortality data of late juvenile stages in *Sitophilus* species.

Comparison of adults

Donahaye (1991) noted that both of his strains of *T. castaneum*, selected for tolerance against hypercarbia and hypoxia, respectively, were significantly heavier than the unselected strain.

When the LT_{99}-values and adult body weights of strains were compared using Spearman's ranks test, a significant correlation between body weight and tolerance to 95% CO_2 (Table 9, Atm. 3) could be found. Among adult weevils, the GB L-strain GB-L2 (originally named "methyl bromide (MB) resistant XXM 65 (51)") proved to be most tolerant to Atm. 3. According to information from the Slough Laboratory, this strain originally came from Canada and was described in Bond and Upitis (1976) as being resistant to MB. When it arrived in the United Kingdom in 1979, it was reported to be about 15 times more tolerant to MB than normal strains; since then no selection has been carried out. As early as 1973, Upitis *et al.* reported increased body weight coinciding with MB-resistance in granary weevils. The increased body weight found then has probably been retained ever since selection was carried out.

The Canadian "CO_2-selected" laboratory strain CN-L2 was the culture described in Bond and Buckland (1979). In their experiment it proved to be the strain the adults of which were the second most tolerant to Atm. 3 (Table 9). Both selected strains seem to have been, at least to some degree, genetically stable over many years without further selection. Moreover, it seems that resistance to MB in *S. granarius* could result in cross resistance to CO_2 even though the experiments of Donahaye (1991) with CO_2-selected *T. castaneum* did not reveal any sign of this.

TABLE 9
Body weight, LT_{99}-values (days) and rankings of adults of ten *S. granarius* strains

Strain	Weight		Atmosphere 1		Atmosphere 2		Atmosphere 3	
	g	Rank	LT_{99}	Rank	LT_{99}	Rank	LT_{99}	Rank
GB-L2	4.66 ± 0.03	1	8.0	2	8.1	2	8.1	1
D-L	4.29 ± 0.13	2	7.3	4	5.6	6	4.5	5
CN-L2	4.19 ± 0.07	3	7.6	3	6.8	4	5.8	2
CN-L1	3.87 ± 0.04	4	6.3	6	7.4	3	5.5	3
GB-L1	3.51 ± 0.04	5	4.3	8/9	2.5	10	3.4	10
D-f	3.35 ± 0.01	6	5.5	7	10.3	1	5.1	4
USA-L	3.33 ± 0.02	7	6.6	5	5.2	7	4.4	6/7
AUS-L	3.05 ± 0.03	8	4.2	10	3.2	8	4.0	8
F-L	2.62 ± 0.03	9	9.0	1	6.6	5	4.4	6/7
AUS-f	2.53 ± 0.02	10	4.3	8/9	2.9	9	3.8	9

Weight of 1000 live adult weevils, based on 3 replicates, and standard deviation. Spearman's ranks test gave the following values: Correlation of mean adult body weight to LT_{99}–Atmosphere 1, $r_s = 0.415$; correlation of mean adult body weight to LT_{99}–Atmosphere 2, $r_s = 0.479$; correlation of mean adult body weight to LT_{99}–Atmosphere 3, $r_s = 0.706$.
Threshold r_s value for significance ($p = 0.05$) is 0.55. Thus, medium adult body weight correlates significantly only to tolerance for the hypercarbic atmosphere.

The adults of the Australian field strain AUS-f, originally named "GSG 66, PH_3-resistant" had the lowest comparative body weight and were also quite susceptible to all tested gas mixtures.

Comparison of juvenile stages

Annis (1991) pointed out that analyses in resistance surveys should be carried out on the most tolerant developmental stage of the most tolerant species because dosage schedules for CA treatments are designed to control these pests. He suggested that the pupal stages of *S. oryzae* and *Trogoderma* spp. should be studied. Exposing groups of pupae of seven different strains of *S. oryzae* for seven generations to hypercarbic atmospheres of 40, 65 or 95% CO_2 in air at 26°C and 65% r.h., Annis found a significant increase in tolerance in one strain. Because of variations in the data, however, this increase was too small to enable its magnitude to be estimated.

In a later study, Donahaye *et al.* (1992) found that partial resistance was displayed by the eggs and larvae of both the high-CO_2 and the low-O_2 resistant strains of *T. castaneum*. However, the naturally most tolerant pupal stage did not display any increased tolerance in these strains selected in the adult stage.

The comparison of *S. granarius* strains is somewhat confusing because the AUS-f strain the adults of which were most susceptible to 95% CO_2 displayed pupal stages which were the most tolerant to this atmosphere (Tables 6 and 7). This could mean that the

relative tolerance of pupal stages is independent of adult body weight. The variation in LT_{99}-values shown in Figs. 1–3 may be an indication of the genetic potential of each stage of development. The maximum ratio of the most susceptible to the most tolerant strain is 1:4 in adults (Atm. 2), but it is 1:2 in pupae (Atm. 3).

CONCLUSION

The development of resistant pest populations can be regarded as a natural response to selection by man's competitors for food resources. This process is comparable to the "arms race" in the co-evolution of wild plants and their pests. To keep the lead in the race with agricultural pests, man will have to minimize the chances for selection.

The striking tolerance of certain pest species to CA's raises the question of whether this could be the result of long-term hermetic storage carried out in many agricultural countries since ancient times. Up till now, no treatment failures due to resistant strains have been reported from practical applications, and no tolerant stages of tolerant species could be selected to a sufficient degree to allow defined resistance factors to be estimated. It should be kept in mind that high m.c. favours the selection of tolerant populations, and such tolerance could be stable for an extended period. More research needs to be done to gain a better understanding of the mode of action of CA's and the potential of arthropods to develop resistance to them. The data available today, however, do not prove that the development of resistance is an immediate threat to the use of CA's for stored-product protection.

In order to minimise the risk of resistance developing, such methods for pest prevention as insect-proof structural design, hygiene, cold storage and product drying should be used together in an integrated stored-product protection system (Fig. 4). Today's informa-

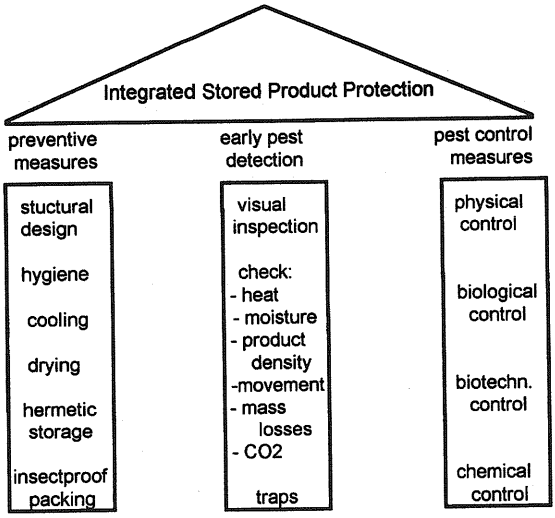


Fig. 4. The three columns of integrated stored-product protection.

tion technology could also support a logistical approach to the handling of stored products, reducing storage durations and shortening the routes between producer, manufacturer and consumer. Early pest detection methods in combination with hygiene and limited control treatments could help to reduce the number of large scale fumigations. The efficacy of each control treatment should be assured, and inadequate treatment methods must be avoided. Surface treatments may be dangerous because insects below the surface would be exposed to sublethal doses. Finally, the different pest-control methods available should be alternated in order to reduce the chance of accidental selection of resistant populations.

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SELECTION OF THE RED FLOUR BEETLE (*TRIBOLIUM CASTANEUM* (HERBST)) FOR RESISTANCE TO A COMBINATION OF PHOSPHINE PLUS CARBON DIOXIDE AND BIOLOGICAL OBSERVATIONS ON THE RESISTANT STRAIN

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ABSTRACT

The main objectives of this work were to study the development of resistance to a combination of phosphine (PH_3) and carbon dioxide (CO_2) in the red flour beetle *Tribolium castaneum* (Herbst) and to investigate some biological characteristics of the resistant strain in comparison with the parental stock.

Sixteen generations of adult *T. castaneum* were exposed in the laboratory for varying exposure periods to a mixture of 40 ppm PH_3 + 46% CO_2 at 26 ± 1°C and 6 ± 1°C in order to select for a resistant strain. Selection pressure was carried out at the median lethal time inducing 50–70% mortality.

Results showed that the lethal time (LT) values recorded to obtain a given mortality were significantly higher at the two test temperatures for the 16th generation of adults than for the parental strain. When compared to the parental stock at the LT_{50} level, resistance to the PH_3–CO_2 mixture increased by 19.4 at 26 ± 1°C and by 18.5 at 6 ± 1°C. This clearly indicated that *T. castaneum* adults have the genetic potential for developing resistance to a PH_3/CO_2 atmosphere. Analysis of the biological characteristics of the resistant strain revealed that it laid significantly more eggs than did the laboratory strain. However, no significant differences were found in either the average pre-oviposition period or the sex ratio. Both the average incubation period and the total developmental period were clearly longer for the laboratory strain than for the resistant strain. The average hatching rate of the eggs and larval mortality were both increased significantly in the resistant strain. The emergence rate of the adults was unaffected, amounting to 100% for both strains.

INTRODUCTION

Some investigators have studied the efficacy of phosphine (PH_3)–carbon dioxide (CO_2) mixtures against stored-product insects, noting the advantages of using such combinations for control (Aliniazae, 1971; Kashi and Bond, 1975; Desmarchelier and Wohlgemuth, 1984; El-Lakwah *et al.*, 1989, 1991b, c, 1992).

A major problem that has developed in many insect control programmes in recent years is the resistance acquired when successive generations are exposed to toxic agents. Survivors of progressive selection can develop characteristics that make the toxicant ineffective or uneconomical.

Resistance to the fumigants methyl bromide (MB), PH_3 (Champ and Dyte, 1976) and ethylene dibromide (Bond, 1973) has been found in field populations of stored-product insects in recent years.

In the laboratory El-Lakwah *et al.* (1991a) developed a PH_3-resistant strain of the red flour beetle *Tribolium castaneum* (Herbst) by exposing successive generations of adults to the median lethal dosage.

The present investigation was conducted in the same way on the same insect both to study the development of resistance to a mixture of PH_3 and CO_2 and to compare the biological characteristics of the resistant strain and those of the parental stock.

MATERIALS AND METHODS

Rearing technique

T. castaneum adults were reared in the laboratory at 26 ± 1°C and 60 ± 5% r.h. The strain had been held in the laboratory at the same temperature without being subjected to any chemical pressure for 6 years before starting the experiments. Approximately 200 7–14-d old adults were added to 50 g wheat flour with 5% dry yeast in a small jar which was then covered with muslin. After 48 h, the adults were sieved out and the cultures then incubated under the above conditions. Newly emerged 7–14-d old adults were used for all stages of this study.

Selection pressure

The adult populations of *T. castaneum* were exposed in the laboratory for the median lethal time (LT) to a controlled atmosphere (40 ppm PH_3 + 46% CO_2) at 26 ± 1°C and at 6 ± 1°C.

The regression line of the laboratory strain (parent stock) was drawn. The LT_{50} of that generation was then extrapolated from the line. When this was done, 240 individuals were exposed to the mixture for the median lethal time, thereby obtaining approximately a 50% kill. Adult insects surviving this selection were reared in turn at 26 ± 1°C and 60 ± 5% r.h. to produce the next generation. The same selection procedure was applied to each generation, rearing being carried out under the same conditions as the laboratory strain. This was done for 16 generations and the susceptibilities of each generation of adults were determined.

Assessment of resistance factor

Mortality results were calculated using the correction of Abbott's formula (1925) and the LT_{50} values of the various generations used to indicate resistance development within successive generations. The increase in resistance was calculated as the ratio between the

LT_{50} values of the selected strain and that of the parental strain, i.e.

$$RR = \frac{\text{The } LT_{50} \text{ of the successive generation}}{\text{The } LT_{50} \text{ of the parental strain}}$$

where *RR* = resistance ratio.

Generation of phosphine

Throughout this work PHOSTOXIN®-pellets (Detia-DEGESCH — Germany) were used to obtain PH_3 gas. Each PHOSTOXIN®-pellet contains approximately 56% aluminium phosphide which reacts with water according the equation:

$AlP + 3\ HOH \rightarrow Al(OH)_3 + PH_3 \uparrow$

A pellet weighs 0.6 g and produces approximately 0.2 g PH_3. The gas was generated by introducing a glass tube (1.5 × 3 cm) containing one pellet and 2 ml water into a Dressel flask connected on one side to a gas reservoir and on the other side to a recirculatory pump (also connected to the gas reservoir). The pump was operated for 0.5 h, after which the flask was detached and the reservoir closed.

Measurement of phosphine concentrations

Concentrations of PH_3 in the gas reservoir were determined using Draeger gas detector tubes (50/a). The required gas concentration was then obtained by diluting the gas, using the Dressel flasks and the recirculatory pump: 1 ppm PH_3 = 1.413 µg/L = 0.001413 mg/L.

Pre-fumigation procedure

Wire gauze 14 × 45-mm cages were filled with about 2 g wheat flour. Batches of 30 *T. castaneum* adults were introduced into each cage which was then covered with rubber stoppers. Three replicates were used in each treatment.

Fumigation procedure

Fumigation experiments were performed at 26 ± 1°C and 6 ± 1°C. The relative humidity during the fumigation was 55–65%. A recirculatory multi-flask fumatorium was set up for the fumigation experiments. To obtain a mixture of 40 ppm PH_3 + 46% CO_2 (v/v), six Dressel flasks were filled with 99% v/v CO_2 and a seventh with 480 ppm PH_3. These flasks were inter-connected in circuit to each other, to a gastight pump and to five additional flasks containing the test insects. The pump was operated for 1 h to circulate the gas mixture inside the flasks.

Post-fumigation procedure

Following exposure to the PH_3–CO_2 mixture, the insects were transferred to glass petri dishes with about 3 g wheat flour and held at 26 ± 1°C and 60 ± 5% r.h. for mortality assessment. Mortality was determined 3 d after fumigation. The percentage mortality was corrected using Abbott's formula (1925).

Effect of selection pressure of PH_3 + CO_2 on some biological parameters of *T. castaneum*

Laboratory experiments were carried out at 30 ± 1°C and 75 ± 5% r.h. to compare some biological aspects of the 12th generation PH_3–CO_2-selected strain with those of the parental stock. In these experiments the following data were recorded: the average number of eggs laid daily by each female, the average total number of eggs laid during 14 d by each female, the pre-ovipostion period, the incubation period of eggs, the percentage of eggs hatching, the developmental periods for the various insect stages, the average duration of larval instars, the average duration of pupal instars and the mean weight of both adults and pupae.

Number of eggs. Tests were carried out to assess how many eggs were laid by the females during a 14-d observation period. Freshly emerged (unmated) males and females were paired in a 3 × 5-cm glass tube containing a small amount of wheat flour; it was then covered with muslin. Every day the number of eggs laid in the flour was counted and the flour replaced. The total number of eggs laid per female during 14 d was recorded. Hatching of eggs was also recorded to determine their viability. Each treatment was replicated four times.

The incubation period and the developmental span. To observe the incubation period of eggs and the developmental periods for the various stages, 1-d-old eggs were placed individually, with a small amount of wheat flour, in a 3 ×-5 cm glass tube which was then covered with muslin. Ten replicates were used for each laboratory and each PH_3–CO_2-selected strain. The incubation period for every egg was recorded. The developmental stages were observed and their developmental periods noted. In addition, the mortality rate for the larvae was calculated.

Statistical analysis

The toxicity data obtained were subjected to Probit analysis (Finney, 1971) using the computer program of Noack and Reichmuth (1978). The biological data were subjected to analysis of variance.

RESULTS AND DISCUSSION

Tables 1 and 2 show the lethal times and parameters of the probit regression-line estimates for different generations of *T.castaneum* adults, selected for resistance to the PH_3–CO_2 mixture at 26 ± 1°C, 6 ± 1°C and 60 ± 5% r.h. The RR's and the lethal response for the various generations at the two test temperatures are given in Table 3 and Figs. 1 and 2. Results showed that the LT needed to obtain a given mortality level was significantly higher for the 16th generation at both temperatures than for the laboratory parent strain. The LT's for 50% kill at 26 ± 1°C were 1.9 h for the parent strain and 36.8 h for the 16th selected generation. Similarly, at 6 ± 1°C the LT's recorded for 50% kill were 4.4 h for the parent strain and 81.2 h for the 16th selected generation

TABLE 1

Lethal times and parameters of probit regression line estimates for adults of different generations of *T. castaneum* (Herbst) exposed to a controlled atmosphere containing 40 ppm PH_3 + 46% CO_2 at 26 ± 1°C and 60 ± 5% r.h.

	Lethal times and their 95 % confidence limits (h)			Parameters of regression line			
Generation	LT_{50}	LT_{90}	LT_{99}	Slope ± SE	*a*	*F*	*R*
Parent (laboratory strain)	1.9 (2–2)	4.2 (4–5)	7.9 (6–10)	3.8 ± 0.35	0.93	4	0.984
2nd generation	3.0 (3–3)	7.7 (7–9)	16.8 (13–12)	3.1 ± 0.18	3.52	6	0.990
4th generation	6.2 (6–7)	11.7 (10–13)	19.5 (16–24)	4.7 ± 0.50	1.29	5	0.849
6th generation	18.8 (16–21)	29.5 (24–37)	42.7 (30–62)	6.5 ± 0.16	–3.30	2	0.965
8th generation	14.7 (11–20)	20.0 (13–30)	25.6 (13–50)	9.6 ± 0.00	–6.22	1	1.000
10th generation	18.5 (15–23)	23.8 (16–35)	29.2 (15–56)	11.7 ± 0.03	–9.85	1	0.993
12th generation	26.0 (23–30)	44.3 (35–56)	68.4 (47–100)	5.5 ± 0.13	–2.82	2	0.981
14th generation	32.7 (28–38)	61.3 (47–80)	102.5 (65–163)	4.7 ± 0.05	–2.10	2	0.990
16th generation	36.8 (31–44)	77.9 (55–111)	144.0 (78–265)	3.9 ± 0.05	–1.16	2	0.988

SE = Standard error of regression line; *a* = axis intercept; *F* = degree of freedom; *R* = correlation coefficient.

TABLE 2
Lethal times and parameters of probit regression line estimates for adults of different generations of *T. castaneum* (Herbst) exposed to a controlled atmosphere containing 40 ppm PH_3 + 46% CO_2 at 6 ± 1°C and 60 ± 5% r.h.

	Lethal times and their 95 % confidence limits (h)			Parameters of regression line			
Generation	LT_{50}	LT_{90}	LT_{99}	Slope ± SE	*a*	*F*	*R*
Parent (laboratory strain)	4.4 (4–5)	11.2 (9–14).	24.4 (17–34)	3.1 ± 0.31	3.01	5	0.988
2nd generation	6.7 (6–7)	15.8 (13–19)	31.6 (24–42)	3.5 ± 0.34	2.14	5	0.989
4th generation	35.9 (24–54)	54.8 (31–98)	77.6 (32–189)	6.9 ± 0.19	–5.80	1	0.983
6th generation	52.2 (40–68)	69.5 (45–106)	87.8 (46–169)	10.3 ± 0.12	–12.68	1	0.979
8th generation	36.9 (21–65)	63.0 (30–135)	97.4 (22–429)	5.5 ± 0.02	–3.66	1	0.994
10th generation	54.4 (50–60)	75.3 (65–87)	98.1 (79–122)	9.1 ± 0.04	–10.79	2	0.998
12th generation	62.5 (58–67)	93.5 (84–105)	130.0 (108–157)	7.3 ± 0.17	–8.12	3	0.984
14th generation	76.4 (70–83)	130.8 (109–157)	202.9 (151–273)	5.5 ± 0.22	–5.32	3	0.966
16th generation	81.2 (75–88)	129.7 (113–148)	189.9 (152–238)	6.3 ± 0.38	–7.05	3	0.954

SE = Standard error of regression line; *a* = axis intercept; *F* = degree of freedom; *R* = correlation coefficient.

TABLE 3

Resistance ratios (RR) at LT_{50} and LT_{90} levels of different generations of *T. castaneum* adults exposed to an atmosphere containing 40 ppm PH_3 + 46% CO_2 at 26 ± 1°C and 6 ± 1°C

	RR* at 26 ± 1°C		RR at 6 ± 1°C	
Generation	LT_{50}	LT_{90}	LT_{50}	LT_{90}
Parent (laboratory strain)	1.0 (1.1–1.1)	1.0 (0.9–1.2)	1.0 (0.9–1.1)	1.0 (0.8–1.3)
2nd generation	1.6 (1.6–1.6)	1.8 (1.7–2.1)	1.5 (1.4–1.6)	1.4 (1.2–1.7)
4th generation	3.3 (3.2–3.7)	2.8 (2.4–3.1)	8.2 (5.5–12.3)	4.9 (2.8–8.8)
6th generation	9.9 (8.4–11.1)	7.0 (5.7–8.8)	11.9 (9.1–15.5)	6.2 (4.0–9.5)
8th generation	7.7 (5.8–10.5)	4.8 (3.1–7.1)	8.4 (4.8–14.8)	5.6 (2.8–12.1)
10th generation	9.7 (7.9–12.1)	5.7 (3.8–8.3)	12.4 (11.4–13.6)	6.7 (5.8–7.8)
12th generation	13.7 (12.1–15.8)	10.5 (8.3–13.3)	14.2 (13.2–15.2)	8.3 (7.5–9.4)
14th generation	17.2 (14.7–20.0)	14.6 (11.2–19.0)	17.4 (15.9–18.9)	11.7 (9.7–14.0)
16th generation	19.4 (16.3–23.2)	18.5 (13.1–26.4)	18.5 (17–20)	11.6 (10.1–13.2)

*Confidence limits of RR based on confidence limits of parent strain.

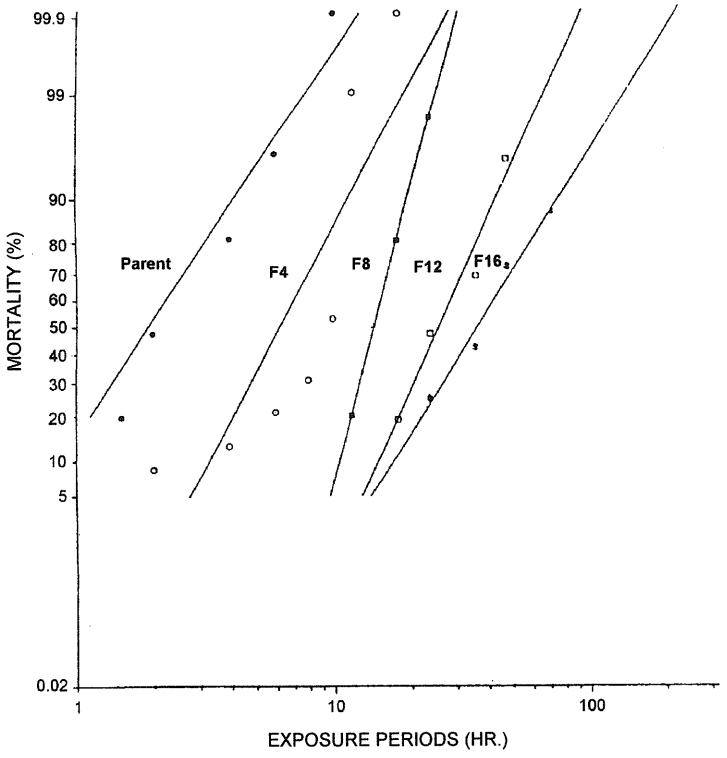


Fig. 1. Lethal response of parent and various strains of *Tribolium castaneum* adults, selected to PH_3–CO_2 at 26 ± 1°C.

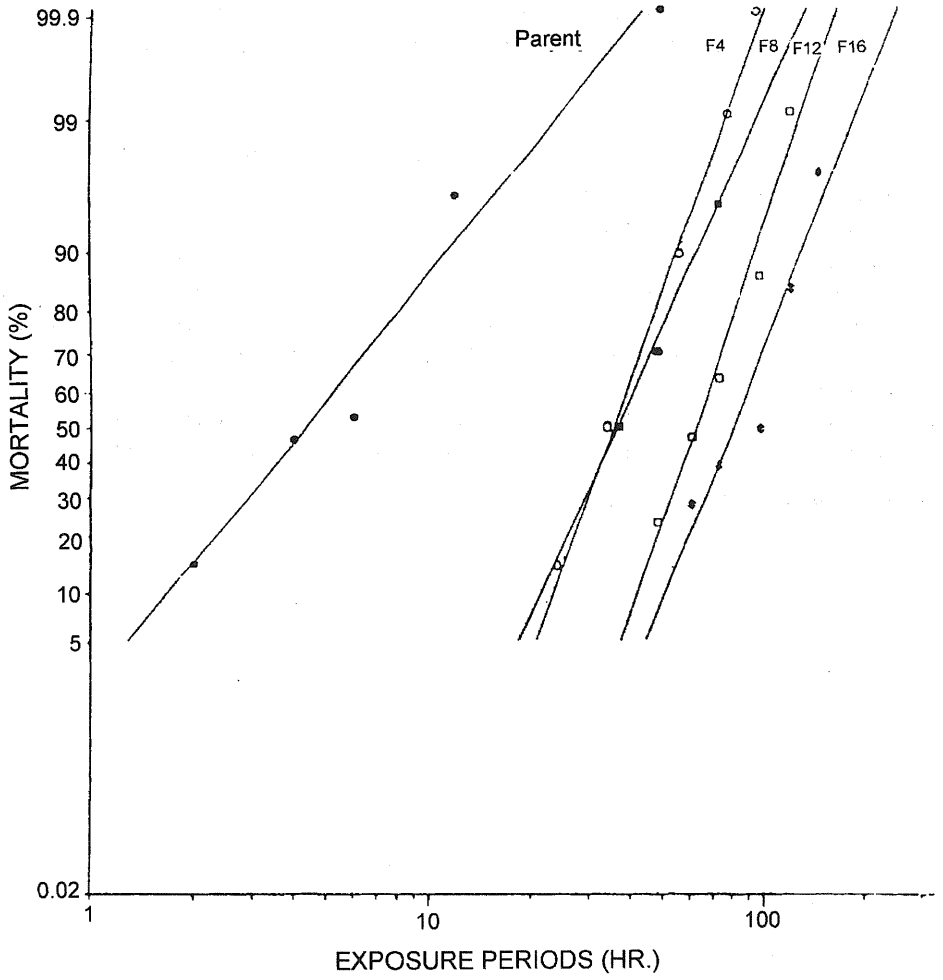


Fig. 2. Lethal response of parent and various strains of *Tribolium castaneum* adults, selected to PH_3–CO_2 at 6 ± 1°C.

(Table 2 and Fig. 2). The same trend occurred at both temperatures at LT_{90} and LT_{99} levels.

At the LT_{50} level, the RR's at 26 ± 1°C, increased from 1.6 (the first generation) to 19.4, and at 6 ± 1°C to 18.5, at the 16th generation (Table 3). These results clearly indicated that *T. castaneum* adults have the genetic potential to develop resistance to an atmosphere consisting of 40 ppm PH_3 + 46% CO_2. They corroborate the findings of other investigators: stored-product insects have the genetic potential to build up resistance to modified atmospheres (Bond and Buckland, 1979; Navarro *et al.*, 1985; El-Lakwah *et al.*, 1995).

Biological characteristics of the selected strain in comparison with the parental stock

The biological parameters of the selected strain of *T. castaneum* examined at the 12th generation are summarized in Tables 4, 5, 6 and 7 and Fig. 3.

Data showed that there was no significant difference between the laboratory and the PH_3–CO_2-selected strain in the average pre-oviposition period or the sex ratio (Table 4). The total developmental period was significantly longer for the laboratory strain (29.167 d) than for the resistant strain (24.076 d). It was also observed that the emergence rate of the adults was unaffected (100% for each strain). The average incubation period was significantly longer in the laboratory strain (4.2 d) than for the selected strain (2.67 d). The mortality rate of the larval instars was significantly higher for the selected strain than for the laboratory strain. The average total duration of the larval instars and the average duration of pupal instars were significantly longer for the laboratory strain than for the selected strain.

During the 14-d observation-period, the average number of eggs laid per female per day was significantly higher for the selected strain than for the laboratory strain (Table 5). The average hatching rate of the eggs significantly declined from about 96% for the selected strain to 81% for the laboratory strain.

A comparison of the average number of eggs laid daily per female by the laboratory strain and the selected strain during an observation period of 14 d is given in Fig. 3 and Table 5. From them it is clear that the selected strain laid an average of 172 eggs during the observation period, significantly higher than the average of 55.8 eggs laid by the parent strain.

TABLE 4

Some biological parameters for the laboratory strain and the PH_3–CO_2-resistant strain (F12) of *T. castaneum* at 30 ± 1°C and 75 ± 5% r.h.

Parameters	Laboratory strain (L)	PH_3–CO_2-resistant strain (F12)	Probability
Average pre-ovipostion period (d)	5.33 ± 0.33[1]	6.33 ± 0.33	0.059
Average no. of eggs per female per day	3.80 ± 0.409	12.40 ± 3.44	0.000**
Average total no. of eggs per female during 14 d	55.83 ± 1.1	172.0 ± 2.44	0.000**
Average hatching rate (%)	81.67 ± 3.34	96.67 ± 1.76	0.015*
Average incubation period (d)	4.17 ± 0.310	2.67 ± 0.213	0.002**
Average duration of larval instars (d)	19.00 ± 0.317	16.40 ± 0.509	0.002**
Average duration of pupal instars (d)	6.00 ± 0.0	5.00 ± 0.0	0.000**
Total developmental periods (d)	29.167 ± 4.68	24.076 ± 4.25	0.000**
Mortality for larval instars (%)	0.0	40	
Emergence rate (%)	100	100	
Sex ratio	1:1	1:1	

* = Differences significant at the 5% level; ** = differences significant at the 1% level. [1]±SD.

TABLE 5
Average daily number of eggs laid per female of *T. castaneum* during 14 d for the laboratory and the PH_3–CO_2-resistant strain (F12) at 30 ± 1°C and 75 ± 5% r.h.

Day	Average number of eggs laid per female per day	
	Laboratory strain (L)	PH_3–CO_2-resistant strain (F12)
1	1.7 ± 0.219[1]	10.2 ± 0.280
2	1.3 ± 0.366	11.2 ± 0.684
3	1.5 ± 0.280	12.5 ± 0.326
4	2.7 ± 0.275	16.0 ± 0.335
5	3.2 ± 0.109	14.5 ± 0.440
6	4.3 ± 0.219	13.5 ± 0.369
7	5.5 ± 0.281	15.2 ± 0.663
8	7.3 ± 0.366	15.5 ± 0.939
9	6.8 ± 0.313	12.7 ± 0.626
10	6.3 ± 0.404	13.8 ± 0.262
11	3.8 ± 0.313	10.2 ± 0.313
12	4.2 ± 0.201	11.7 ± 0.219
13	4.2 ± 0.393	8.7 ± 0.139
14	3.0 ± 0.238	8.0 ± 0.478
Average total number of eggs laid during 14 d	55.8 ± 1.1	172.0 ± 2.44**

**Differences significant at the 1% level. [1]±SD.

From Table 6 it can be seen that eight larval instars were recorded for the selected strain whereas there were only seven larval instars for the laboratory strain. This table shows that the laboratory-strain first, second, fifth and sixth larval instars continued for a significantly longer time than did those of the selected strain; the total duration of the larval instars was 19 d for the laboratory strain and 16.4 d for the selected strain, this difference being significant.

The mean weights of adults and pupae of the various *T.castaneum* strains are given in Table 7; the adults and pupae of the PH_3–CO_2-resistant strain were significantly lighter than those of the laboratory strain and of PH_3-resistant and CO_2-tolerant strains reared in the laboratory over several years.

Summary

The PH_3–CO_2-selected strain laid significantly higher numbers of eggs than the laboratory strain, and their hatchability was higher, but their incubation period was lower. Larval mortality was significantly higher in the selected strain. The duration of the 1st, 2nd, 5th and 6th larval instars was significantly shorter for the selected strain, and the

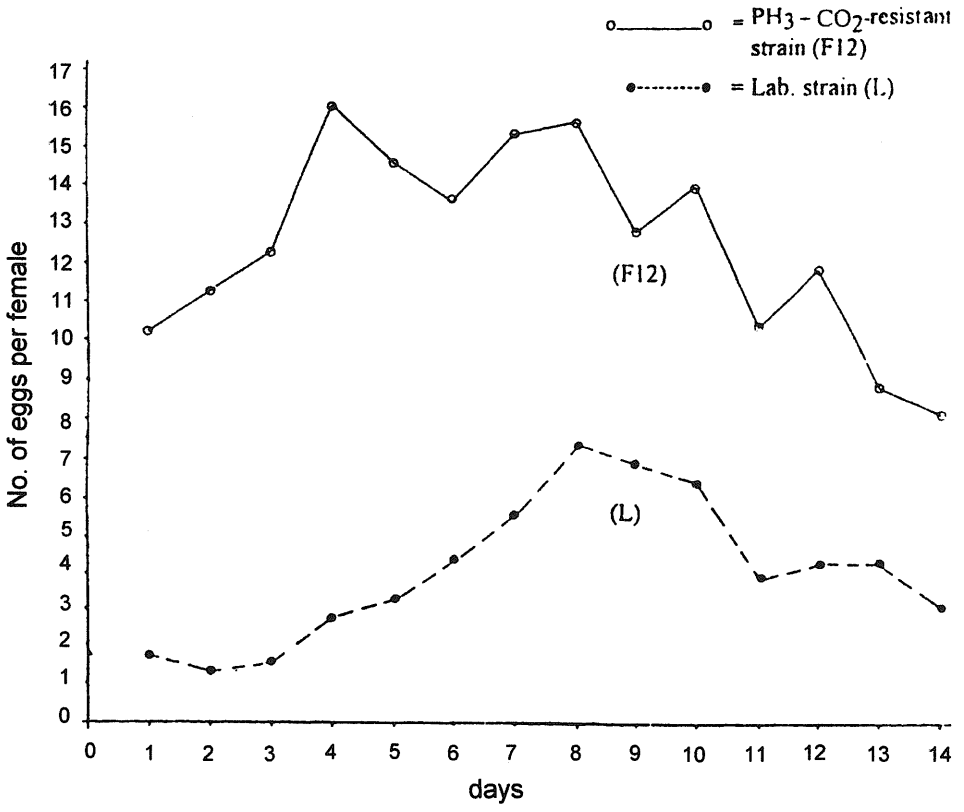


Fig. 3. Average number of eggs laid daily per female by a laboratory strain and a PH_3–CO_2-selected strain of *Tribolium castaneum* at the 12th generation.

average number of larval instars was significantly higher. No significant differences between the two strains in the other biological parameters were found.

These findings agree with those of Abdel-Salam and Nasr (1967), Spratt (1979), El-Sayed (1981) and El-Lakwah *et al.* (1991a). Abdel-Salam and Nasr (1967) observed an increase in egg laying by *Spodoptera littoralis* exposed in the laboratory to sublethal doses of insecticides. El-Sayed (1981) mentioned that resistance to fenitrothion was associated with a significant decrease in all tested biological aspects of *Callosobruchus maculatus* except the average total number of eggs laid per-female, which was significantly larger than that of the laboratory strain.

El-Lakwah *et al.* (1991a) found that the females of a PH_3-resistant strain of *T. castaneum* laid a significantly higher number of eggs during an observation period of 14 d than those of the parent stock. The hatchability of eggs for the PH_3-resistant strain declined and their incubation period was considerably prolonged. No other significant biological differences were found between the two strains.

TABLE 6
Average duration and total period of the larval instars for the laboratory strain (parent) and the PH_3–CO_2-resistant strain (F12) of *T. castaneum* at 30 ±1°C and 75 ± 5% r.h.

Larval instars	Average duration of larval instars		Probability
	Laboratory strain (L)	PH_3–CO_2-resistant strain (F12)	
First	2.60 ± 0.377[1]	1.00 ± 0.0	0.003**
Second	3.80 ± 0.375	2.40 ± 0.246	0.014*
Third	2.00 ± 0.0	1.80 ± 0.201	0.346
Fourth	2.00 ± 0.0	1.80 ± 0.201	0.346
Fifth	2.60 ± 0.246	2.00 ± 0.0	0.040*
Sixth	4.80 ± 0.580	2.40 ± 0.246	0.005**
Seventh	1.20 ± 0.969	2.80 ± 0.491	0.179
Eighth	0.0	2.20 ± 1.02	0.063
Average no. of larval instars	6.40 ± 0.2	7.60 ± 0.2	0.008**
Average total period for the larval instars (d)	19.0 ± 0.317	16.40 ± 0.509	0.002**

** = Difference significant at the 1% level; * = difference significant at the 5% level. [1]±SD.

TABLE 7
Mean weight of the adults and pupae of the various *T. castaneum* strains

Strain	Weight (mg)	
	Adults	Pupae
Laboratory strain	2.3	2.9
PH_3-resistant strain	2.5	2.9
CO_2-tolerant strain	2.3	2.5
PH_3–CO_2-resistant strain	2.0	2.3

ACKNOWLEDGEMENT

This work was performed in the framework of Research Project No. 66 (Evaluation of MB alternatives) at the Faculty of Agriculture, Moshtohor, Zagazig University. It was supported by a grant from the Egyptian National Agricultural Research Project (NARP) at the Ministry of Agriculture and Land Reclamation.

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PHOSPHINE MEASUREMENT AT ENVIRONMENTAL LEVELS BY GAS CHROMATOGRAPHY AND PORTABLE METHODS

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ABSTRACT

Effective methods for generating standard concentrations of phosphine in air in the ppb range have been developed. They have been used to evaluate modified commercial devices which may prove useful for field measurement of low levels of phosphine.

Our analyses are based on gas chromatography with phosphorus-specific flame photometric or nitrogen/phosphorus-specific thermionic detectors. These detectors have recorded detection limits in the picogram (ng L^{-1} or ppb) range for phosphine. Several auxiliary techniques for detecting low phosphine concentrations have, in our laboratory, been tested against the standard GC analyses. Some of these involve novel uses of sensors in which they function as GC detectors, and others involve the amplification of existing electrochemical and opto-chemical sensors.

INTRODUCTION

As air pollution is taken more seriously as a threat to human health, industries which emit substances into the atmosphere are finding it in their own best interest to determine whether their activities contribute to the problem. Recent Australian guidelines on environmental levels of phosphine (PH_3) are so low, from 4 to 20 $\mu g\ m^{-3}$, or 3 to 13 parts per billion v/v (ppb), that sensitive analytical methods are required to make field measurements. The perceived threat of bans on using PH_3 (Banks, 1994), unless it can be shown that concentrations near grain stores do not exceed levels about 100 times lower than those in work-places, has prompted our efforts to develop field measurement methods. Trace-level PH_3 analysis is therefore becoming important for ensuring the continued use of this fumigant by the grain industry.

The proposed International Standards Organisation ISO 14000 series of Environmental Management Standards (Standards Australia, 1995) emphasises both pollution management on the part of industry and open communication with workers and the public, rather

than stressing regulatory prescriptions for detection and correction. This will encourage industry, in consultation with other parties, to participate in the management of emissions. The relationship among the regulatory authorities, the community and the PH_3-using industries hinges on realistic assessments of both the level of PH_3 in the environment and the origin of the chemical. The grain industry is only one of several sources of PH_3 in the environment (Vinsjansen and Thrane, 1978; Devai *et al.*, 1988; Gassmann and Glindemann, 1993; Devai and Delaune, 1995). Not only is trace analysis of PH_3 difficult, but questions also arise concerning sample instability and interference from other substances. These problems have led to disagreement about non-industrial PH_3 sources (Burford and Bremner, 1972).

PH_3 concentrations can be considered, from the point of view of measurement means and aims, under three broad categories: fumigation concentrations, work-place concentrations and environmental concentrations. Fumigation concentrations typically range from hundreds or thousands of parts per million by volume (ppm) (for metal phosphide applications, especially in sealed storages) to tens of ppm (for more modern methods such as SIROFLO®). The principal concern in monitoring fumigation concentrations within storages is how to achieve effective pest control at an economic price. Work-place concentrations, set by regulatory bodies, mandate levels ranging from about 1 ppm, which is the Short Term Exposure Limit (STEL), to the Threshold Limit Value (TLV) of 0.1 ppm in Germany and 0.3 ppm in other countries. Work-place concentrations are the concern of employers and employees within PH_3-using industries. The category of environmental concentrations can be defined in general terms as concentrations that occur outside the work-place. They are typically lower than work-place concentrations. A pragmatic lower bound is set by the effective limit of gas detection, in the order of 1 ppb or lower, depending on the technique used. Environmental levels are an issue which may affect the community as a whole; this means that the concerns, such as those arising from sources of PH_3 and levels and durations of exposure, are not easily defined in a manner to which all involved parties can agree.

Australia's National Health and Medical Research Council (NHMRC) Standing Committee on Toxicity (SCoT) examined PH_3-toxicity data and recommended an environmental action level of 4 $\mu g\ m^{-3}$ ($\approx$3 ppb) (NHMRC, 1992). These levels were based on sub-acute animal studies which found a no-effect level of 0.3 ppm. This figure was divided by ten to allow for species differences and by a further factor of ten as an added safety margin to allow for variability in the human population. However, the SCoT recognised the difficulty of measuring 3 ppb with hand-held instruments and went on to recommend a short-term intervention level of 20 $\mu g\ m^{-3}$ ($\approx$15 ppb). These draft guidelines have not yet been put into practice but they serve as harbingers of the regulatory requirements which may be expected in the future.

Since the application of gas chromatography to PH_3 analysis in the 1960's, detection limits have fallen from about 1 μg of PH_3 to 0.1–10 pg. It is notable that wide variations in the reported limits of detection remain and that the limits have not fallen consistently over the period.

Laboratory analysis

It was essential to have a reliable laboratory analytical system for generating and quantifying standards and samples in the range from 1 to 100 ppb in order to evaluate tools which may be useful as field methods for detecting PH_3 in this range. The packed columns used for analysis of higher concentrations gave unacceptable results when PH_3 concentrations were low and sample sizes large due to failure to separate oxygen (O_2) in the sample from PH_3. The use of a megabore capillary column at lower oven temperatures allowed the separation of O_2 and PH_3 without the penalty of peak broadening.

For environmental analyses, the gas chromatography systems which we have used are: carrier — nitrogen (N_2); column — GSQ (J&W), DB Wax (J&W), length 30 m, i.d. 0.53 mm; oven temperature — 30–50°C; detector — Flame Photometric (FPD) (Tracor) or Thermionic specific (TSD or NPD) (Varian, and SRI Inc. stand-alone). Chromatographs were a Tracor MT150, a Varian 3400 and a Varian Aerograph. PH_3 (*ca.* 85%) source determinations were carried out on a Gow-Mac Gas Density (GADE) detector fitted to a Tracor MT150 chromatograph. Reference gas cylinders (BOC Australia spectra seal b standard) were assayed, for comparison, on a GC/FPD, with quantitatively diluted samples of PH_3 source which had been assayed by GC/GADE.

Our early investigations into establishing the linear range and detection limit of our GC detectors focused one of the problems of working with low concentrations of gas. A series of static PH_3 concentrations were prepared by serial dilution and analysed by GC/NPD on a DB 624-megabore column. Excellent linearity of response over three orders of magnitude was observed, but at concentrations of the order of 10 ppb and below there was considerable deviation from a linear response (Fig. 1) due to the effect of O_2 (in the sample) co-eluting with PH_3. At higher PH_3 concentrations, the response caused by the PH_3 masked the small contribution from the O_2.

The stability of PH_3 samples at low concentrations is a matter of some debate. Some researchers (e.g. Vinsjansen and Thrane, 1978) found that samples in gastight bags were stable for days or weeks, but other reports (Frank and Rippen, 1987) agree with our experience that PH_3 samples can degrade in a matter of hours or days. PH_3 can also break down on surfaces such as brass or be absorbed into teflon and silicone rubber (Waterford and Winks, 1986). If one accepts that the stability of PH_3 at low concentrations is not assured, the question of how to maintain reliable standards arises. These concerns became critical as measurement of lower concentrations was attempted. We solved this problem by using continuous flow standards.

First, a cylinder of a mixture of PH_3 in N_2 was accurately assayed as described above. The PH_3 concentrations in these cylinders are typically in the range 1–50 ppm, and, in the experience of this laboratory, remain stable for periods of years.

Secondly, two electronic mass flow controllers (MFC's) were calibrated using either a certified bubble flowmeter or a certified digital flowmeter. Our experience with MFC's has been that they maintain excellent flow rate control and linearity between calibrations. Figure 2 is a calibration chart of a MFC. In this case, the maximum flow was 25 ml min^{-1}. When calibrating a mass flow controller, particular attention was paid to the extremities of

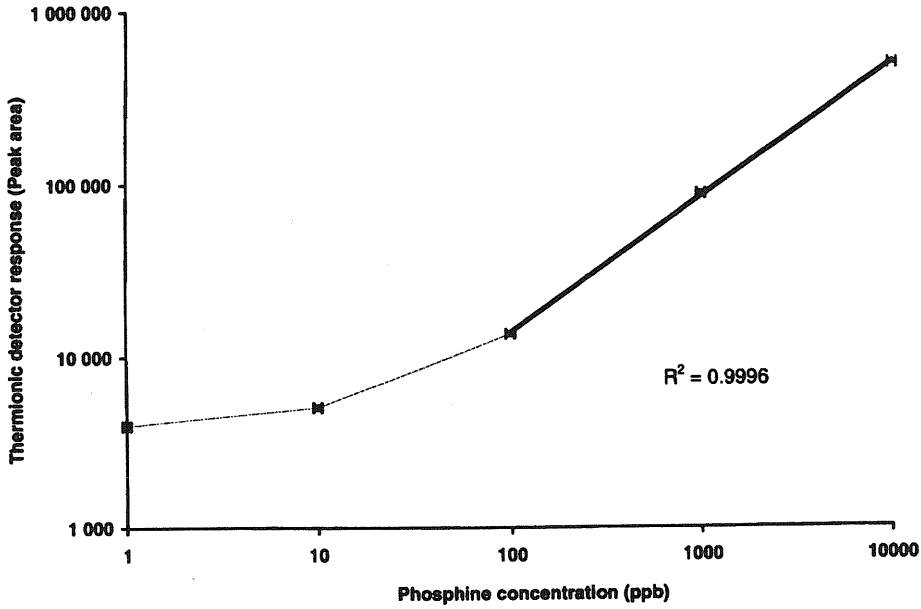


Fig. 1. An example of a linearity assessment (GC/NPD, DB 624 column). At lower concentrations the presence of oxygen in the samples gave spuriously high responses.

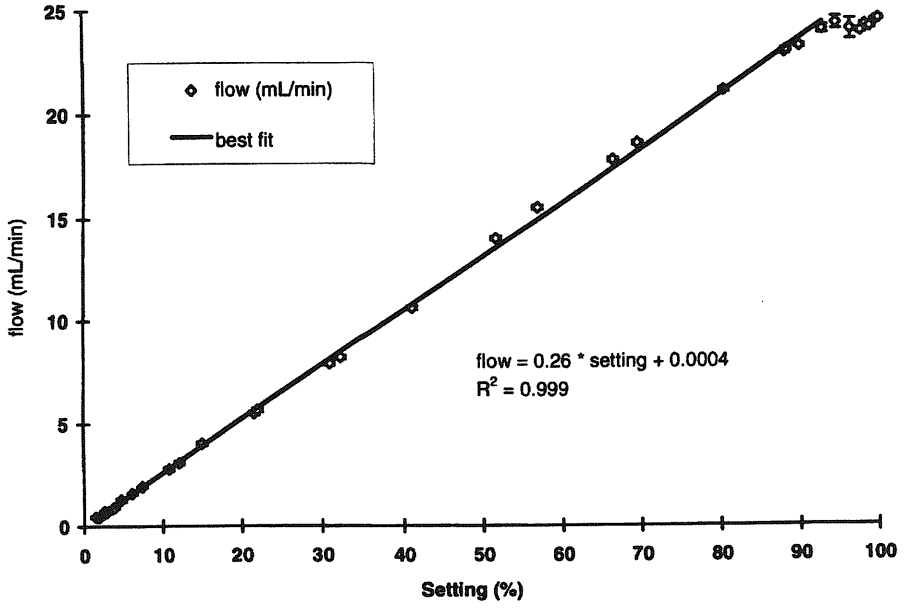


Fig. 2. Calibration chart of a mass flow controller (MFC) used for production of continuous flow standards by quantitative dilution.

the flow range (i.e. below 5% and above 90% of the maximum flow) because deviations from linearity most often occur in those ranges. In the example shown, readings above 92% were not used in the calculation of the line of best fit because they deviated from the line. Thus, in practice, this MFC could not be used at settings above 92%.

The next step involved blending a low flow from the PH_3 standard cylinder with a higher flow of air which had been filtered to remove both hydrocarbons and any ambient PH_3 and then humidified (Winks and Hyne, 1994) to about 55% r.h. In this manner dilution ratios between 5 (maximum PH_3 flow, minimum air flow) and 10,000 (minimum PH_3 flow, maximum air flow) were obtained using a 25 ml min^{-1} MFC for PH_3 and a 3,500 ml min^{-1} MFC for air. With a standard cylinder containing 25 ppm PH_3, this equates to a concentration range of 5 ppm to 2.5 ppb.

Quantitative dilution by mass flow has several advantages. MFC's are pressure-independent and thus better than either regulators or mechanical diluters. The calibration of flow rates assures accurate dilution. Continuous flow avoids surface reaction/absorption problems and provides unlimited volume for testing cumulative (or long time period) field monitoring methods. Humidified air can be blended with PH_3/N_2 for verisimilitude, i.e. the properties of the standard mixture are as close as possible to those of an actual environmental air sample. Concentrations can be set in seconds and become stable in minutes.

The linearity of the GC/FPD response to a range of PH_3 blends produced by the method described above is shown in Fig. 3. Three manual injections of 2.0 ml were made at each

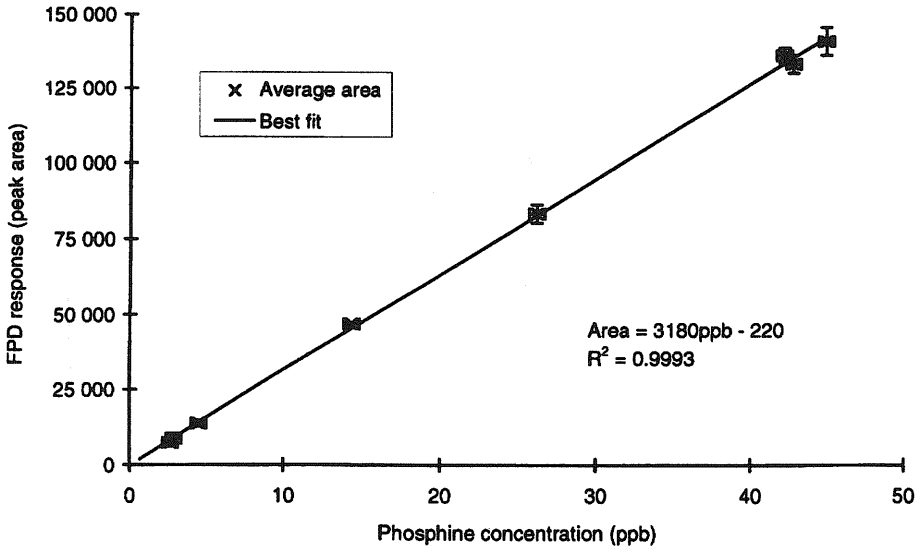


Fig. 3. Linearity of GC/FPD measurements of phosphine in air produced by quantitative dilution. The minimum resolvable peak under these conditions has an area of about 3000 mV sec, so the limit of detection in this case was about 1 ppb.

concentration, and the average peak area was plotted. The error bars show the standard deviations of the readings. Excellent results ($R^2 = 0.9993$) were obtained over a range of 2.5–45 ppb. The linearity of the GC determinations validates the accuracy of the MFC-produced dilutions created by the blending of the PH_3/N_2 and air streams. The small vertical error bars, even at the lowest concentration (2.6 ppb), indicate the reproducibility of the measurements.

The responses of the GC detectors to the size (volume) of the injected sample were also varied to determine at what point they began to deviate from linearity. In practice there is a maximum sample volume which may be introduced before the column becomes overloaded (leading to peak distortion) and pressure, flow or flame chemistry fluctuations affect the detector. Generally, injection volumes of more than 1 ml are not used for megabore (0.53-mm i.d.) columns, but in the interests of increasing sensitivity, even to the extent of exceeding the bounds of linearity, we investigated samples of the maximum size which could be injected.

In this experiment, we used a stream of 22 ppb PH_3 in air and manually injected samples onto a J&W brand GSQ column with N_2 carrier gas running at a head pressure of 18 psi. The FPD responses, in terms of peak height and peak area, are shown in Fig. 4. The relationship between peak area and injection volume was linear from 200 µl to 3 ml, and occasionally even to 4 ml (not shown). However, the larger injections produced chromatographic anomalies such as split peaks. This is reflected by the lack of increase of

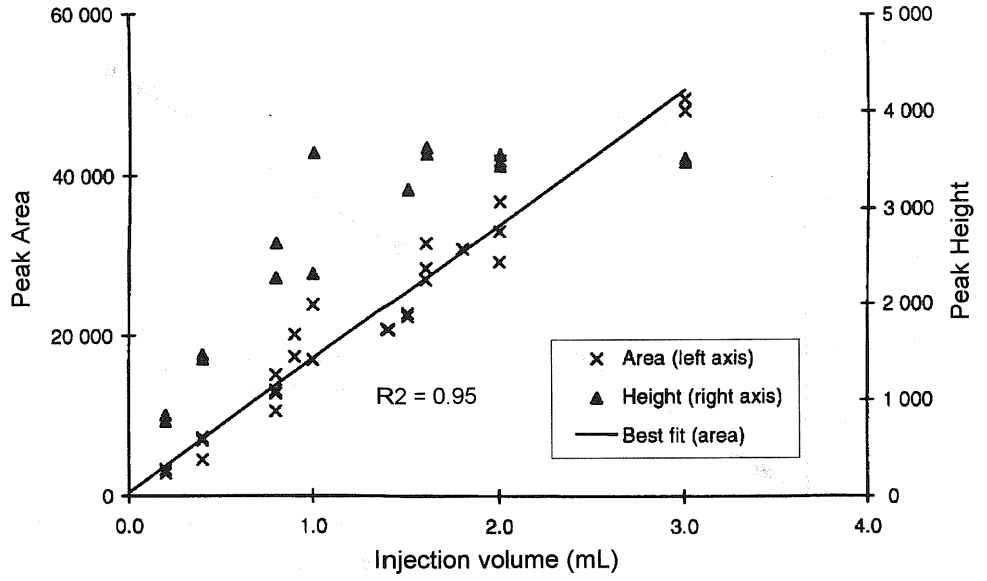


Fig. 4. Response of the GC/FPD to variations in the volume of sample. A linear response in peak area (crosses, left axis) was found in the range 200 ml–3 ml, but the peak height (triangles, right axis) was not linear above about 1.25 ml, which indicates column overloading.

peak heights where injection volumes were larger than about 1.25 ml. The use of increased injection volumes is justified when concentrations near the limit of detection are encountered and detection, rather than quantitation, becomes the goal.

An example of a GC analysis near the limit of detection of our GC/FPD is shown in Fig. 5. In this case we used a GSQ column at 45°C and a manual injection of 3.0 ml of a MFC blend of 1.5 ppb PH_3. The trace shows a large response, due to O_2, followed by the small PH_3 peak (marked). The second injection, of the same volume of laboratory air, shows no PH_3 peak.

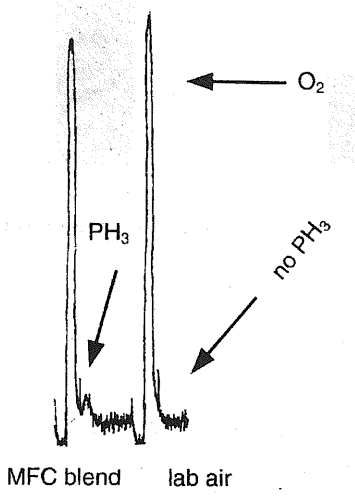


Fig. 5. Example of a GC/FPD analysis of a)1.5 ppb phosphine in air, and b) lab air. Large peaks are due to oxygen and small peak (marked) is phosphine.

Field methods

A paper-tape PH_3 monitoring device, the Autostep, manufactured by the GMD Corp. of the USA, measures PH_3 concentrations in the range 5–400 ppb. An internal pump draws sample air through a section of paper-tape chemically treated to discolour on contact with PH_3. A photosensor continuously monitors the darkness of the stain. A quantitative reading requires a sampling period of 4 min when concentrations are less than 100 ppb. Readings are expressed as graduated bars, with a resolution (or block size) of 5 ppb. At concentrations higher than 100 ppb, i.e. when the photosensor detects a fully-developed stain in less than 4 min, the device automatically steps the paper-tape to the next unused portion and begins again. We found that the device gave consistent readings, although it was less accurate at lower concentrations than at higher ones.

We modified the device by disconnecting its built-in pump and attaching a pump capable of drawing sample at ten times the normal rate, or 1 L min^{-1}. The expectation was that the device's sensitivity would be increased tenfold so, for example, a concentration of 10 ppb would be read as if it were 100 ppb, and 1 ppb would be read as 10 ppb. Figure 6

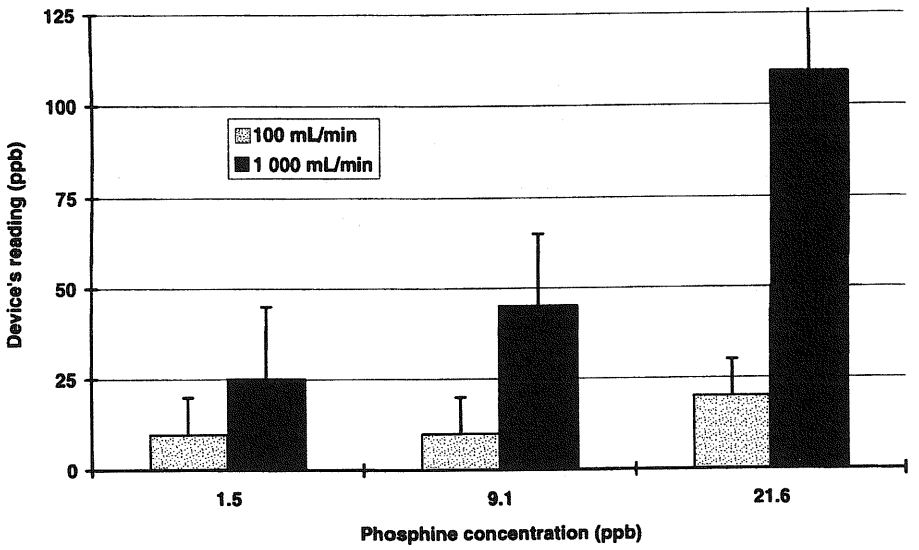


Fig. 6. Measurements of phosphine samples at three concentrations between 1.5 and 21.6 ppb using a GMD Autostep paper-tape phosphine monitor. The light bars show the readings of the unmodified device, and the darker bars show the readings obtained by increasing the sampling rate by a factor of ten.

shows the device's response to a range of concentrations from 1.5 ppb to approx 22 ppb. The device gave anomalously high responses to the 1.5 ppb sample at both flow rates, but at 9.1 and 21.6 ppb, it gave accurate readings with its own pump and higher readings by a factor of five (rather than the expected ten) at the higher sample flow rate. The attenuated response at the higher flow rate was possibly due to either air leakage around the paper strip or insufficient contact time. The paper-tape device holds promise as a fairly simple semi-quantitative method of field monitoring.

As one attempts to measure lower concentrations of PH_3 in the air, the likelihood of false positive readings becomes more prevalent with most measuring techniques. The physical separation of sample components by chromatography circumvents many sources of false positive readings where it can be shown that the likely interfering species are separated from PH_3. The main drawbacks to the use of GCs in the field are the capital cost of the equipment, the bulkiness of the various carrier and detector gases and the power requirements.

We have been developing a solid state detector (SSD) system which is highly sensitive to PH_3 but has no gas requirements (apart from compressed air generated on site) and very low power usage. It is based on a sensor that has been examined for use in fumigation monitors (Ryan *et al.*, 1994) and is presently in an early prototype stage attached to a laboratory GC; further work may see it become the basis of a compact, simple hybrid portable field "GC" monitor. The detector itself is not specific to PH_3, but by using a gas chromatography column to perform separation of the constituents of field samples, real-time quantitative PH_3 measurements in the low ppb range appear to be feasible.

An example of the output of the GC/SSD is shown in Fig. 7. Retention time (i.e. time elapsed since the injection of the sample) is shown on the horizontal axis and the detector-amplified response (in volts) on the vertical axis. The dashed trace shows the detector's response to a few µl of 25 ppm PH_3 in N_2 standard which identifies the compound's retention time as about 1 min under these conditions. The solid traces show the detector's response to 1-ml injections of 95 ppb (black line) and 38 ppb (grey line). In each case there is a large negative peak, centred on 30-sec retention time, which is due to the O_2 in the sample. In both cases a clear peak at the same retention time as the standard is present. Injections of PH_3-free air show no peak at this retention time (not shown in this diagram). It must be pointed out, however, that the baseline of the detector is not stable; there are several fluctuations of a similar magnitude to the PH_3 peaks. Baseline stability must be improved before the detector's limit of detection can be lowered by the 1–2 orders of magnitude that are required to successfully monitor PH_3 at the levels required by the NHMRC guidelines.

In earlier work (Pratt *et al.*, 1994) we found that some commercially available PH_3 monitors based on electrochemical cells responded in a linear fashion to PH_3 concentrations throughout the 'workspace' range (100 ppb–1 ppm). It was therefore thought that, with appropriate amplification, some of them could be used to monitor field concentrations in the 1–100 ppb range. However, the devices we tested (Amahsco 0–5 ppm GEM, MSTox 9001) were both too sensitive to fluctuations of humidity, air pressure and

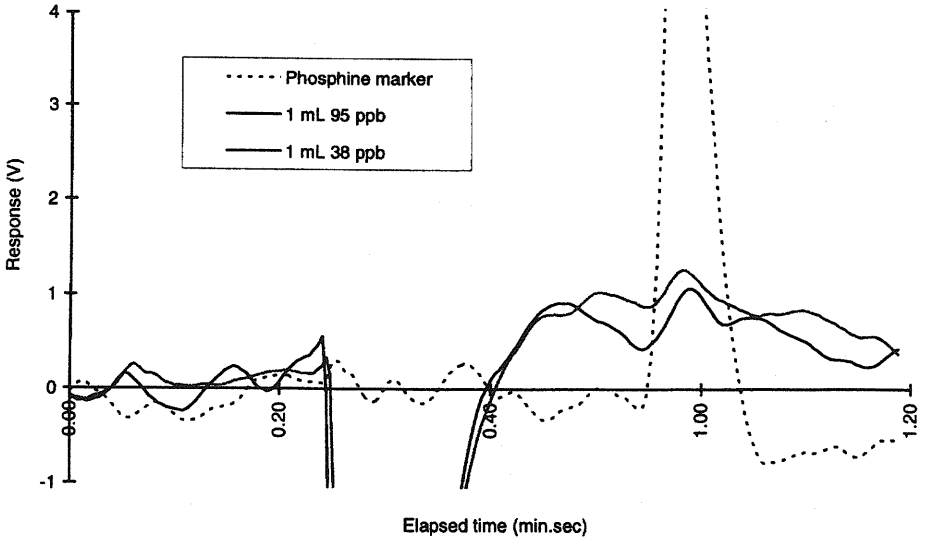


Fig. 7. Gas chromatograph/solid state detector traces of 95 and 38 ppb phosphine samples in air. Injections of 1 ml, large negative peak at 30 sec is oxygen, small peaks at 1 min are due to phosphine. Dashed trace is detector's response to 4 ml injection of 25 ppm phosphine in nitrogen (therefore no negative oxygen peak).

temperature, and too cross-sensitive to other gases such as H_2S, CO and SO_2, for use in monitoring PH_3 below 10 ppb. In addition, the long response time (in the order of 2 min for a 90% response) made it hard to distinguish between responses to gas concentrations and the other influences mentioned above. This is in no way a criticism of the suitability of these or other electrochemical monitors for work-place or fumigation monitoring, for which they are designed, where the PH_3 concentrations of interest are high enough to produce a clear response.

CONCLUSIONS

The grain industry's need to measure environmental levels of PH_3 seems inevitable. Laboratory detection of parts per billion is feasible using standard apparatus, but scrupulous attention must be paid to the quality of gas standards at these levels. We have found that a continuous flow of freshly-diluted PH_3 in air provides reliable gas concentrations of virtually unlimited volume and duration from which comparisons with unknown samples can be made. In view of the variability of the stability of PH_3 in the laboratory environment, we feel that field measurements of PH_3 must be made as soon as possible after sampling. This necessitates taking the laboratory to the site and will be the subject of further work.

ACKNOWLEDGEMENTS

The work described in this paper was funded in part by the Grains Research and Development Corporation, project CSE16M. We gratefully acknowledge their support. The Ian McLennan Achievement for Industry Award, which was awarded to SGRL, allowed SJP and others to travel to the conference. The authors also wish to thank Jim Desmarchelier for his invaluable chromatographic advice.

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DOES UNDERDOSING SELECT FOR RESISTANCE TO PHOSPHINE?

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ABSTRACT

Farmers in eastern Australia are increasingly reliant on phosphine to control insect pests. Many fumigations undertaken on farms and by produce merchants are done in unsealed storages of all types. These fumigations vary considerably in doses achieved and hence in efficacy. The aim of this work was to determine what effect current small-scale fumigation practices have on selection for resistance to phosphine. Five small-scale fumigations in a variety of situations were monitored to determine doses typically achieved in practice. Meanwhile, the responses to phosphine of adults, eggs and pupae of phosphine-resistant, heterozygous and susceptible *Sitophilus oryzae* and *Tribolium castaneum* were measured in the laboratory. A range of phosphine concentrations were assayed for exposure periods of 3 and 6 d. The responses to phosphine of each life stage and each species varied considerably, as did the dominance of the resistant phenotype. A wide range of concentration × time profiles was observed in the field fumigations, none of which, however, would have controlled all stages of all species. Analysis of the responses of resistant, susceptible and heterozygous individuals showed that any fumigation that does not achieve a dose sufficient to kill all life stages of resistant insects (underdosing) will select for resistance. The significance of this in the management of phosphine resistance must be considered.

INTRODUCTION

Market pressure for reduced pesticide residues in Australian grain is increasingly being met through the use of phosphine (PH_3) fumigation. Pest control in most of Australia's grain crop is now achieved using this method. Heavy reliance on PH_3 for insect control, however, means that there is enormous selection pressure for insects to evolve resistance. Although the resistance to PH_3 so far detected in Australia is relatively weak, the possibility that a stronger resistance will develop, as has occurred in the Indian sub-continent (Taylor, 1989), is very real.

In eastern Australia small-scale fumigations, those undertaken on farms and by produce merchants, are carried out under a wide range of conditions. Sealed silos are

sometimes used, but fumigations are often undertaken in unsealed storages such as bag-stacks, covered with plastic sheets or tarpaulins, and metal bins. Although in Western Australia sealed silos are used on farms, many of them are poorly maintained (Newman, 1994).

Current small-scale fumigation practices are expected to vary both in their efficacy, with some practices giving incomplete control, and in the selection pressure they exert for resistance. Practices that select for PH_3 resistance should be discouraged to ensure the long-term efficacy of this fumigant.

The aim of this work was to determine what effect current small-scale fumigation practices have on selection for resistance to PH_3. To achieve this, a range of typical small-scale field fumigations was monitored and the data obtained compared with the laboratory responses of insects to a range of PH_3 concentrations and exposure times. The species tested were *Sitophilus oryzae* and *Tribolium castaneum*; tests were conducted on adults, eggs and pupae of resistant and susceptible strains and on insects heterozygous for resistance.

MATERIALS AND METHODS

Field fumigations

Fumigations on farms were monitored to determine what dosages were typically achieved in practice. The fumigations monitored included a standard above-ground galvanised-steel silo, aluminium phosphide PH_3-generating tablets placed near the fan of an aeration system, a farmer-built sealed silo, a commercially built sealed silo in which the seal had failed, and a plastic-sheeted bag-stack. All fumigations were undertaken by the usual operator in the usual manner.

A number of gas sampling lines (1.8-mm internal diameter nylon pressure tubing) were inserted at a range of points inside each fumigation enclosure before dosing with PH_3. During each fumigation a small amount of gas was pumped out of the enclosure and the concentration of PH_3 at each point was measured using PH_3-sensitive indicator tubes (Dräger).

Laboratory assays

Resistant and susceptible strains. About 30 field strains each of *S. oryzae* and *T. castaneum* were collected from farms and grain merchants' premises in central and southern Queensland and assayed for resistance to PH_3 using the FAO 20-h exposure method (FAO, 1975). Those adults with the highest levels of PH_3 resistance were selected over several generations until there was no further increase in resistance. At this point the resistant strains were substantially homozygous for resistance.

The susceptible insects used were reference laboratory strains that had not been exposed to insecticide or fumigant selection for 20–30 years.

Preparation of eggs, pupae and adults for assay. Pupae and eggs of *S. oryzae* were fumigated in whole grain. Adults of both species, and immature stages of *T. castaneum*,

were sieved from the culture medium and placed in small containers in the fumigation chamber with 0.5 g of wholemeal flour or cracked grain.

Eggs and pupae of *S. oryzae* were obtained by placing 100 adults in small jars containing 68 g wheat for 2 d at 30°C. Adults were then removed and the lidless jars of grain were either placed in the fumigation chamber (desiccator) for treatment of eggs or returned to a 30°C room. Pupae of *S. oryzae* were fumigated for 27 d (R-strain) and 29 d (S-strain) after the 2-d oviposition period. Dissections of whole grains revealed that, at 30°C, about 90% of the insects had pupated within these times.

Eggs and pupae of *T. castaneum* were sieved from the flour after the adults were allowed a 24-h oviposition period at 30°C. Eggs were used at 1–2 d, and each assay of pupae contained a range of ages. Fifty eggs or pupae were placed ready for fumigation in each of four plastic, 50-ml open-topped measuring cups with 0.5 g flour. Adults of both species were assayed at 2–4 weeks after emergence.

Obtaining F_1 progeny for assay. Virgin adult *S. oryzae* were obtained by isolating individual infested grains in gelatine capsules. After emergence the adults were sexed according to the shape of the rostrum (Halstead, 1963). Reciprocal crosses of the resistant and susceptible parent strains were made by adding 50 males and 50 females of the appropriate strain to 68 g whole wheat.

The oviposition period was 2 d. F_1 eggs, pupae and adults were obtained from these cultures as described above.

Pupae of resistant and susceptible *T. castaneum* were sieved from flour and sexed (Halstead, 1963). Reciprocal crosses of parental strains were made by placing 100 male and 100 female pupae in flour. About 1 week after eclosion, the adults were removed from the culture medium and placed onto fresh flour for an oviposition period of 1 d. F_1 eggs, pupae and adults were removed from the flour as described above.

Phosphine susceptibility tests. Response to PH_3 was measured by exposing insects to a range of concentrations of fumigant at 25°C and 55% r.h. in desiccators as described in the FAO method (FAO, 1975). Exposure periods were either 3 or 6 d with a post-fumigation recovery period of 14 d for eggs and adults and 21 d for pupae.

Four batches of each assay were undertaken. Batches of adults consisted of 50 insects confined within glass rings on a filter paper base supplied with 0.5 g wholemeal flour or cracked grain. Numbers of eggs or pupae in each batch were as described above. The response of insects to PH_3 was subjected to probit analysis (Finney, 1971) where control insects could be counted directly, i.e. adults of all species and eggs of *T. castaneum*. Wadley's method (Finney, 1971) was used where controls were estimated, i.e. eggs and pupae of *S. oryzae*.

PH_3 was generated from a commercial aluminium phosphide formulation and collected over acidified water. A gas density balance chromatograph (Varian Aerograph Model 90-P) was used to determine the concentration. Dichlorofluoromethane was used as the carrier gas. PH_3 was injected using gas-tight syringes through a rubber septum in the lid of the desiccator to give the required concentration.

RESULTS

Phosphine fumigations in farm silos

Fumigation 1 was in an unsealed bolted galvanised-steel 1000-t silo fitted with aeration ducting. At the time of this fumigation, the silo was almost full of sorghum leaving a 3-m headspace. Grain temperature ranged from 27 to 41°C with headspace temperature from 37 to 75°C. PH_3 was applied by adding aluminium phosphide tablets to the grain as it was being augured back into the silo. The operater added the tablets at a rate of 3 tablets t^{-1} so that the applied dose of PH_3 was 3 g t^{-1}, double the recommended rate. Concentrations of PH_3 were monitored inside the silo at three points on the surface of the grain and three additional points at a 3-m depth. The concentration of PH_3 at the surface did not exceed 60 ppm and was usually less than 30 ppm. PH_3 concentration at 3 m below the surface peaked at about 150 ppm after almost 2.5 d but declined rapidly over the following 3 d. Gas lines placed inside the aeration ducts and the auger boot indicated that gas was lost rapidly from the silo at these points.

Fumigation 2 was designed to test the efficacy of applying PH_3 through the aeraton fan — a fairly common practice among farmers. Six sampling points were set up before harvest inside a 100-t steel above-ground silo. After the silo had been filled with wheat, the farmer placed 90 aluminium phosphide tablets, equal to 90 g PH_3, in an open-topped steel can (about 500 ml in capacity) that was suspended at the outside opening of the aeration fan. Aerator suction was recorded as 7.2 m/sec while wind velocity was 0.8 m/sec. Grain temperature was 35°C. All the PH_3 generated from the tablets was lost from the silo within 48 h, and the concentration did not exceed 4 ppm.

In fumigation 3, stacks of bagged seed (about 10 t) were stored inside a raised wooden shed with a floor area of about 7 × 5 m. Before fumigating, gas lines were installed at three points in the bag-stack and at two points above the position where the tarpaulin was to be placed. The farmer then spread 200 aluminium phosphide tablets on the bag-stack and covered the area with a plastic sheet. Windy conditions prevailed throughout the time of the fumigation with wind speed varying between 1.2 and 1.6 m/sec. Little or no PH_3 was detectable after 24 h. At the centre of the bag-stack the PH_3-concentration peaked at 380 ppm after 9 h, but the gas was rapidly lost. The PH_3 concentration at two other points monitored under the tarp did not exceed 50 ppm.

Fumigation 4 was in a farmer-built, sealable, bolted-steel silo of 25-t storage capacity (for wheat). There was no pressure relief valve. Before harvest eight gas monitoring lines were placed inside the silo at various points. The farmer added the recommended rate of 50 aluminium phosphide tablets (50 g PH_3) to sorghum as it was augured into the silo. Gas concentration reached a peak at about 2 d and then decreased rapidly. However, even at the sample point showing the lowest range of concentrations, PH_3 concentration remained above 100 ppm for 6 d, i.e. from day 1 to day 7.

Fumigation 5 was in a commercially sealed 1,700 bushels (46.32 t) silo. Although this silo had a pressure release valve, it could not be used to test gastightness. The silo was filled with sorghum and three gas sample lines were inserted into the grain from

the top hatch. Samples were taken at the grain surface and at 2 and 3 m below the surface. Fifty aluminium phosphide tablets were added by lifting the hatch and spreading them across the surface of the sorghum. This dose rate is slightly more than half that currently recommended. PH_3 concentrations rose to a peak of about 600 ppm in 2 d and then rapidly declined. At the surface of the grain the gas concentration fell to below 100 ppm at about 6 d, while at the 2- and 3-m points the concentration reached 100 ppm at about 8 d. Gas could be smelt near the hatch during the fumigation, so the hatch seal was probably leaking. This would explain the lower gas concentration at the surface.

Laboratory assays

S. oryzae. At 3 d the most tolerant life stage of both the R- and S-strains was the pupa, with $LD_{99.9}$ values of 2,160 ppm (3.0 mg/L) and 144 ppm (0.2 mg/L), respectively. In addition, one strain (LS2), which showed resistance in the pupal stage but not as eggs or adults, had a $LD_{99.9}$ of greater than 3,000 ppm (4.25 mg/L). At 6 d eggs were the most tolerant stage giving $LD_{99.9}$ values of 625.5 ppm (0.9 mg/L) and 66.2 ppm (0.09 mg/L) for the R- and S-strains, respectively. Resistance to PH_3 in *S. oryzae* was almost completely recessive in the eggs at both the 3 and 6 d exposures.

T. castaneum. At the 3-d exposure period eggs were about 10 times more tolerant than either pupae or adults for both the R- and S-strains. $LD_{99.9}$ values for eggs were 733 ppm (1.02 mg/L) for the R-strain and 63 ppm (0.088 mg/L) for the S-strain. Similarly, at 6 d the eggs were the most tolerant stage with $LD_{99.9}$ values of 220 (0.3 mg/L) and 50 ppm (0.07 mg/L) for the R- and S-strains, respectively. Resistance was almost completely recessive in adults and pupae at both the 3 and 6 d exposure periods. In eggs resistance was expressed as incompletely recessive at 3 d and semi-dominant at 6 d.

DISCUSSION

Efficacy of current phosphine fumigation practices in small-scale storages

Fumigations in the best unsealed storage produced enough PH_3 to kill some stages of susceptible insects, i.e. adults of *S. oryzae* and adults and pupae of *T. castaneum.* The highest doses achieved in this silo would also have resulted in 100% mortality of resistant *T. castaneum* adults and up to 80% kill of adult, resistant *S. oryzae*, giving the impression of a successful fumigation. However, about 90% of *S. oryzae* and 100% of *T. castaneum* eggs, and about 80% of *S. oryzae* and 25% of *T. castaneum* pupae, would have survived the fumigation.

The fumigations using the aeration fan and the bag-stack covered with plastic sheeting produced concentrations of PH_3 that would kill at least a proportion of susceptible adult insects, again giving the appearance of an effective fumigation. However, PH_3 doses would not kill resistant adults and were not sufficient to kill either eggs or pupae of even susceptible insects.

Dosages achieved in the sealed silos were significantly higher than those in the best unsealed silo. Even the silo that received only half the recommended number of PH_3 tablets gave a result superior to that in the unsealed silo. The underdosed sealed silo held enough PH_3 over a 6 d period to kill all stages of susceptible strains of both species, adults and pupae of resistant insects and the resistant pupae of the LS2 strain of *S. oryzae*. In the farmer-sealed silo, which received the correct number of PH_3 tablets, the lowest measured concentrations of PH_3 reached were still sufficient to kill all stages of susceptible insects and all stages of resistant insects except eggs and LS2 pupae of *S. oryzae*.

To achieve a successful fumigation, all life stages of the most tolerant species must be killed during the exposure period. The concentrations of PH_3 necessary to give 99.9% mortality of the eggs and pupae of the resistant strain of *S. oryzae*, the most tolerant species, in 6 d at 25°C are illustrated in Figs. 1 and 2 against the concentrations achieved in the best unsealed silo and the two sealed silos. Although resistant pupae were killed in sealed silos (Fig. 2), the concentrations of PH_3 for the required exposure time were not sufficient to control eggs (Fig. 1). The minimum concentration achieved in the unsealed silo, barely adequate to control all adults, would be ineffective against eggs and pupae.

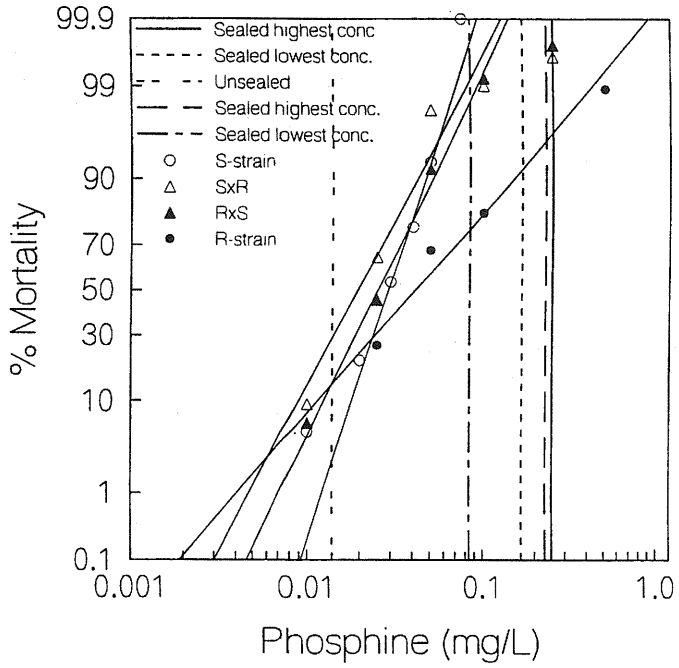


Fig. 1. Response to phosphine of susceptible, resistant and heterozygous eggs of *Sitophilus oryzae* compared with the maximum and minimum concentrations of phosphine that occurred at 6 d in two sealed silos and the maximum concentration in the best unsealed silo.

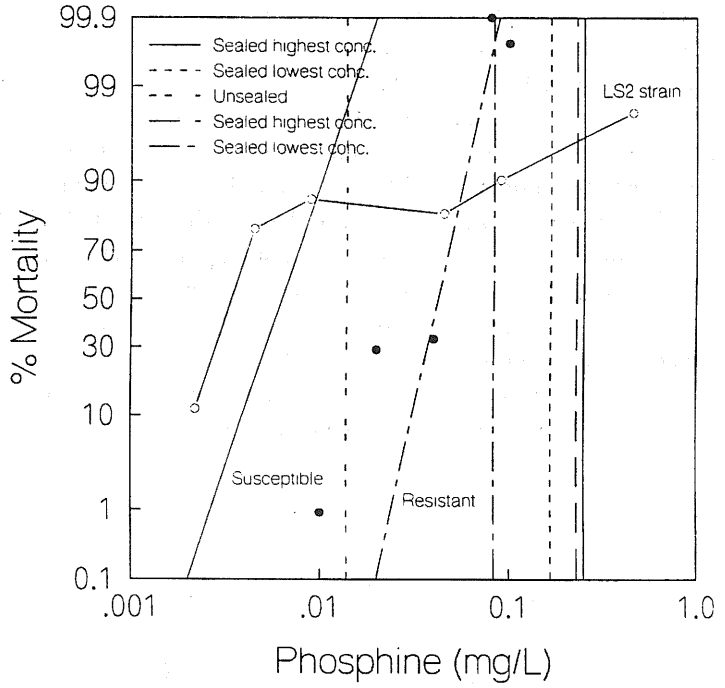


Fig. 2. Response to phosphine of susceptible and resistant pupae of *Sitophilus oryzae* compared with the maximum and minimum concentrations of phosphine that occurred at 6 d in two sealed silos and the maximum concentration in the best unsealed silo.

Selection for resistance to PH_3 under current fumigation practices

Selection for any trait requires that individuals possessing that trait are favoured; i.e. they produce more progeny than individuals not possessing the particular trait. To select for resistance to a toxicant such as PH_3 the chemical must be used in such a way that insects possessing the resistance gene(s) can survive and multiply while insects not possessing the resistance gene(s) will be killed or produce fewer progeny. This means that doses applied must be sufficient to kill all resistant insects. Lower doses must necessarily favour resistant insects by killing most of the susceptibles and allowing a high proportion of resistant individuals to survive. A caveat to this argument is that high doses may readily select for any new, stronger resistance that may be present in the population.

We have shown that even very poor fumigations producing low gas concentrations for short periods, such as those using an aeration fan or performed under a loose cover, will select for resistance by killing only susceptible adult insects (and probably larvae). On the other hand, these fumigations only select for resistance in a proportion of the population, allowing eggs and pupae of both resistant and susceptible insects to survive so there is no

selection effect on these life stages. Selection for resistance is also likely to occur in the most effective fumigations, i.e. the two sealed silos and the unsealed silo. The selective effect of these fumigations on the eggs and pupae of *T. castaneum* are illustrated in Figs. 3 and 4, respectively. In the first example (Fig. 3) a proportion of both homozygous resistant and heterozygous (R × S, S × R) *T. castaneum* eggs would survive fumigations in the sealed silos, but susceptibles would not survive. In this situation, development of resistance would be quicker in the sealed silos than in the unsealed silo because the unsealed silo also allowed survival of about 80% of susceptibles. These would dilute the resistance frequency in succeeding generations. In the second example, susceptible and heterozygous pupae of *T. castaneum* were controlled by fumigations in sealed silos (Fig. 4). However, the concentration of PH_3 reached over the 6 d fumigation in the unsealed silo was ideal for selecting for resistance in this species. It killed all susceptibles and heterozygotes (R × S, S × R) and allowed only homozygous resistant insects to survive.

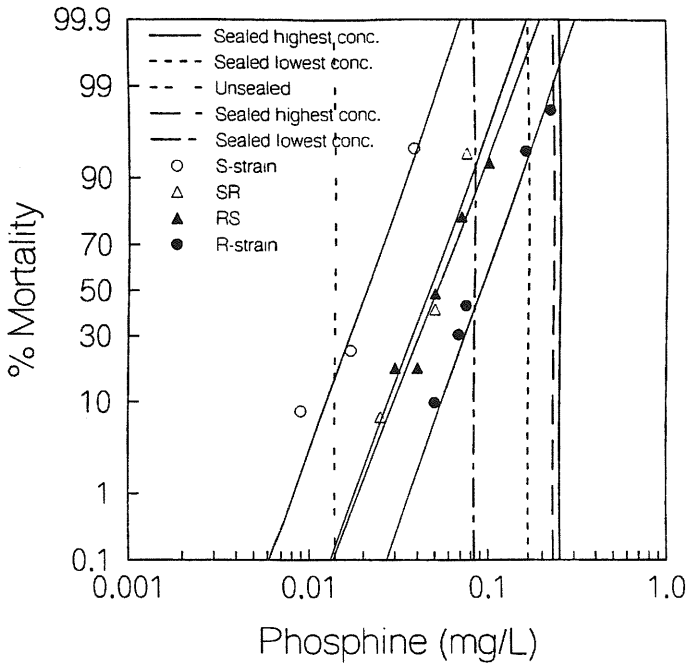


Fig. 3. Response to phosphine of susceptible, resistant and heterozygous eggs of *Tribolium castaneum* compared with the maximum and minimum concentrations of phosphine that occurred at 6 d in two sealed silos and the maximum concentration in the best unsealed silo.

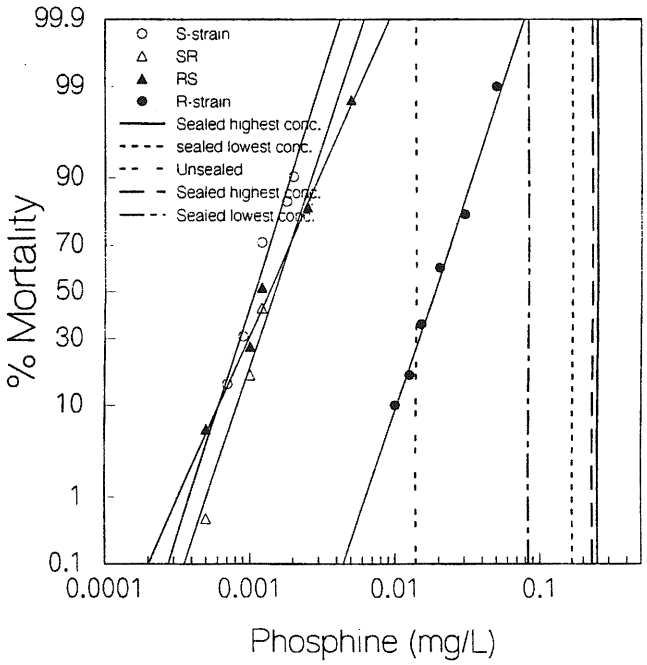


Fig. 4. Response to phosphine of susceptible, resistant and heterozygous pupae of *Tribolium castaneum* compared with the maximum and minimum concentrations of phosphine that occurred at 6 d in two sealed silos and the maximum concentration in the best unsealed silo.

CONCLUSION

The two examples cited above and the results for *S. oryzae* (Figs. 1 and 2) illustrate that selection for resistance will vary from species to species and among life stages, depending on the expression of resistance and the dosage of PH_3 encountered. In general, however, a very poor fumigation will not select for resistance as quickly as a fumigation that is close to successful. Nevertheless, any fumigation that achieves less than the recommended dose level (concentration × time) is likely to select for resistance in at least one of the insect pest species involved.

ACKNOWLEDGEMENT

We are grateful to Grains Research and Development Corporation for their financial support of this research.

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LIMITATIONS FOR INFESTATION CONTROL IN COOLED BULK GRAIN AND A STRATEGY TO OVERCOME INHERENT SEALING AND GAS DISTRIBUTION PROBLEMS USING PHOSPHINE

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ABSTRACT

The tolerance of five important grain pests to phosphine (PH_3) at 10°C and below was assessed in the laboratory. Older stages of *Sitophilus granarius* were highly tolerant of the combination of cold and exposure to PH_3 and survived exposures to concentrations above 0.7 mg/L at 5–7.5°C for over 3 weeks. Therefore, fumigation with PH_3 at temperatures below 10°C can only be recommended if *S. granarius* is absent. *Oryzaephilus surinamensis, Cryptolestes ferrugineus, Tribolium castaneum* and *Ahasverus advena* were all killed by a 12-d exposure to 0.1 mg/L PH_3.

A sensor-controlled automated dosing system originally developed for use with methyl bromide mill fumigations has been modified for use in PH_3 fumigations of bulk grain. A new sensor based on an electrochemical cell has been incorporated into the system to monitor PH_3 concentrations within the ranges encountered in commercial fumigations. The dosing system has been tested both in the laboratory and in recent field trials on bulk grain. The system can potentially maintain adequate PH_3 concentrations throughout the long exposure times required for such treatment at low temperatures by countering the gas losses caused by both adverse weather conditions and inherent sealing problems.

INTRODUCTION

The introduction of modern effective and accurate sampling techniques within the grain industry has led to the frequent detection, during trading, of insect pests. Three major pest species, commonly encountered as adults wandering on grain even at temperatures below 10°C, are the beetles *Oryzaephilus surinamensis* (L.), *Cryptolestes ferrugineus* (Stephens) and *Sitophilus granarius* (L.). Two other beetles, *Tribolium castaneum* (Herbst) and *Ahasverus advena* (Waltl), may also be seen. Once infestations have been identified, often the only practicable course of action for treating a whole bulk *in situ* is to recommend fumigation with phosphine (PH_3). PH_3 is a highly toxic gas liberated from commercially obtainable aluminium or magnesium phosphide preparations in the presence of moisture. These preparations are available in a variety of sizes from 0.6-g pellets, which liberate 0.2 g

gas, to 3,400-g bag-blankets, which liberate over 1 kg gas. PH_3 is released over a number of days, how many depends upon the temperature within the structure.

There are several factors which can contribute to the success or failure of bulk grain fumigations.

Leakage

Many storage structures are not designed or constructed with fumigation in mind. In some, because there is no space between the bulk and the walls of the building, only the grain surface can be sheeted. Even with a well-sheeted grain bulk, gas diffuses easily through aeration ducts and cracks in the walls, as well as permeating through the sheet itself. In addition, smaller bulks of grain (below 1,000 t) have proportionately large surface-to-volume ratios, permitting a greater percentage of fumigant loss per unit of time (Bell *et al.*, 1991).

Poor distribution

Uneven placement of formulations, especially surface treatments, can lead to high concentrations of gas in some areas and low concentrations in others due to poor penetration, particularly where the grain is deep and temperatures low.

Phosphine toxicity

In general long exposures to low concentrations have been found to be more effective than short exposures to high concentrations (Howe, 1973; Hole *et al.*, 1976; Bell, 1979). This is primarily because longer exposures allow time for the naturally tolerant stages of the life cycle, the pupae and eggs, to develop into the more susceptible larval and adult stages. Different species vary enormously in their level of tolerance.

Phosphine resistance

Resistance is known in all the species in the present study with the exception of *A. advena*, which has not hitherto been investigated. The import of products from overseas, where there is a higher incidence of resistance, is a particular concern.

Temperature

Low temperatures cause a slow release of PH_3 from metal phosphide formulations. In poorly sealed structures this can result in a significant loss of gas before it has a toxic effect on the pest. Hole *et al.* (1976) concluded that many insect species were more tolerant to PH_3 at lower temperatures, and that at 15°C and below effective control could only be achieved by arranging long exposure periods. Due to the lack of information available on the control of insects below 10°C, fumigation schedules do not make recommendations for treatment at such temperatures. High doses are currently applied to increase the chances of success, and there is some concern that PH_3 residue levels will exceed the MRL (maximum residue limit) of 100 μg/kg. However, the susceptibility of some species to cold may permit a reduction in the dosage applied,

provided that there is an efficient dosing and gas distribution system, particularly if the final stage of infestation consists of adults only because of the effect of cold on immature stages. Hence any risk of exceeding MRL's could be avoided without loss of efficacy, and low-temperature treatments could not only be recommended with confidence but also seen to be effective.

The current programme was undertaken to investigate the effect of PH_3 at temperatures down to 5°C on beetles commonly occurring in grain bulks in order to facilitate planning field trials on dosing strategies.

MATERIALS AND METHODS

Tests on insects

Two strains of each of the five species cited above were tested. One was a laboratory strain reared for many years and known to be susceptible to the commonly-used contact insecticides and fumigants, and the other was a strain recently collected from a natural infestation and put into culture. Of these, the new *T. castaneum* strain, as judged by the standard FAO test (Anon, 1975), was resistant to PH_3.

Insects for fumigation were reared at 25°C (*O. surinamensis, S. granarius* and *T. castaneum*) or 30°C (*A. advena* and *C. ferrugineus*) and 60–70% r.h. Preliminary ranging tests were performed in 6-L calibrated glass desiccators fitted with a stainless steel mesh platform and a magnetic stirrer. Thereafter, a total of 20 tests was carried out at 5, 7.5 and 10°C and 60% r.h. and at concentrations ranging from 0.05–1.62 mg/L, on immature stages and adult insects. For each strain in each test, in addition to controls, a total of seven exposure periods was planned (at the test temperature and at 25°C) with three replicates per exposure. Cultures were set up by adding 50 adult beetles to 350-ml glass jars containing a precise quantity of a suitable food mix (Clifton *et al.*, 1995). The seeded cultures were sealed by means of double filter paper tops secured with molten paraffin wax, and they were then held at their breeding temperatures until the original adults were removed 3 weeks later. At this time a second set of cultures was prepared in the same manner as the first for each strain. The life-cycle was thus divided into two halves; the first set provided older stages (predominantly pupae and older larvae), and the second set provided younger stages (predominantly younger larvae and eggs). The adult beetles were removed from the 'younger' cultures a few days prior to the test. All the cultures were then simultaneously conditioned down in temperature, in steps of 5°C for periods of 24 h, until they reached 15°C. Here they remained for 48 h before being placed directly at the test temperature where they were conditioned for a further 48 h before testing.

Prior to 'stepping down', the adult insects to be fumigated were prepared by placing 50 beetles in 120-ml jars, each of which contained a small spoonful of food mixture. Each jar was sealed by means of a nylon top secured in place by an aluminium screw ring. As was done with the immature stages, a total of seven exposure periods and two sets of controls, with three replicates of each, were included in each test.

Fumigation procedure

Fumigations were performed in 1,700-L stainless steel chambers. Each chamber was fitted with a 15-cm diameter port and a row of 2-cm diameter ports. A specially designed polythene sleeve was attached to the internal surface of the chamber surrounding the large port through which it protruded. By means of a bag attachment, it was thus possible to transfer jars into and out of the chamber with minimal loss of gas.

Chambers were dosed for each test by adding pellets of a commercial aluminium phosphide preparation. The chambers were dosed at least 5 d prior to the fumigation, allowing time for the complete decomposition of the pellets at the low test temperatures. During the fumigation test period, gas samples were taken at regular intervals by means of a fine bore nylon sampling line run from each fumigation chamber to the gas chromatograph. Gas concentrations within the chamber were calculated based on 'standard' gas samples from calibrated cylinders. These were used to calculate Ct products.

All fumigated insect material remained at the test temperature until the cultures with the longest exposure had been removed from the chamber. After airing for a period of 2 d, all the cultures were returned to 25°C and 60% r.h. (25°C and 70% r.h. for *A. advena*) in 5°C steps. Immature insect cultures were sieved and examined on a weekly basis to assess survival rates by counting the live adults which emerged. Counting commenced on the older cultures during the week following the fumigation test. Counts for the younger cultures started approximately 3 weeks later, allowing time for the younger stages to develop into adults. The mortality of adult samples was assessed after 14 d. Where possible, the data obtained was subjected to probit analysis.

The automated dosing system

A prototype automated dosing device, developed for use with methyl bromide in flour-mill fumigations, was adapted for use with PH_3 by fitting an electrochemical cell-based sensor and then reprogramming fumigant concentration ranges and set points. The sensor was calibrated in the laboratory by monitoring a range of PH_3 concentrations set up in chambers and comparing them using a gas chromatograph fitted with a phosphorus filter and a flame photometric detector, the CSL (Central Science Laboratory) standard device used in all recent field trials involving PH_3. The system was then used to control gas concentrations in trial fumigations of bulk grain.

Maintenance of adequate gas levels in field trials

The first field trial was performed to investigate the efficacy of the sensor-controlled dosing system described above in maintaining PH_3 concentration levels in a 470-t bulk of wheat held on a drying floor and cooled to 10 ± 2°C. The bulk was sealed with laminate sheeting and, utilizing the ventilation channels in the floor, dosed with Detia Gas ex-B at approximately 2 g/t. Three days later the fumigation was placed under the control of the automated dosing system, which sampled the bulk from six gas sampling lines placed at selected positions in the bulk. It then opened a dosing valve, from cylinders of 2.6% vv

PH_3 in carbon dioxide, to direct the gas to positions with concentration levels below 0.2 mg/L, the level chosen for maintenance.

After reprogramming the dosing system, a second trial was performed at a different site on a 270-t bulk of barley cooled to below 10°C. On this occasion the sides of the stowage bay were lined with polythene before harvest. After harvest the bulk and lateral ventilation ducts were sheeted, as in the first trial (the latter via the under-floor plenum duct) and provided with an initial dose in the form of four strings of ten Detia bags inserted into four of the ducts before sealing. From the third day the fumigation was placed under the control of the automated dosing system operating from eight sensor-linked dosing points.

RESULTS

Tests on insects

A summary of exposures recommended to the UK Home Grown Cereals Authority (Clifton *et al.*, 1995) for the control of each species is presented in Table 1.

Immature stages of both strains of *A. advena* proved highly susceptible to PH_3 and were severely affected by exposure to low temperature. At 7.5 and 10°C, no stage

TABLE 1

Estimated dosages required for control of five species of stored-product beetles at 5–10°C

Species	Temperature (°C)	Dose (mg/L)	Exposure (d)[1]
A. advena	10	0.10	2
	5 or 7.5	0.05	2
T. castaneum (laboratory strain)	10 (or below)	0.10	2
O. surinamensis	10	0.10	4
	7.5	0.10	4
	5	0.05	4
T. castaneum (PH_3-resistant strain)	10 (or below)	0.30	8
C. ferrugineus	10	0.10	12
		0.30	8
	7.5	0.10	12
		0.30	8
	5	0.10	12
		0.80	6
S. granarius	10	1.00	18
	7.5	1.00	>23
	5	1.00	>28

[1]Extra time has to be allowed for the decomposition of a solid formulation and the distribution of the gas through the grain bulk.

survived as long as 40 h. In the absence of PH_3, at 5°C younger stages were all killed within 7 d and at 7.5°C only a few (10–15%) of the older stages survived as long as 9 d.

The two strains of *T. castaneum* differed widely in their tolerance to PH_3, and the strain recently collected from the field gave a positive result when tested for resistance to PH_3 by the FAO resistance test method (Anon, 1975). A total of 176 of 700 adults survived the discriminating concentration of 0.04 mg/L at 25°C for a 20-h exposure. All stages of the laboratory strain succumbed after a 24-h exposure to 0.11 mg/L of PH_3 at 10°C, but older immature stages of the field strain required 7 d at 0.29 mg/L for control.

Like *A. advena* and the laboratory stock of *T. castaneum*, the strains of *O. surinamensis* showed a high susceptibility to PH_3 at all the low temperatures tested, particularly as eggs or younger larvae. At 10°C all immature stages were killed within 3 d at concentrations near 0.1 mg/L, and at 7.5°C there were only small levels of survival of older stages after 4–5 d exposures at 0.05 mg/L. At 5°C all immature stages died after a 4-d exposure at 0.05 mg/L. A Ct product of 7–8 mg h/L was sufficient for complete control of all stages at each low temperature tested.

A much higher level of tolerance to PH_3 at low temperatures was apparent in all stages of both strains of *C. ferrugineus*. Older immature stages were tolerant of prolonged exposure at low temperature in the absence of PH_3. At 10°C immature stages of the field strain showed a higher level of tolerance to PH_3 than did the laboratory strain, older larvae and pupae surviving up to 8-d exposure at 0.1 mg/L and 4 d at 0.36 mg/L. Younger stages of both strains were all killed within 3 d at the higher concentration. At 7.5°C, a 12-d exposure at 0.11 mg/L was required for complete kill of the field strain, and 8 d for the laboratory strain. At 5°C with a much increased PH_3 concentration of 0.78 mg/L, some older immature stages of the laboratory strain and adults of the field strain survived a 4-d exposure.

Older immature stages of *S. granarius* were highly tolerant and survived exposures of up to 3–4 weeks at low temperatures at PH_3 concentrations above 0.6 mg/L. Younger immature stages were, however, susceptible to cold; over three quarters of the total in control cultures died after 2–3 week exposures at 10°C, and they were very easily killed at 10°C by the lowest Ct products at the shortest exposures tested. As a result they were omitted from most tests at 5 or 7.5°C. Adults of *S. granarius* were relatively susceptible to PH_3 at 5–10°C. They were considerably less tolerant than those of *C. ferrugineus*.

Large scale tests on the automated dosing system

Through much of the first trial, high winds hit the north side of the store resulting in the repeated activation of the two dosing points nearest this area. This led to freezing at the gas regulators on the cylinders, and the restricted flow meant that gas concentrations remained low. In such situations it was concluded that reprogramming was necessary to reduce both the dose pulse duration and the interval between pulses to ensure that the required amount of gas was administered. Better results might have been obtained if either the north wall had been lined with polythene before loading the grain or a grain wall had been installed behind the outside wall to permit sheeting to ground level.

In the second trial, somewhat better results were obtained even though the bulk (270 t)

was considerably smaller, resulting in a more adverse surface-area-to-volume ratio. The gas concentrations obtained at various positions in the bulk are presented in Table 2. For comparative purposes, results obtained from monitoring a commercial fumigation of a larger bulk (2,000 t) of barley using conventional PH_3 formulations, but with the provision of a low-volume gas recirculation facility, are also presented. It can be seen that the commercial treatment compared extremely well with the trial treatment for the first week or more of the fumigation but thereafter lost ground as gas slowly leaked away. However, the gas concentrations and Ct products achieved in both treatments were sufficient to control the grain pests present.

DISCUSSION

There were wide differences in the PH_3 tolerances of the five species tested, with *A. advena* being the least and *S. granarius* the most tolerant. In every case the younger immature stages, comprising eggs and younger larvae, proved highly susceptible to PH_3 at the low temperatures tested. The older immature stages, comprising older larvae and pupae, were almost always the stages of highest tolerance.

Many immature stages, particularly eggs, were killed by cold in the absence of PH_3. Fields (1992), in his comprehensive review of the effect of extreme temperatures on insects, states that eggs are usually the most cold-susceptible stage. In the present study, eggs of *O. surinamensis* proved particularly susceptible. Mullen and Arbogast (1979) found that exposures of under 3 d at 5°C killed 95% of young eggs exposed. Jacob and Fleming (1986) found that complete kill of all age groups of eggs of this species was obtained after a 4-d exposure at 5°C.

A. advena, *O. surinamensis* and the laboratory strain of *T. castaneum* were all controlled by the low dosage of 0.1 mg/L held for 4 d at all temperatures tested. It is thus possible that low doses of PH_3 can be recommended for grain infested only with these species as long as it is certain that other species are not present. In practice the evolution of gas from metal phosphide formulations is slow at these temperatures, and this should be borne in mind when setting treatment times. Allowance must also be made for the time required for the gas to distribute itself throughout the bulk.

A very wide difference in tolerance was apparent between the two strains of *T. castaneum*; the field strain in fact was diagnosed as resistant. All stages of this strain, including adults, showed increased tolerance, necessitating a concentration of 0.3 mg/L to be held for 8 d to obtain complete control at the temperatures tested. Resistance to PH_3 is becoming increasingly common among stored-product beetles, and its presence means that both concentration level and exposure time need to be increased when PH_3 is used.

As reported by other workers (Smith, 1970; Fields, 1992), *C. ferrugineus* showed high tolerance of cold, the adult stage being particularly tolerant. This is perhaps surprising as the species requires temperatures in excess of 22°C for population increase, a threshold higher than that for the other species (Howe, 1965). When exposed to PH_3 at 5°C and 0.78 mg/L, adults were the most tolerant life stage in at least one strain. The very high Ct

TABLE 2
Concentrations (mg/L) at different times and Ct products (mg h/L) (last column) of PH_3 obtained at various positions in a 270-t bulk of wheat using a metered dosing system

Position and depth in grain	32 h	3 d	6 d	8 d	11 d	Ct after 15 d
Rear corner						
bottom	0.12	0.01	0	0.25	0	51.3
middle	0.10	0.05	0	0.16	0	40.1
surface	0.20	0.10	0	0.13	0.01	29.4
Centre						
bottom	0.30	0.33	0.29	0.14	0.20	120.3
middle	0.24	0.14	0.50	0.29	0	75.7
surface	0.14	0.31	0.28	0.10	0.19	76.4
Between centre and back, middle	0.19	0.22	0.45	0.36	0.14	84.2
Right-hand side, middle	0.14	0.18	0.28	0.29	0.17	70.7
Top of slope centre, middle	0.33	0.47	0.57	0.17	0.32	103.0
Top of slope left-hand side						
bottom	0.27	0.22	0.52	0.23	0.28	86.4
middle	0.32	0.38	0.51	0.22	0.26	105.2
surface	0.33	0.37	0.52	0.28	0.23	109.1
Top of slope right-hand side, middle	0.47	0.33	0.40	0.39	0.27	118.9
On slope, middle	0.22	0.24	0.43	0.42	0.33	99.8
Mean ± SE	0.24 ± 0.10	0.24 ± 0.13	0.34 ± 0.21	0.25 ± 0.10	0.17 ± 0.12	83.6 ± 28.4
Means for commercial treatment of 2000-t bulk of barley	0.47 ± 0.17	0.30 ± 0.06	0.30 ± 0.06	0.16 ± 0.11	0.07 ± 0.05	98.5 ± 17.3

products which appeared to be required for control, however, may well have been the result of the inefficiency of such a high concentration of PH_3 in short exposure periods. Bell (1979) showed that for diapausing larvae of *Ephestia elutella* (Hubner) at 20°C, PH_3 concentrations above 0.49 mg/L did not significantly affect the exposure time required for 99% mortality and the level of kill attained at higher concentrations was largely determined by exposure period. In further experiments over a range of temperatures, PH_3 concentrations between 0.05 and 0.10 mg/L gave the greatest efficiency of fumigant action (Bell, 1992). At 5–10°C, an 8-d exposure at 0.3 mg/L should achieve complete control of *C. ferrugineus* in the absence of resistance.

S. granarius has for some time been recognised as the most PH_3-tolerant stored-product species and is responsible for the higher dosages and exposures currently recommended for grain treatment (Winks *et al.*, 1980; Anon, 1984). The present results for this species at 10°C agree closely with the earlier results of Hole *et al.* (1976), showing some survival even after exposures longer than 16 d. This survival is due to a highly tolerant phase early in the pupal period (Howe, 1973) which, at 25°C, may only last 4–5 d. The threshold temperature for development of the species is 13–15°C (Evans, 1977, 1983; Howe, 1965). Development of some stages may proceed slowly at lower temperatures, and transition to the next stage will not occur. Hence phases of tolerance are enormously extended, and this is reflected in the fumigation results obtained here. The tolerance of these stages and adults to cold is high, and this species has no difficulty in overwintering in cooled grain (Armitage and Llewellin, 1987).

To combat resistance and control naturally tolerant pests such as *S. granarius*, some means of lengthening the exposure in PH_3 fumigations,, particularly at low temperatures, is clearly required. Conventional formulations and application techniques, given the inherent sealing problems and vulnerability to adverse weather conditions which apply to bulk grain storage, are at present unable to provide a complete answer. However, much can be accomplished by the continuous introduction of gas during a fumigation, such as is possible if a cylinder-based supply of PH_3 is available. The metered dosing system described here offers the additional advantage of being able to selectively introduce gas to those regions of the bulk where gas losses are highest, thus reducing the chances of localised pest survival.

ACKNOWLEDGEMENTS

This work was supported by grants from the UK Home-Grown Cereals Authority, London. The authors wish to extend their thanks for assistance and technical support to Mr S. Cardwell and Miss N. Savvidou.

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RESISTANCE OF *RHYZOPERTHA DOMINICA* (COLEOPTERA: BOSTRYCHIDAE) TO PHOSPHINE IN THE PHILIPPINES

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ABSTRACT

The study was conducted to assess phosphine (PH_3) resistance of the lesser grain borer, *Rhyzopertha dominica* (F.), in the Philippines and to determine both the PH_3 concentration and exposure time required to control the resistant strains.

Five field strains and one laboratory strain of *R. dominica* were tested using the FAO discriminating dose test for adult insects. Three of the field strains were collected from the National Food Authority (NFA) warehouses in selected sites where PH_3 is commonly used. The sites represented the three major islands in the Philippines, Metro Manila (in Luzon), Cebu City (in the Visayas) and General Santos City (in Mindanao). The other field strains were collected from private warehouses in South Cotabato and Rizal.

High levels of resistance (>400×) were observed in the field strains collected from the NFA warehouses in Cebu City, Metro Manila and General Santos City. In contrast, low levels of resistance were detected in the strain from a private warehouse in South Cotabato (1.13×) and in the laboratory strains (1.65×). The strain from Rizal was still susceptible to PH_3.

The Cebu City strain was found to be the most resistant. It was subjected to a series of concentrations in order to determine both the PH_3 concentration and the minimum exposure period required for effective control. A concentration of 0.89 mg/L for 7 d at 25 ± 2°C provided the desired control. Based on this result, a dosage rate of 2 g/m^3 for a minimum 7-d exposure at 25°C and above is recommended for use in industry.

INTRODUCTION

The lesser grain borer, *Rhyzopertha dominica* (F.), is both the most abundant insect in stored maize and paddy in the Philippines and the most difficult to control (Sayaboc *et al.*, 1990). This can be attributed to both its tolerance of high temperatures and its ability to survive in grains with low moisture content (m.c.). *R. dominica* is known to be resistant not only to organophosphorous compounds such as pirimiphos methyl and malathion (Sayaboc

and Acda, 1990) but also to PH_3 (PH_3) (Gibe *et al.*, 1995). PH_3-resistance in stored-product insects was first reported in the Philippines in the government owned National Food Authority (NFA) warehouses in Metro Manila and Cebu City in 1995 (Gibe *et al.*, 1995). In those warehouses a high level of resistance was detected in *R. dominica* strains. The development of resistance in these insects is attributed to continuously exposing them to repeated inadequate PH_3 treatments. Resistance at low levels was also present in *Sitophilus zeamais*. Other major pests, such as *S. oryzae* and *Tribolium castaneum*, were found to be susceptible to PH_3.

The development of high levels of resistance in *R. dominica* prompted us to test resistance in other locations (in both government and private warehouses) in order to further evaluate resistance levels and determine the PH_3 concentration and exposure period required to control adult insects of tolerant strains.

MATERIALS AND METHODS

Insect collection

Field strains of *R. dominica* were collected by sieving infested grains from the NFA and private warehouses. At NFA, collections were made in areas where fumigation is commonly practiced. These represent the three major islands in the country: Metro Manila (in Luzon), Cebu City (in the Visayas) and General Santos City (in Mindanao). In private warehouses, insects were obtained from South Cotabato and Rizal. The collected insects were reared in the laboratory using the methods of the Entomology Branch of the Queensland Department of Primary Industries (QDPI), Australia. In addition to those collected, fenitrothion-selected *R. dominica*, bred at the NAPHIRE laboratory for more than 10 years, was also used as a test insect.

Resistance test

The strains were tested using the FAO recommended method and concentration (Anon., 1975). PH_3 was generated from a commercial aluminum phosphide formulation and collected over acidified water.

Adult beetles (1–2 weeks old) were confined within glass rings on a filter paper base inside gastight dessicators. The required concentration of PH_3 was injected from a gas-tight syringe through a rubber septum in the dessicator lid. Insects were exposed to a range of four concentrations for 20 h at 25 ± 2°C. After exposure, insects were held in culture medium. Mortality was assessed after 14 d. Percent mortality were later subjected to probit analysis.

The strain with the highest level of resistance among the strains tested was subjected to further tests. In this case, the Cebu strain was the most resistant in the dosage range used. Toxicity tests on adult *R. dominica* aged 1–2 weeks were carried out at 25°C and 70% r.h. in dessicators, according to the FAO method (Anon., 1975). The insects were exposed for 20 h, 3 d and 7 d. Mortality assessments were carried out 14 d after each designated exposure period.

RESULTS AND DISCUSSION

High levels of resistance were detected in strains collected from Cebu City, Metro Manila and General Santos City; the strains from Rizal and South Cotabato provinces, collected in private warehouses, were found to be susceptible (Table 1). In this experiment, the Rizal strain was used as a reference strain.

Table 2 shows the different dosages of PH_3 that provided 100% kill of the highly resistant strains of *R. dominica*. The concentration at which survival was observed is also presented. The Cebu strain had the highest dosage requirement (12.04 mg/L to obtain a

TABLE 1
Response of adult *R. dominica* exposed to PH_3 for 20 h based on mortality assessed after 14 d

Strain	Slope (±SE)	LC_{50} (mg/L) (95% FL)	$LC_{99.9}$ (mg/L) (95% FL)	RF
Cebu City	3.61 (±0.5	2.43 (1.788–2.960)	17.452 (10.857–47.85)	514.81
Metro Manila	2.56 (±0.4)	1.14 (0.763–1.475)	18.458 (10.07–61.41)	544.48
General Santos City	2.15 (±0.3)	0.62 (0.393–0.811)	16.827 (9.997–41.796)	496.37
South Cotabato	5.19 (±0.5)	0.0097 (0.0088–0.011)	0.0384 (0.0314–0.0514)	1.13
Laboratory strain	6.00 (±0.7)	0.017 (0.015–0.019)	0.056 (0.0434–0.086)	1.65
Rizal (susceptible)	4.70 (±0.4)	0.0075 (0.0067–0.0082)	0.0339 (0.0276–0.045)	–

TABLE 2
Dosages allowing survival and giving control of adults of *R. dominica* at 20 h exposure with their corresponding mortality (%)

	Dosage (mg/L)	
Strain	With survival	Complete control
Cebu City	8.0 (92.79)	12.04 (100)
Metro Manila	4.0 (95.00)	8.0 (100)
General Santos City	4.0 (93.33)	8.0 (100)
Laboratory strain	0.03 (92.97)	>0.03
Rizal (susceptible)		0.03 (100)

100% kill) while the Metro Manila and General Santos City strains required 8.0 mg/L. The Cebu strain survived the same dosage with 92.79% mortality.

Mills (1986) recommend a concentration of >0.80 mg/L for a 7-d exposure at 25°C to control highly resistant adults of *R. dominica*. This would give a Ct product of >134.4 mg L/h, which can be considered marginal when compared with the recommended concentration of 150 mg L/h for 7 d at 25°C given by Winks *et al.* (1980). In this study, a concentration of 0.89 mg/L for a 7-d exposure provided the same control (Table 3). Tyler *et al.* (1983) stated that a Ct product of 150 mg L/h for a minimum of 3 d is adequate. This is theoretically acceptable; however, in practice, this may not be appropriate. The tolerance of the egg and pupal stages is considerably greater than that of the larval and adult stages, and it is advantageous to lengthen exposure periods to give time for tolerant stages to develop into less tolerant ones (Winks, 1987).

TABLE 3
Dosages allowing survival and giving control of adults of *R. dominica* (Cebu strain) expressed in % mortality

Exposure at 25°C	Ref. strain	Concentration (mg/L)			
	0.03	0.71	0.89	1.0	1.5
20 h	100	–	–	–	–
3 d	–	–	–	98.33	100
7 d	–	99.12	100	–	–

Winks *et al.* (1980) found a Ct product of 150 mg L/h for at least 7 d sufficient to control all resistant species of insects; Mills (1986), however, found this concentration marginal for the resistant *R. dominica*, which is also true of our results (Table 4). Winks expected a Ct product of 150 mg L/h to be achieved in a well sealed enclosure over 7 d at a dosage rate of 1.5 g/m^3. However, a slight increase in the dosage rate to 2.0 g/m^3 for 7-d exposure is actually required to attain effective control.

TABLE 4
Phosphine concentrations needed to control populations of resistant *R. dominica* at 25°C

Exposure period	Ct (mg L/h)	
	Present work	Mills (1986)
3 d	108	104.4
7 d	149.5	134.4

PH_3 fumigation continues to play a major role in controlling stored-product infestations. It can still achieve effective control provided that adequate gas concentrations can be maintained and that the exposure period is extended so that tolerant stages of the insect can develop into susceptible stages within the exposure period.

The National Food Authority (NFA) has been using PH_3 for the past 30 years and still relies on it. PH_3 is currently applied at 2–3 g/m^3 for a 3-d exposure period. Based on this experiment, no change need be made in the rate. However, the exposure period should be extended to a minimum of 7 d.

ACKNOWLEDGEMENT

The authors would like to thank Dr Mervin Bengston, Chief Entomologist, QDPI, for his valuable technical assistance and suggestions in carrying out this study. Thanks are also extended to the National Food Authority and to private grain handlers in Rizal and South Cotabato for allowing the team to collect insects from their respective warehouses. This study is a part of ACIAR Project 9009 and was funded by the Australian Center for International Agricultural Research (ACIAR).

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THERE IS NO RESISTANCE OF STORED-PRODUCT MOTHS AGAINST TREATMENT WITH CARBON DIOXIDE UNDER HIGH PRESSURE!

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ABSTRACT

Several moth species of the family Pyralidae, such as *Ephestia* spp. and *Plodia interpunctella*, regularly infest various stored products. Warehouse keepers and food factories throughout the world suffer from losses ranging in the billions of DM annually. In addition to direct losses, further financial loss is caused both by claims on the part of consumers, retailers and importers for replacement and by the need to dispose of infested commodities. The current strategies for overcoming this problem include intensive hygienic measures and the treatment of all raw products entering the storage structure. The high turnover of trade and production and, in some cases — such as herbs and spices — the high value of the commodity, require quick disinfestation procedures that do not damage the goods. A recent new approach is treatment with carbon dioxide (CO_2) under high pressure (about 20 bar) over 1–3 h. Nearly all developing stages of insect pests and mites fail to survive such treatment.

Official authorization according to the plant protection law exists in Germany for the use of CO_2 in this type of application. The results presented here deal with the possible build-up of resistance in the eggs of *P. interpunctella*. Twelve generations stemming from eggs of adults surviving this treatment were exposed at the 50% lethal dose (LD_{50}) level. Only a slight, insignificant change in mortality occured during this 9-fold replicated series of experiments.

INTRODUCTION

A very recent innovative, and feasible, approach to the control of stored-product insects by combining chemical and physical methods was developed by Stahl *et al.* (1985a, b). Supercritical carbon dioxide (CO_2) had been previously used for extracting chemical compounds from drugs, so techniques for handling this gas under pressure were already well-developed for routine procedures. The use of combinations of fumigants, both excluding and including CO_2, under pressure, for controlling insects was reviewed by Ferguson and Hawkins (1949). The surprise was that CO_2 had a much more rapid effect than many other gases under increased pressure (Anon., 1989a, b; Gerard *et al.*, 1988a, b and 1990;

Mitsura, 1973; Pohlen *et al.*, 1989; Rau, 1985). Presumably, this is due to its solubility in water or other liquids, which, combined with changes in pH and the lesion of cell membranes during decompression of the treated and infested products, accounts for its rapid lethal effect (Gerard *et al.*, 1988a; Nakakita and Kawashima, 1994; Prozell and Reichmuth, 1991; Reichmuth, 1991). Caliboso *et al.* (1994) and Ulrichs (1994) observed ruptures of cell membranes of *Lasioderma serricorne* after exposure to CO_2 at a high pressure (20 bar) with subsequent rapid depressurisation. Eggs of *Plodia interpunctella* failed to survive treatment at 20 bar for 15 min with subsequent decompression within 1 min (Reichmuth and Wohlgemuth, 1994).

In Germany, the USA and Japan, where steel chambers of up to 30 m^3 are in commercial use, this new technique is generating much interest. Patents for bigger chambers for treatment of bulk products have been registered (Corinth and Reichmuth, 1991).

Some failures to completely control all insects or mites might be explained by either too short an exposure time, especially when treated goods are held at low temperatures, or lack of even and rapid distribution of the CO_2, especially when bulk commodities (such as several kg of palleted dried figs in cardboard cartons) are treated. Prozell addressed this problem at this meeting of the CAF Conference (Prozell *et al.*, 1997).

On the other hand, due to their short life cycles, insects are known to be able to develop resistance to nearly all the toxic substances to which they are exposed. This raises the question of whether unsuccessful treatments can be linked to the development of resistance. This paper reports on work done to determine whether eggs of the Indian meal moth *P. interpunctella*, which are known to be the most tolerant developmental stage to treatment with CO_2 under high pressure (Reichmuth and Wohlgemuth, 1994), develop resistance when exposed for several consecutive generations to treatments that produce 50% mortality (LD_{50} conditions).

MATERIALS AND METHODS

Insects were taken from the stock culture of the Institute for Stored-Product Protection of the Federal Biological Research Centre for Agriculture and Forestry in Berlin, where they had been reared for more than 25 years at constant conditions of 25°C and 65% r.h. under a 16:8 light:dark photoperiod. One-day-old eggs of *P. interpunctella* were collected after exposing several hundred adult moths of both sexes overnight under culture conditions in an oviposition chamber made of 2-mm-aperture steel-wire mesh. Prior to exposure the eggs were aged 14 ± 12 h. Previous experiments (Reichmuth and Wohlgemuth, 1994) showed this age group to be the most tolerant, with LD_{50} achieved in 5 min at 20 bar and 25°C. Table 1 shows the experimental design for one generation of eggs treated for selection.

Four petri dishes (3-cm diameter, 1-cm rim), each containing 100 eggs, were stacked in the fumigation chamber, as described by Reichmuth and Wohlgemuth (1994) and Ulrichs (1994), and treated with CO_2 for 5 min at 20 bar. An additional 100 eggs of the same age from the same generation served as control to establish if hatch occured normally. This experimental set of treated eggs was repeated four times for a total of 1,600 treated eggs and

TABLE 1
Experimental design for one generation of eggs treated for selection

	Number of eggs in each set of replicates			
	Set 1	Set 2	Set 3	Set 4
Replicate 1	100	100	100	100
Replicate 2	100	100	100	100
Replicate 3	100	100	100	100
Replicate 4	100	100	100	100
Untreated	100			

Experiment 1 (four sets repeated nine times) comprises the trials with eggs of the first generation from parents of the unselected stock culture. *P. interpunctella* eggs, 14 ± 12-h-old were exposed for 5 min at 20 bar CO_2.

100 control eggs. After treatment, the four lots of treated eggs and the control eggs from the fifth petri dish which did not undergo pressure and CO_2 treatment were all transferred into small gauze cages with substrate. Subsequently, all were checked for larval hatch. Trials were repeated with a set of 1,700 eggs from each of nine generations. The surviving larvae from untreated eggs were discarded. The surviving larvae from the 5-min treatment were reared to maturity and delivered the eggs for the next set of 1,700 eggs for the experiment on the second generation. After treatment of 11 sequential generations had been carried out in this manner, an additional series of experiments comprising threefold repeated investigations with 100 eggs was carried out for each sample, exposure time and replicate at five different exposure periods (2, 4, 5, 8 and 16 min) to determine precisely if a pronounced tendency to increased tolerance could be observed. A set of eggs from the 12th generation of the unselected stock culture was similarly tested.

RESULTS AND DISCUSSION

In all experiments, the 100 untreated eggs, laid by parents that had been selected, developed normally. There was an average hatch rate of 97%.

The mortality rates of the 9-fold repeated experiments on the eggs after 5-min exposure to 20 bar CO_2 at 25°C are summarised in Table 2, the last column of which shows the averages of the nine replications. Figure 1 shows the average results, including standard deviations, for the 12 subsequently selected generations. The deviations ranged from 5.1 to 9.4%. The average mortality of 30.19%, after 12 successive selections, with an upper limit of +7.39% lies within the lower part of the range of average mortality of the stock culture (40.47 – 7.91%).

An examination of the standard deviations reveals that for the most part the average mortality results can not be distinguished significantly from each other. Over the whole range of 12 generations, after an initial increase there was a slight tendency to decrease in the sensitivity of the eggs to successively repeated CO_2 high-pressure treatment. This

TABLE 2

Mortality results (±SD) of investigations with 11 sequential generations of 1,600 × 9 eggs each of *Plodia interpunctella,* all treated at 25°C with 20-bar CO_2 for 5 min corresponding to LD_{50}

	Percentage mortality of eggs of *P. interpunctella*									
	Replicate									Averages
Generation of eggs	1	2	3	4	5	6	7	8	9	1–9
Experiment 1 (F_1) (eggs of stock culture)	34.9 ±5.9	35.4 ±7.9	39.4 ±10.3	34.4 ±6.9	42.1 ±8.0	42.1 ±8.3	41.2 ±6.0	42.8 ±10.9	51.9 ±7.0	40.47 ±7.91
Experiment 2 (F_2) (LD_{50} survivors of F_1)	49.6 ±10.6	69.6 ±8.2	38.7 ±6.1	54.3 ±8.4	45.7 ±8.5	37.4 ±6.1	42.0 ±10.1	49.4 ±13.5	44.8 ±13.3	47.94 ±9.42
Experiment 3 (F_3) (LD_{50} survivors of F_2)	42.6 ±7.7	40.4 ±7.1	47.9 ±9.8	51.6 ±7.6	39.0 ±9.5	45.9 ±9.5	45.8 ±6.9	35.5 ±6.6	51.9 ±7.7	44.51 ±8.04
Experiment 4 (F_4) (LD_{50} survivors of F_3)	56.8 ±10.8	43.8 ±7.6	46.6 ±7.6	38.8 ±8.9	48.6 ±7.7	57.1 ±11.3	49.6 ±8.2	60.4 ±7.9	48.9 ±5.8	50.07 ±8.42
Experiment 5 (F_5) (LD_{50} survivors of F_4)	41.1 ±6.5	38.0 ±6.4	50.2 ±7.5	40.1 ±7.0	39.4 ±8.0	46.7 ±6.8	40.4 ±7.3	49.6 ±7.9	52.4 ±10.0	44.21 ±7.49
Experiment 6 (F_6) (LD_{50} survivors of F_5)	28.8 ±6.6	37.3 ±7.1	35.4 ±9.7	42.6 ±7.0	38.2 ±9.6	44.9 ±9.9	38.5 ±9.5	43.6 ±8.2	42.9 ±5.3	39.13 ±8.10
Experiment 7 (F_7) (LD_{50} survivors of F_6)	35.3 ±5.4	40.3 ±5.0	34.8 ±10.1	19.4 ±4.3	24.9 ±4.5	21.3 ±4.8	25.9 ±3.0	30.8 ±4.7	20.3 ±4.4	28.11 ±5.13
Experiment 8 (F_8) (LD_{50} survivors of F_7)	36.5 ±7.2	27.4 ±5.9	25.4 ±5.5	30.0 ±6.0	29.0 ±4.2	23.6 ±5.2	30.8 ±4.4	29.6 ±4.0	20.6 ±5.2	28.10 ±5.29
Experiment 9 (F_9) (LD_{50} survivors of F_8)	34.4 ±4.3	29.9 ±6.5	47.5 ±3.9	31.2 ±4.6	31.5 ±5.4	31.0 ±6.6	27.2 ±6.7	32.7 ±8.5	36.8 ±3.8	33.58 ±5.59
Experiment 10 (F_{10}) (LD_{50} survivors of F_9)	32.1 ±4.8	42.1 ±6.1	34.9 ±6.2	39.8 ±7.2	38.7 ±5.6	36.5 ±6.7	31.3 ±5.0	31.7 ±4.1	33.6 ±7.3	35.63 ±5.89
Experiment 11(F_{11}) (LD_{50} survivors of F_{10})	25.4 ±3.2	39.4 ±4.3	27.4 ±9.3	31.3 ±5.1	48.1 ±7.0	41.3 ±8.1	29.4 ±6.3	44.4 ±6.8	48.7 ±4.9	37.27 ±6.11
Experiment 12 (F_{12}) (LD_{50} survivors of F_{11})	27.6 ±8.2	25.2 ±6.4	26.2 ±5.2	36.4 ±9.8	39.9 ±9.4	31.0 ±11.4	26.6 ±5.7	26.4 ±5.6	32.4 ±3.7	30.19 ±7.27

[1]After 5-min exposure to 20 bar CO_2 at 25°C.

tendency fluctuated during the process of selection and is therefore not enough to reach the conclusion that there was a quick build-up of resistance. It would be of interest to check further selection pressure and investigate the stability of the increased tolerance without further selection. From the results of the first three selected generations it can be concluded that the chosen sublethal conditions of 5 min at 20 bar of CO_2 were slightly below the real LD_{50}. Figure 2 supports this statement. After 12 selections the eggs respond

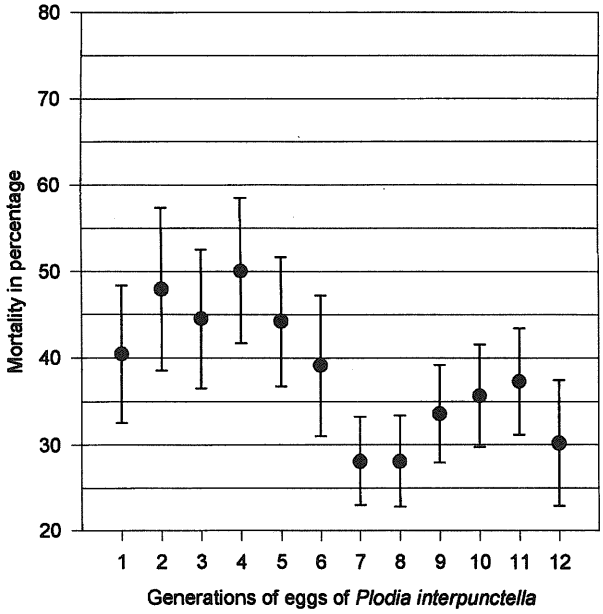


Fig. 1. Repeated selection using 14,400 eggs per generation of *Plodia interpunctella* with CO_2 at 20 bar at 25°C for 5 min treatment (vertical bars are SD values).

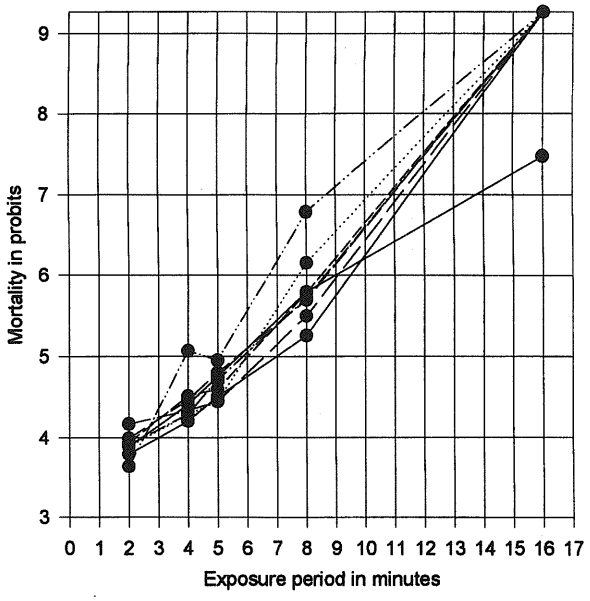


Fig. 2. Dose mortality response to CO_2 at 20 bar at 25°C of 13,500 eggs of the 12th generation of selections of *Plodia interpunctella.* n = 9.

to treatment with CO_2 at 20 bar in a similar manner as does the stock culture. The LT_{50} (probit 5) at about 6 min is slightly longer than, but still very close to, the 5 min of the stock culture. Standard deviations are not included in this graph.

These findings lead to the conclusion that the use of high pressure CO_2 treatment to control stored-product pests is not endangered by the threat of quick insect-resistance build-up. Where eggs of stored-product pests are exposed for several generations to sublethal conditions, subsequent treatment at normally lethal conditions still causes complete mortality.

ACKNOWLEDGEMENT

My assistant, Mrs Agnes Paul, is hereby thanked for her excellence, patience and accuracy in doing tedious laboratory work.

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THE CURRENT STATUS OF METHYL BROMIDE AND PHOSPHINE FUMIGATION IN POLAND

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ABSTRACT

Insects and mites are common pests of stored products in Poland. They not only cause economic loss of agricultural commodities in storage but, due to quarantine, their presence restricts the export of grain, dried fruits, mushrooms, vegetables and other products. These losses and restrictions are eased by the application of fumigation treatments. Only two fumigants are registered in Poland: methyl bromide (MB) and phosphine (PH_3). Methyl bromide (METABROM 98; METHYL BROMIDE) is used widely as a fumigant for soils, commodities and structures. However, MB is an effective ozone-depletor and will be banned in Poland at some future date. PH_3 will continue to play a major role in the fumigation of agricultural commodities, and Poland is becoming increasingly dependent on this fumigant for stored-product pest control. The following PH_3-releasing products are now registered in Poland: PHOSTOXIN (tablets), QUICKPHOS (pellets), DELICIA-BEUTEL (bags), DELICIA-GASTOXIN (tablets) and DEGESCH PLATES or STRIPS. For PH_3 to remain available as a fumigant, it is essential that any risk, such as PH_3-resistance in pests, that could lead to its failure be identified and eliminated. Therefore, a research program has been undertaken to detect and monitor PH_3-resistance in Poland. The information obtained will be used to predict the future impact of resistance on storage industries and to develop tactics and strategies aimed at managing or delaying the development of resistance.

INTRODUCTION

Insects and mites are common pests of stored agricultural products in Poland. In some years, the acarid mites (Acaridae) alone are responsible for losses as high as 400,000 t of stored grain (Golebiowska and Nawrot, 1976). In general, 5–10% of stored commodities are destroyed by pests each year (Boczek and Ignatowicz, 1980). Stored-product pests not only cause economic loss of agricultural commodities in storage, but, due to quarantine,

their presence also restricts the export marketing of grain, dried fruits and vegetables and other agricultural products. These losses and restrictions are eased in Poland by the use of fumigation treatments with toxic gases.

All fumigants, regardless of formulation, are highly toxic and hazardous to use, and in Poland they are classified as restricted-use pesticides (Class I toxicity). A special certification, granted by the State Inspection of Plant Protection, is required from purchasers before these fumigants can be sold to them. Thus, the fumigants are only available to persons who have been certified and trained in their use (Monitor Polski, 1965). This training is periodically updated to ensure the qualifications of the person conducting a fumigation.

There are several firms and companies certified to conduct fumigation treatments of stored products in Poland, and the following firms belong to the largest fumigation companies: Agropest (Lodz), Solfum Ltd. (Lodz), Agrochemikal-Pest Ltd. (Bydgoszcz), Zaklad Zwalczania Szkodnikow Zywnosci (Wroclaw) and Straz Portowa (Szczecin). The Agropest company, governmentally owned since March 31, 1950, has branches and cooperating works in several regions (Bialystok, Lodz, Warszawa, Krakow, Katowice, Rzeszow, Poznan, Gdynia, Radom and Klutajny near Olsztyn), and its activity covers all Polish territory. Sulfum Ltd., a private company, has branches in Opole, Gdansk and Stargard Szczecinski. In the 1990's, there emerged many small private companies which only use spraying with contact insecticides for controlling insect pests in apartments and houses.

The granary weevil *(Sitophilus granarius)*, rice weevil (*S. oryzae)*, flour beetles *(Tribolium* spp.*)*, the Mediterranean flour moth *(Ephestia kühniella)* and the acarid mites (Acaroidea) are the pests most often controlled with fumigants. The following stored products are most often fumigated: grain, processed food products, animal feed, seed for sowing, tobacco and coffee beans. 100,000 to 200,000 t/year of grain as well as structures are for the most part fumigated by Agropest, which uses approximately 3 t of metal phosphides and 60 t of MB.

Fumigation is not always practical in Poland. Grain stored in poorly sealed wooden buildings is covered with special gastight sheets. This procedure is very expensive and time-consuming. The increased amount of fumigant required and the poor control often achieved make this practice cost-prohibitive. Poor control often results in rapid resurgence of infestation which becomes just as large and damaging as before the fumigation. Therefore, small farmers, whose small, primitive storages are improperly sealed, are rarely clients of fumigation companies. One solution to this problem might be cooperatively owned and managed storages with drying facilities, but this solution is not acceptable to Polish farmers because of their bad experiences with the so-called cooperatives forced on them by the former Communist government (see also Sinha *et al.*, 1991).

Effective fumigation is possible in large grain-storage premises with good storage practices. Such storages belong to the central handling system (silos) or to flour mills, tobacco works and food processing factories.

PAST FUMIGANT USAGE IN POLAND

After World War II, the following fumigants were used or tried in Poland: hydrogen cyanide, ethylene oxide, ethylene dibromide, ethylene dichloride and carbon tetrachloride.

In the 1950s, CARTOX (imported from West Germany) which contained 10% ethylene oxide and 90% carbon dioxide was used widely, but in 1956 it was superceded by ROTANOX, which was produced in Poland. ROTANOX was used for fumigation of structures, grain in silos, grain products in flour mills, food storages, food processing factories and, ultimately, even for desinfestation of medicinal herbs and food products in both Mallet fumigation chambers and flat storages. However, ethylene oxide was determined to be an effective carcinogenic agent and was therefore banned in Poland, in 1987, for treatment of food products. Ethylene oxide is still used in limited amounts for the fumigation of museum collections (books, carpets and wooden exhibits) and the sterilization of medical supplies (syringes and needles).

Ethylene dichloride was used only for fumigating the underfloor space of storages where other fumigants and contact insecticides were either not effective or it was impossible to apply them. No more than 10 t of ethylene dichloride was used each year. This fumigant was finally replaced by contact insecticides (DDVP) in 1982/1983.

Hydrogen cyanide was once imported from Czechoslovakia as CYJANOFUM. It consisted of calcium cyanide packed in metal tins which on reaction with water produced hydrogen cyanide. Several tons of CYJANOFUM were used yearly. In the 1960's/1970's, CYCLON was imported from West Germany in the form of filter paper imbued with liquid hydrogen cyanide which, after opening the tins in a store, quickly changed into gas form. Nine t/year of hydrogen cyanide was used for fumigation of structures and empty storages, for desinfestation of seed materials and for rodent control. It was banned in Poland in 1978–1979 both because of a possible threat to fumigators and for psychological reasons.

In the 1960's and 1970's, a wide range of gases was used in fumigation for stored-product protection in Poland (Table 1). However, in past decades there have been many pressures on particular chemicals, ranging from development of resistance by the target pests and carcinogenic concerns to increasing demands for reregistration to maintain use.

TABLE 1

List of gas chemicals labelled for stored-product protection in Poland at the indicated years

Years	Gases used as fumigants for stored products in Poland[1]
1960's	hydrogen cyanide, ethylene dichloride, ethylene oxide, MB, PH_3
1970's	hydrogen cyanide (1978–1979), ethylene dichloride, ethylene oxide, MB, PH_3
1980's	ethylene dichloride (1982–1983), ethylene oxide (1987), MB, PH_3
1990's	MB, PH_3
2000's	MB (?), PH_3

[1]Years given in the parentheses denote when fumigating stored products with a particular gas was banned.

As a result, fumigation is becoming threatened. Now only two fumigants remain in widespread use — phosphine (PH_3) and methyl bromide (MB) (Table 2). Even these remaining materials are under pressure because of carcinogenic concerns and ozone depletion (Alavanja *et al.*, 1990; Garry *et al.*, 1989; Garry *et al.*, 1990; Ignatowicz, 1993).

TABLE 2
Fumigants registered for fumigation of stored agricultural products in Poland (February 8, 1995)

Fumigant preparation	Dosage of preparation	Commodity; remarks
MB	according to instructions	grain, food products, animal feed, medicinal herbs,
METABROM 98	30–50 g/t	fabric bags; not for sowing materials or brewery
	30–50 g/m^3	barley;
	20 g/m^3	grain, food products, animal feed; dried fruits and
	50–100 g/m^3	vegetables, medicinal herbs, food products; empty storages; fabric bags in gas chambers.
DEGESCH PLATES/	1–3 plates/30 m^3	grain and mill products, animal feed containing
STRIPS	1–3 strips/600 m^3	high levels of proteins and lipids; in storages, silos
	1–3 plates/15 t	and on ships.
	1–3 strips/300 t	
DELICIA-BEUTEL	2 bags/t	grain in flat storages; grain on ships.
	1.7 bag/t	
DELICIA-GASTOXIN	4–6 tablets/t	grain and raw materials for animal feed in silos;
	4–6 tablets/m^3	grain, sowing materials, packed mill products,
	4–10 tablets/t	animal feed, raw materials for animal feed in flat storages; on ships.
PHOSTOXIN	4–6 tablets/t	grain and raw materials for animal feed in silos;
	4–6 tablets/m^3	grain, sowing materials, packed mill products,
	4–10 tablets/t	animal feed, raw materials for animal feed in flat storages; on ships.
QUICKPHOS	20–30 pellets/t	grain and raw materials for animal feed in silos;
	20–30 pellets/m^3	grain, sowing materials, packed mill products,
	20–50 pellets/t	animal feed, raw materials for animal feed in flat storages; on ships.

METHYL BROMIDE AND PHOSPHINE USES IN POLAND

MB is used in Poland as a fumigant for soils, commodities and structures. It is labelled for disinfestation of grain, food products, animal feed, medicinal herbs and empty fabric bags, but not for seed materials and malting barley. It is currently vital for the economic viability of certain agricultural markets and for quarantine treatment of products involved in

international trade. MB was introduced in Poland in 1964. However, long before its registration in Poland, the first field trials with MB on grain were conducted to determine and confirm its effective doses and exposure times. Initially, the use of MB was restricted to fumigation of imported grain and other agricultural products at the ports of entry. Later, its application was extended to treatment of grain and mill products, packagings (empty bags), structures, food product works and empty storages. Since 1989, it has been labelled for fumigation of medicinal herbs and soils in greenhouses and open fields.

At present, MB is successfully used by most of the fumigation companies. Up to 220 t (170 t for fumigation of structures and agricultural products and 50 t for soil fumigation) has been used each year in Poland. The Agropest company uses it for disinfestation of stored agricultural products (8–15% of tonnage) and structures (85–92% of tonnage). Solfum Ltd uses it for soil fumigation (60% of tonnage) and disinfestation of stored and transported agricultural products (40% of tonnage).

By all accounts, MB has been a very cost effective and beneficial fumigant. However, about half of the MB used as a fumigant is released into the atmosphere where it reacts with stratospheric ozone. MB has been classified as an effective ozone depletor, and because of this it will be banned at some future date (Watson, 1992; Ignatowicz, 1993). At the most recent Montreal Convention held in Vienna (1995), the developed countries decided on the following MB phase-down schedule: 25% by 2001, 50% by 2005 and 100% by 2010. The group of developing countries agreed to freeze MB use by 2002; after that year they will be limited to the 1995–1998 average yearly tonnage (ca. 220 t in Poland).

Metal phosphides will continue to play a major role in the fumigation of commodities in Poland. DELICIA (imported from East Germany) was the first preparation used in Poland which contained calcium phosphide as its active ingredient. It was packed in white paper bags within sealed metal tins containing a moisture sorbent. Before being placed in the grain, calcium phosphide was transferred into green paper bags which easily absorbed water from the surroundings. DELICIA was used only for fumigation of grain. In the 1960's, GASTOXIN was imported from East Germany in the form of tablets of aluminium phosphide (56–57%) packed in cylindrical aluminium tubes (20 tablets in a tube); there were 16 tubes in each metal tin. In the 1970's, fumigation treatments with GASTOXIN were extended to agricultural food products and seeds, and it was also used for the control of rodents. About 10 t/year of GASTOXIN were used.

At the same time, the assortment of metal phosphides was enlarged by the addition of PHOSTOXIN pellets and tablets, and later further enlarged by the addition of MAGTOXIN (a.i. magnesium phosphide). In the 1980's, another phosphide pesticide, QUICKPHOS, a product of India, was also registered. In the 1990's, two products of Detia Degesch (Laudenbach, Germany) — DEGESCH PLATES and DEGESCH STRIPS — were introduced. They contain magnesium phosphide (56%) incorporated into a plastic polyethylene matrix pressed out in the form of a flat plate and then packed in aluminium foil (Table 2).

Globally, in recent years, PH_3 usage has increased considerably to more than 4,060 t/year. It has been estimated that 50% of all the grain treated with pesticides is fumigated with PH_3; 15% is still fumigated with MB; and 35% is protected with chemical

insecticides (protectants) (Mueller, 1992). Only 60% of all metal phosphides in the United States are used for fumigation of bulk grain, whereas in Poland more than 85% of metal phosphides are used for this purpose. In 1995 the Agropest company used 60 t of MB for structures and stored products and 3 t of metal phosphides for grain fumigation. Solfum Ltd. used 110 t of MB for fumigation of soil, stored products and ships and 2 t of metal phosphides for fumigation of agricultural products.

DETECTION, MONITORING AND MANAGEMENT OF PH_3-RESISTANT STRAINS OF STORED-PRODUCT PESTS IN POLAND

Poland and other countries are becoming increasingly dependent on PH_3 for stored-product pest control, both on-farm and in the central handling systems. PH_3 has many benefits over other fumigants, including ease of use, good penetration and low cost. There are few alternatives which have the same advantages, particularly with the inevitable withdrawal of MB owing to its ozone-depleting properties. In order for PH_3 to remain available to the industry as a fumigant, it is essential that any risk that could lead to its removal be identified and eliminated.

Entomologists involved with storage are now concerned about the potential of stored-product pests to develop PH_3 resistance. The FAO Global Survey of Pesticide Susceptibility indicated the potential for a PH_3-resistance problem and considered that assessment of the status of PH_3 resistance in stored-product pests should be classed as most urgent (Champ and Dyte, 1976). Then, in the 1980's and 1990's, resistance to PH_3 was detected in several species of stored-grain pest insects in many countries (Subramanyam and Hagstrum, 1996).

Several surveys of PH_3 resistance have been performed in the US, the UK, Canada, Australia, India, the Philippines, Indonesia and Brasil (Subramanyam and Hagstrum, 1996). However, no studies have yet been initiated to monitor PH_3 resistance in stored-product pests in Poland. Therefore, the USDA-Warsaw Agricultural University research program on detection, monitoring and management of PH_3-resistant strains of stored-product pests has been implementated in Poland.

The initial stage of the program will involve studies on baseline responses of stored-product pests in this country to PH_3. Laboratory strains and field populations of stored-product pests will be tested with a range of concentrations of PH_3. Concentrations that could be used with a 20-h exposure to discriminate between susceptible and resistant individuals, based on these tests, will be determined to facilitate separation of susceptible and resistant individuals in later work. Other concentrations will be determined to discriminate between the detected level of resistance and possible higher levels of resistance.

As result of this work, susceptible reference strains will be maintained, bioassay methods for rapid diagnosis of resistance will be adopted, and resistant strains from field samples will be established. These will allow the full potential of resistance to be studied and also provide material for genetical examination. Information obtained at this stage will be used in surveys on PH_3 resistance in field strains of pests.

One threat to the grain industry is delivery to the central handling system of PH_3-resistant strains of pests, which may develop in farm storages because of fumigations in unsealed and poorly sealed storages. It has been shown that storages in this condition are unable to meet the exposure periods required for effective control. There is much evidence to suggest that the development of PH_3 resistance is associated with poor fumigation techniques (Price, 1986; Tyler *et al.*, 1983). Inefficient use of sealed silos rather than fumigation in unsealed silos, however, poses a greater threat through facilitating the development of higher levels of resistance (Emery, 1994).

In order to accurately determine the extent and level of PH_3 resistance in grain pests in the central handling system's storages and on farms in Poland, a random survey will be made. The farm survey will determine the extent of PH_3 resistance in Poland and facilitate the establishment of a resistance management plan which can be used to limit the development and spread of PH_3-resistant strains of grain pests.

PH_3 fumigation is a treatment commonly used to control pest infestations in stored commodities in Poland. However, control failures have been reported with increasing frequency and now comprise about 0.5% of all treatments. Control failures following fumigation may not always be caused by resistance. In many cases where survivors are found following treatment, the reason is improperly done fumigation, as when structures are inadequately sealed between the silo wall and the roof. The other causes of control failure include high humidity of the stored product resulting in poor gas distribution, a late decision for fumigation and low temperatures during winter treatments. The acarid mites (at hypopus stage) are relatively resistant to PH_3, and often recommended doses fail to control them. However, the frequency of existing resistance mechanisms in the pest population increases concomitantly with increasing use of PH_3 fumigation. Some grain handlers therefore probably no longer use PH_3 because it does not seem to work.

Following a properly conducted fumigation, control failures due to resistance depend upon both the presence of genes which confer resistance and the degree to which resistance is conferred by those genes. Zettler (1991) stated that the percentage of survivors of the discriminating dosage tests reflects only the frequency of genes for resistance in that population, telling nothing at all about the level of the population's resistance. Genes which confer resistance may occur frequently in a population without causing control failures. Thus, a strain with a high frequency of resistance might still be susceptible to normal treatments with the pesticide to which its genes confer resistance. Conversely, a strain with a low frequency of resistant individuals could be difficult to control if the few genes for resistance conferred extremely high levels of resistance. Thus, while the laboratory discriminating dose test is beneficial in detecting the early development of resistance, it plays little or no role in predicting control failures in the field.

It would be extremely useful to correlate control failures with a simple diagnostic assay for resistance. More studies are needed to evaluate not only the results of the discriminating dose tests but also such other factors as the level or intensity of resistance in a particular population. The results may explain the cause of the control failures with PH_3; they will certainly help in making pest control decisions.

The last stage of this study will be to establish the cross-resistance characteristics of resistance. This will involve selecting the resistant population in the laboratory with the failing chemical (PH_3) until all (or most) individuals in the population are homozygous for the resistance mechanism. Such selection is necessary because new resistances can be associated with a loss of fitness so that resistance levels may be lost during culturing, selection of the resistant population allows the full potential of the resistance to be measured, and selection provides the material needed for more detailed genetic studies (Collins, 1990).

The response of the resistant strain to a wide range of chemicals, including new fumigants such as carbonyl sulphide and protectants registered for use on grain, such as ACTELLIC 500 EC or K-OBIOL 25 EC, will then be tested. The results will show which insecticides are jeopardised by resistance and which are not; they will also provide a reasonable indication of the field doses necessary both to kill adults and to prevent the development of progeny (i.e. if any cross-resistance is provoked by PH_3-resistance).

Results of this project are expected to provide immediate benefit to Polish pest-management programs, none of which presently incorporate resistance management. Thus, pest control efficiency should increase immediately and, with resistance management practices in place, improved control efficiency will be maintained in the future despite increasing levels of resistance. Finally, the development of pest management programs in relation to worldwide resistance will have a positive influence on grain exports/imports.

ACKNOWLEDGEMENTS

The paper was prepared with the financial support of the US–Poland Maria Sklodowska-Curie Joint Fund II (Project MR/USDA-96-235). The authors acknowledge this financial support.

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SESSION 9

QUARANTINE AND REGULATORY ISSUES PERTAINING TO THE USE OF CONTROLLED ATMOSPHERES AND/OR FUMIGATION

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Valérie Ducom, *France*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 539–546

A NEW APPROACH TO ADOPTION OF POTENTIAL ALTERNATIVE QUARANTINE TREATMENTS WORLD-WIDE

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ABSTRACT

The recent GATT agreement concerning the way plants and plant products are traded is expected to lead to more transparent and scientifically justified quarantine regulations on a worldwide basis. Following the guidelines set by the FAO for regulating quarantine specifications and entry conditions, many countries are now starting to develop justifiable Import Standards. These must be based on the principles of Pest-Risk Analysis, a three-stage process involving the production and categorisation of pest lists, scientific and realistic assessment of pest risk from both economic and biological viewpoints and development of transparent risk management requirements. New treatments and technologies arise from the need to satisfy risk management requirements. Whether the implementation of new technologies is delayed by the actions of quarantine agencies depends on the quality of scientific research and the clarity with which the justification for new treatments is presented. This paper illustrates the new challenges to trade in stored products by describing the approach being taken by New Zealand. In response to the FAO initiatives, New Zealand has mandated standards for the import of fresh produce, nursery stock, grain, seed for sowing and processed products, and standards for Pest-Risk Analysis (PRA) as well. While PRA for items of fresh produce and nursery stock is well advanced, there are still many problems to overcome in applying PRA principles to the import of stored products.

INTRODUCTION

There is increasing awareness that all quarantine decisions which affect the movement of plants and plant products among countries must be based on sound scientific advice and, therefore, be fully justifiable and transparent. The objective of this approach is to prevent quarantine regulations from being used as barriers to trade. Importing countries should state quite clearly the pests and diseases they wish to exclude ("quarantine pests"), publish specifications which reflect their tolerance to the listed pests and propose scientifically

justifiable risk-management options (entry conditions). The exporting country has the responsibility of responding to the specifications by informing the importing party what systems and/or treatments will be applied to the product to ensure that the specifications are met. Actions must also meet the criteria of openness and full scientific justification. Quarantine authorities should be prepared to consider the principle of equivalence (i.e. that there may be several treatments of equivalent merit) and not adopt a dogmatic approach to risk management.

In 1995 the Food and Agriculture Organisation of the United Nations produced draft standards as guidelines for the regulation of quarantine specifications and entry conditions (FAO, 1995). In response to this initiative, many countries, including New Zealand, are now starting to develop justifiable Import Standards for the full range of plants and plant products. These must be based on the principles of Pest-Risk Analysis (PRA) which demand the production and categorisation of pest lists, the realistic scientific assessment of pest risk from both the economic and biological viewpoint and the development of transparent risk management options. While the initial thrust is to apply the new methods to the trade in fresh produce, it is inevitable that they will also be applied to the movement of all forms of plant products, including such stored products as grain, seeds and food items. Whether the implementation of these new technologies is delayed by the actions of quarantine agencies will depend on the quality of scientific research and the clarity with which the justification for new treatments is presented. This paper explores the new challenges to the trade in stored products by describing the approach being taken by New Zealand in response to the FAO initiatives.

DEVELOPMENT OF STANDARDS

Quarantine authorities have always imposed conditions, usually in the form of government regulations simply stating the required entry conditions, on the import of plant products. How these were derived was not usually disclosed, and they were based on subjective perceptions of pest risk rather than on formal assessments. Entry conditions ranged from one extreme (no treatment or inspection at all) to the other (total prohibition). Once the need to justify entry conditions was realised, it became evident that full documentation in the form of a standard would be required. At the same time it was seen that, if entry conditions were to be scientifically justified, there would be a need to declare a quantifiable tolerance based on as objective an assessment of risk as was possible. The idea of so-called zero tolerance, leading to the prohibition of certain products, also became untenable in the new atmosphere of trade's being allowed to proceed without artificial "quarantine" barriers.

The New Zealand Ministry of Agriculture Regulatory Authority has progressed along this path, formally declaring tolerances (specifications) for pests and including them in standards for all forms of plant and plant-product imports. This, one of the main differences between the old and the new systems, was made possible by the parallel development of a quantified pest-risk analysis system.

DEVELOPMENT OF PEST-RISK ANALYSIS

New Zealand is a small isolated island nation which has managed to avoid many of the world's more serious pests. There was concern in the 1980's that New Zealand was becoming more vulnerable to pest invasion because of both rapidly increasing plant-product imports and the growth in tourism. This led scientists to question the inspection systems then in place and to explore methods of quantifying the risk of importing hosts of serious pests. The work was started with fruit flies because New Zealand is the only major fruit-producing country that has remained free of important economically deleterious fruit-fly species. The concept of a maximum pest limit (*MPL*) (as a means of setting a realistic limit — or tolerance — to the number of pests on imported produce) was developed as an alternative to an overly restrictive import policy (Baker *et al.*, 1990). This led to the development of a Pest-Risk Analysis Standard using the *MPL* as a specification (Baker *et al.*, 1993). Improvements to the first model have just been published and incorporated into a revised standard (MAFRA, 1995; Whyte *et al.*, 1996). Using the two PRA standards, scientists at the New Zealand Plant Protection Centre have processed 40 pest-risk assessments to date, expanding the concept beyond fruit flies to include other insects, plant pathogens, weed seeds and mites. The most recent assessment was of a serious pest of stored seeds and grain, *Trogoderma variabile.*

A PRA entails three distinct requirements for any product being considered for import: production of a pest list, some form of pest-risk assessment leading to a specification and formulation of risk management options.

Pests are classified as quarantine or non-quarantine according to the FAO (1995) definition of a quarantine pest ("A pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled"). The pest list clearly informs the exporter which pests are to be excluded from the product (quarantine pests) and which will be allowed in with the product (non-quarantine pests). It does not, however, indicate the level of tolerance which the importing country is willing to accord each pest. This can only be done by completing a pest-risk assessment. Two levels of pest-risk assessment are recognised: simple and full. Clearly, a fairly simple assessment is required for the majority of quarantine pests because of the vast number of them associated with some products. The compromise is to declare a general level of tolerance to the majority of quarantine pests and perform a full assessment only on those likely to cause serious problems. In New Zealand, for example, a general specification for quarantine pests is set at 0.5%, i.e. pest infestations up to the level of 0.5% are tolerated for most pests in imported produce. A full pest-risk assessment can be carried out and a different specification arrived at for the serious pests. Once specifications are known, risk management options allowing import but excluding pests, within an accepted level of risk, can be calculated. Naturally, there is no such thing as a zero risk unless all trade and travel is banned completely; quarantine authorities must therefore be aware of the risks they are prepared to accept with any import system.

A full pest-risk assessment consists of a biological assessment plus an economic impact assessment. The purpose of the biological assessment is to determine the probability that an infested host would result in the establishment of a pest population. This is known as the probability of introduction (Φ) and is a function of a number of conditions which must be fulfilled if the entry and establishment of a pest would be successful. The reciprocal of the probability of introduction, $1/\Phi$, denotes a threshold for establishment in terms of the number of infested units leading, on average, to a single establishment. The derivation of Φ is obtained by considering the factors which affect the number of surviving pests from an infested host as well as the factors affecting whether conditions will be suitable for survival. The former conditions refer to the survival of individuals, and the latter to abiotic factors. The calculation of Φ also depends on the mode of reproduction of the pest organism. A full explanation of the use of Φ, with derivation of the formulae used in its calculation, is given by Whyte *et al.* (1996). Once Φ is known, the *MPL* is readily calculated and transformed into an import specification. The specification for import is $M_s = MPL \times 100\%$.

RESPONSE TO SPECIFICATIONS

A specification is declared so that justifiable risk management options can be decided on. The simplest form of risk management is inspection of the product on arrival, and this is appropriate for pests having a *MPL* of 0.005, provided the pest is easily detected. In this case the specification would be declared as an infestation level not exceeding 0.5%, and the risk management would consist of an inspection of 600 items of the product (units) with an acceptance number of 0. This is considered the limit of normal inspection procedures as inspecting larger numbers of units is extremely time consuming and, therefore, expensive. For pests where the specification is less than 0.5%, some form of offshore treatment is the most appropriate form of risk management. This may take the form of production through a quality system, selection of a non-host cultivar, a declaration of area freedom, an intensive inspection during plant production or a treatment such as fumigation, pesticides, heat, cold or irradiation. If the *MPL* is extremely low (e.g. M_s around $1 \times 10^{-3}\%$, as it is for some fruit fly species) often a combination of risk management options is required.

To avoid costly delays and the potential loss of exported products, it is essential that the exporting country develop treatments, in consultation with the regulatory organisation, in response to the specifications. This will ensure that any newly developed treatment or technology is at the level appropriate to satisfy the quarantine security of the importing country. In the past many treatments were developed at inappropriately high levels of efficacy, often made to satisfy a perceived need for efficacy levels around probit 9, an overkill in most instances, which involved vast, expensive trials using 100,000 or so test insects. With a set specification, scientists are able to calculate the specific level of efficacy required and, consequently, the number of test subjects needed to satisfy the quarantine authorities. It is also important that the research scientists conduct their experiments in a fully transparent manner, using documented procedures and

showing sources and estimates of variability as well as manifesting the willingness to declare "raw" results if required. While most of the New Zealand work on quarantine security has involved fruit flies, the principles used can be applied to other pests and in other situations. The concepts which are the basis of New Zealand's quarantine security using quantified systems are based on the publications of Baker *et al.*, 1990; Cowley *et al.*, 1991; Harte *et al.*, 1992; Cowley *et al.*, 1992; Cowley *et al.*, 1993; Baker *et al.*, 1993; Harte *et al.*, 1995 and Whyte *et al.*, 1996.

APPLICATION TO STORED-PRODUCT PESTS

Although the principles of PRA apply to all forms of imports, progress with implementation has been easier with fresh produce than with some other products. This is because the basic item, the "unit", is readily defined for most forms of fresh produce as a single fruit or vegetable. It is also assumed that a pest establishment must come from a single infested unit, as the probability that several infested units will be disposed of together is generally considered extremely small for fresh produce. The unit is important in the calculation of the *MPL* as the total number of units imported has a bearing on the chances of pest establishment. The more often a product is imported (i.e. the greater the number of units) the greater the chance of infested items also being imported. This is clearly shown in the formula used to calculate the *MPL*:

$$MPL = 1 - \sqrt[Nm]{\frac{1 - A_R}{\Phi}}$$

where N = number of units per consignment; m = number of consignments per year; A_R = maximum acceptable risk; Φ = the probability of introduction.

Defining the number of units expected for any product is therefore crucial for calculating the *MPL* and hence the specification. For stored products a number of different definitions of "unit" are possible. With pathogens it is quite feasible to consider a single seed as a unit because one infected seed could produce sufficient spores to establish a disease. A typical *MPL* calculation for a seed-borne pathogen is illustrated by the case of *Ustilago zeae* (NZPPC, 1995). Here *Nm* was calculated, from knowledge of past import levels, as 6×10^8 units per year and Φ, from a detailed study of many biological and abiotic factors, as 2×10^{-4}. If $A_R = 0.05$ (a level of risk frequently used), then the specification ($M_s = MPL \times 100\%$) of $4 \times 10^{-5}\%$ is obtained. A specification of such low magnitude indicates a high-risk pest for which quite stringent quarantine measures are justified. It would be difficult to prove in this instance that a treatment had the required efficacy (which, in this case, must equal the *MPL*) to reduce the level of disease to the required level. However, a combination of treatments could be shown to reduce the level of infection below the value of the *MPL*. Alternatively, a declaration by the exporting state or country of freedom from the pest is likely to be acceptable as a risk management option. Similar results have been obtained with other seed borne pathogens such as

Clavibacter michiganensis subsp. *nebraskensis* (Goss's wilt on maize) and *Tilletia controversa* (dwarf bunt on wheat).

An attempt to calculate the *MPL* for *Trogoderma variabile* (warehouse beetle) has highlighted some of the problems encountered with stored-product insect pests. This insect is a common pest of seeds and grains but may also be sustained by such high protein sources as dead animals and food items. Thus, while the biological characteristics and the abiotic factors leading to establishment can be readily assembled to provide a calculation of Φ, the debate over the correct meaning of the "unit" for this case study continues. It is probable that the issue is similar for most stored-product insect pests because they roam freely over many host items instead of being associated with a single entity such as one seed or fruit. It is quite likely that for items exported in packets or sacks the single packet or sack constitutes the unit. However, for products exported in bulk it is more practical to consider either the entire consignment or one heap of produce on a warehouse floor as a unit. At present this issue is side-stepped by taking a given weight of produce as a unit. At present, for example, the *MPL* for quarantine insect pests in imported grain is listed as 0.9 per kg (i.e. less than 5 per 5 kg, the standard quantity used for sampling). However, this definition of a unit implies that the pests are confined to individual 1- or 5-kg lots, whereas in reality they are free to roam from one lot to another.

Another problem characteristic of stored-product consignments is the nature of pest-distribution within the product. With fresh produce it is usually safe to assume that distribution is relatively homogeneous since all components of a single consignment will have been through a uniform system of grading and packaging. Whyte *et al.* (1995), however, showed that this was certainly not the case with the distribution of weed seeds, which showed significant heterogeneity, within grain shipments. It is likely that the distribution of insects in bulk shipments also shows heterogeneity. The demonstration of heterogeneity would result in more stringent rules for inspection of products for quarantine pests. For quarantine weed seeds in grain shipments to New Zealand, for example, a maximum limit of three per kg has been set. Based on a random (Poisson) distribution, finding eight or fewer quarantine seeds in a 5 kg sample would give 95% confidence that the mean is less than the *MPL*. However, because the distribution is clumped rather than random, it was necessary to suggest that a lower acceptance number of two seeds in 5 kg was more appropriate. It is important to note that the *MPL* was not changed; the acceptance number needed to ensure compliance, however, was tightened significantly.

DISCUSSION

This paper has illustrated how, in the future, quarantine authorities may set specifications for all plant and plant-product imports, including stored products. Using the PRA system would prevent arbitrary decisions about risk management leading to misunderstanding the intent of the quarantine regulations. It should also ensure that both importers and exporters clearly understand what the requirements are and are assured that they are justified on scientific grounds. Some fundamental problems remain to be solved before the PRA system

described here can be fully applied to stored-product pests. In particular, a "unit" must be defined, and the problem of uneven distribution of pests within a shipment must be solved. These are the issues needing attention.

With the eventual phasing out of methyl bromide as a standard fumigation treatment for many products, there will be increasing research into new treatments and technologies. It is vital that such new research take place in consultation with quarantine authorities in order to ensure that treatment efficacies adequately meet their needs. This would not only guarantee that the new treatments are optimal but would also prevent unnecessary effort from being expended to produce treatments which exceed required efficacies. The desirable sequence of events in producing a new treatment of known efficacy consists of seven interchanges between the exporting country (A) that wishes to export a product and the importing country (B). First, A provides B with a list of pests known to infest the product. Then B categorises each pest on the list as a quarantine or a non-quarantine pest. B then provides the specifications (M_s) and suggests possible risk management options. If a new treatment is required, A consults with B over protocols for experimentation. A then conducts the necessary research, bearing in mind that the efficacy must be sufficient to ensure that the *MPL* is not exceeded. A reports the results of this research, including estimates of variability, to B, and B incorporates the new treatment into the schedule of risk management.

The keys to success for both parties are that consultations be open and frank and that discussions take place between their scientists as well as between their quarantine officials. The aim must always be to promote rather than stifle trade while at the same time preventing, as much as possible, the expansion of pest distribution.

ACKNOWLEDGEMENTS

I am very grateful to my colleague, Dr Carolyn Whyte at the New Zealand Plant Protection Centre — Lynfield, for many valuable discussions and suggestions during the preparation of this paper. I also appreciate the invitation from Dr Patrick Vail, Laboratory Director, USDA, Fresno, California, to participate in this conference.

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THE IMPACT OF DOMESTIC AND INTERNATIONAL REGULATORY CONTROLS ON THE COMPETITIVENESS OF AMERICAN AGRICULTURE

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ABSTRACT

Artificially produced methyl bromide (MB), a versatile, highly effective, fast-acting fumigant, is employed by man in a number of important ways to kill organisms destructive to plants. A wide spectrum of commodities is treated with MB. The compound is unique in providing a wide range of pest control techniques that may be applied to a broad spectrum of both food and non-food commodities, can be used for fumigation of both large and small quantities of materials, and, when applied properly, leaves no residues of toxicological significance. This compound has recently come under scientific scrutiny and been identified as a potentially potent ozone-depleting chemical. As a result, countries operating under the Montreal Protocol, an international treaty for regulating ozone-depleting substances, will be restricting, and, in many cases, eliminating its use altogether. For example, the United States Environmental Protection Agency has proposed a total phase-out by 1 January 2001. Its limited use and/or potential phase-out will have severe economic implications unless alternatives which are equally efficacious, economical and available are developed.

There may be many important uses for which, due to the complexity of researching a wide array of alternatives, the number of steps involved in making efficacious alternatives ready for use and the limited amount of time involved, effective alternatives will not be available by the time the phase-out takes effect. This eventuality would impose significant competitive disadvantages on American agricultural production and trade. The provisions of the United States Clean Air Act are more stringent than those of the Montreal Protocol which govern the regulatory controls in other countries of the world. This disparity has been the subject of considerable debate, in both the United States agricultural community and the United States Congress, regarding the economic and competitive position of American agriculture.

INTRODUCTION

Since 1991, methyl bromide (MB) has come under scientific scrutiny, and it has been identified as a potentially potent ozone-depleting substance (ODS). As a result, countries operating under the Montreal Protocol (MP) will be restricting or eliminating its use

altogether. For example, the United States Environmental Protection Agency (USEPA) has published a final ruling that will terminate total production and consumption of MB in the US by 1 January 2001 (US Federal Register, 1993). Developed countries operating under the MP will be eliminating MB production and consumption in the year 2010 (UNEP, 1995). Imposing limitations and/or a total phase-out on the use of MB will have severe economic effects if viable alternative treatments are not available.

In addition to its worldwide use, MB is one of the few fumigants left for insect disinfestation. It is the only remaining fumigant for commodity treatment for quarantine. Commodity treatments account for approximately 10% of the total use of MB in agriculture (Methyl Bromide Science Workshop Proc., 1992). There are no alternative treatments available which would provide the same physical and chemical characteristics as MB and be as useful in broad-scale commodity treatments, including quarantine treatments, which require fast action (fumigation times of 2–24 h depending on commodity) and ease and flexibility of application, as well as gaseous efficaciousness at a broad range of temperatures.

THE MONTREAL PROTOCOL

In 1985 the Vienna Convention, under the auspices of the United Nations Environment Program (UNEP), laid down the framework for addressing substances which deplete the ozone. In response to the growing evidence that chlorine and bromine could destroy stratospheric ozone on a global basis, the international community in 1986 negotiated the MP (UNEP, 1986). The MP limits the production and consumption of specific sets of ODS's. Significant scientific advances have continued; reports indicate that the rate of ozone depletion is more rapid than previously believed and that "anthropogenic sources of MB are significant contributions to stratospheric ozone depletion." (UNEP, 1992a; WMO, 1994).

At the fourth meeting of the Parties to the Montreal Protocol, in Copenhagen, Denmark, 23–25 November 1992, additional adjustments to the Protocol were made, including an amendment regulating use of MB in developed countries. The amendment proposed to add MB to the list of controlled substances with an assigned ozone depleting potential (ODP) of 0.7, to freeze production and consumption in 1995 at the 1991 levels, to exempt quarantine and preshipment uses from the 1991 freeze in production and consumption, to conduct a two-year in-depth study of MB uses and alternatives and to re-evaluate the science and the in-depth study in 1995 (UNEP, 1992b).

At the seventh meeting of the parties, in Vienna, Austria, 5–8 December 1995, actions were taken to strengthen the overall controls for MB. These actions included a change in the listed ODP from 0.7 to 0.6, a developed-country phase-out on 1 January 2010 for production and consumption, which will be preceded by two interim reductions (25% on 1 January 2001, and an additional 25% on 1 January 2005), and a developing-country freeze, commencing 1 January 2002, on production and consumption at the average of the 1995–1998 production levels. These actions did not affect

the 1992 Copenhagen exemptions for quarantine and preshipment applications (UNEP, 1995).

US CLEAN AIR ACT

Section 602(e) of the USCAA states: "Where the ozone-depletion potential of a substance is specified in the Montreal Protocol, the ozone-depletion potential specified for that substance under this section shall be consistent with the Montreal Protocol" (USCAA, 1990). Therefore, the action taken under the Montreal Protocol provided the legal basis for USEPA to publish, in the US Federal Register of December, 1993, rules for use of MB (US Federal Register, 1993). These actions included listing MB with an ODP of 0.7, freezing production and consumption on 1 January 1994 at the 1991 levels, classifying MB as a Class I ozone depleting chemical, terminating production and consumption on 1 January 2001 and not requiring MB-treated products to be labeled.

Comparison of actions for MB under the USCAA and the MP

Regulatory provisions for MB under the USCAA are more stringent than are those contained in the MP. The US pushed very hard at the seventh meeting of the parties for actions consistent with the USCAA to be taken; this would have provided a level playing field for all parties. However, most countries, particularly developing countries, were not prepared to go that far. Their decision was based primarily on the importance of MB to their economies and to the lack of available alternatives. After considerable debate, developed countries agreed to a phase-out of MB on 1 January 2010, with two 25% interim reductions, in 2001 and 2005, for a total of 50%.

In spite of the additional actions taken by the Protocol to strengthen MB controls, a large regulatory gap remains between the Protocol and the USCAA. The Protocol exempts quarantine and preshipment uses; the USCAA authorizes no exemptions. Other, more obvious, difference are the distinction between Class I and Class II ozone-depleting substances and the mandatory phase-out dates required under the USCAA. Class I and Class II ODS's are based on the numerical number of their respective ODP's, and the threshold number which separates the two classes is 0.2. Thus, chemicals with ODP's greater than 0.2 are Class I; those with less than 0.2, Class II. Class I ODS's must be phased out 7 years subsequent to the listing date; Class II ODS's by the year 2030. The Montreal Protocol does not list ODS's by classes and lacks mandatory phase-out dates, which are determined via consensus vote by the Parties.

Impact on US agriculture

Because of the important uses of MB and its role in agricultural production and trade, the phase-out of MB in 2001 under Title VI of the USCAA is of vital significance. This US law is more restrictive than the provisions for MB that govern the rest of the world under the MP. In particular, the MP allows longer phase-out schedules and includes provisions for exemptions for essential uses. The differences between the

domestic and international regulations has caused profound concern among agricultural producers and processors and those engaged in international trade. US farmers are concerned that, if adequate alternatives are not available, when the US phase-out takes effect, they will be put at a significant competitive disadvantage in international agriculture and trade.

MB is particularly important for quarantine treatments because it is effective against a large variety of both indigenous and non-indigenous pests and can be easily and economically applied to both small and large shipments as well as to storage. US regulations require that a wide array of imported food and non-food commodities be fumigated with MB as a condition of entry. In addition, a number of commodities exported by the US must be fumigated with MB in order to comply with quarantine requirements of recipient countries. A quarantine use of MB critical for US agriculture is its role as the only practical emergency treatment for commodities moved out of areas quarantined for outbreaks of such exotic pest insects as the Mediterranean fruit fly.

The primary uses for MB are as a soil fumigant and in intensive production of such high value crops as strawberries, tomatoes, cucumbers, peppers, melons and eggplants. The 1993/94 production value of these six commodities, using MB for pre-plant treatment, was US$2.4 billion (USDA, 1993–4). In addition to these six commodities, a 1993 USDA assessment report listed an additional 15 for which MB was also important in production (USDA, 1994).

Stored agricultural food products include a wide variety of dry foodstuffs (principally cereals, grains, oilseeds and legumes), grain products, dried fruits and nuts, such other durable products as timber and timber-containing products and various artifacts. These products, often stored for long periods of time, are treated with MB for control of a number of domestic pests. Insect and mite pests can breed on these materials during storage. Pests may also be present at the time of harvest, and they persist in storage or during transportation. Control of pests infesting stored commodities is essential in keeping commodity losses to a minimum, maintaining quality, preventing damage and preventing the spread of pests between countries. In 1993/94 the estimated value of dried fruits and nuts alone was in excess of US$4 billion.

Structural fumigation of food production and storage facilities (mills, food processing plants and distribution warehouses), non-food facilities (dwellings and museums) and transport vehicles (trucks, ships, aircraft and rail cars) rely heavily MB for control of a large number of pests. It is used either on an entire structure or on a significant portion of a structure. Fumigation is utilized whenever the infestation is either so widespread that localized treatments may result in re-infestation or within the walls (or in other inaccessible areas).

Agricultural exports consistently make a large positive contribution to the US balance of trade. The USDA Economic Research Service's statistics for the fiscal year 1993/94 showed the value of US exports of apples, cherries, peaches/nectarines and strawberries to the world market was US$650 million. The figure for cotton was US$2.3 billion; for oak logs, US$130 million; and for walnuts (in shell),

US$86 million. The market values for export of these commodities to countries requiring MB treatment totaled US$282.8 million (US$101, US$106, US$24 and US$1.8 million, respectively) (USDA, 1993–4).

The current extent and importance of MB use and the potential impact that the 2001 phase-out poses for American agriculture necessitate a major effort to ensure that American farmers can continue to raise and market their crops. USDA has directed its resources and expertise, with the support of Congress and in cooperation with growers, to conducting an ambitious research program for identifying and developing alternatives for control of the pests currently controlled by MB.

SUMMARY

USDA has placed a high priority on dealing with agricultural concerns while contributing to the protection of the global environment. There are three areas where USDA is working to develop solutions that meet both of those needs in dealing with the MB issue (Elworth, 1995, 1996). These are discussed below.

Research

The Agricultural Research Service (ARS) of the USDA has for many years devoted significant research resources to investigating approaches that can potentially replace MB. Since the USEPA announced the phase-out for MB, the ARS has increased its efforts to find alternatives through research on a variety of approaches. These include new cultural practices, improved host-plant resistance to pests and diseases and biological control systems using beneficial microorganisms, as well as less harmful fumigants. For postharvest treatment, the alternatives being investigated include the creation of pest-free agricultural zones, physical methods such as hot or cold treatment or storage in modified atmospheres, alternative fumigants, MB trapping and recycling technologies, biological control and systems approach.

Spending for ARS research on MB alternatives increased from US$7.4 million in fiscal year 1993 to the US$13.9 million included in the current appropriation for fiscal year 1996. This spending supports 42 scientist-years involving 46 projects. This research is augmented by research from both grower groups and the EPA.

The USDA recognizes that there are a number of real-world factors that affect our ability to find alternatives. We recognize that alternatives will have to be found for a wide variety of crop applications spread over a diverse set of geographic conditions and that no single practice will substitute for all those uses in all those conditions. We also know that a genuine alternative for farmers must be efficacious, cost effective, logistically possible and available for efficient incorporation into standard agricultural practices. In addition, the approval process for a new use or new product requires time for the registrants to conduct and submit the necessary studies that the EPA must review by the latest standards. Finally, securing the approval of importing countries for the quarantine practices has typically taken years of negotiation.

The Montreal Protocol

The USDA has actively participated in the development of positions to be taken by the United States Government in international deliberations by the parties to the Montreal Protocol. The USDA has worked within the delegation to help level the playing field in the international arena for US producers, by pressing at the Vienna meeting for a global phase-out which would require all countries to meet the same standards. Although the parties did not ultimately support that position, important progress was made. Developed countries agreed to a phase-out schedule, and a freeze on developing-country use was adopted. Although these measures fell short of the US position, they represent a universal commitment to international controls and are the first steps toward a world-wide phase-out.

Administrative solution

Despite the progress made internationally, there remains a disparity between the US-CAA controls on MB in the US and the controls affecting the rest of the world under the MP. As a result, the USDA is very concerned that, if adequate alternatives are not available, when the US phase-out takes effect in 2001 US farmers will be put at a significant competitive disadvantage in international agriculture and trade. The Clinton administration has indicated its willingness to work with Congress and other stakeholders in crafting a reasonable solution which would be limited to resolving the concerns about the competitiveness of US agriculture and trade by means of assuring the continued availability of MB where, because of the lack of acceptable alternatives, it is needed.

If we are to come to a successful and responsible solution, some important principles must be incorporated into any legislation. First of all, such legislation must protect American agriculture and trade from being put at a competitive disadvantage. Secondly, it must provide sound protection of the global environment. Thirdly, it must retain the incentives for research on alternatives. Fourthly, it must not result in a cumbersome or unworkable administrative process. Finally, it must not undercut international agreements.

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THE POTENTIAL OF SYSTEMS APPROACHES FOR QUARANTINE

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ABSTRACT
(Full paper not available)

A systems approach is a set of safeguards and investigation measures which individually and cumulatively provide a reduction in plant-pest risk. This approach to management of plant-pest risk provides an alternative to postharvest quarantine treatment for obtaining phytosanitary security. Examples of successful systems approaches for movement of quarantined commodities are discussed in the context of potential loss of methyl bromide.

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 557–563

THE EFFICACY OF METHYLISOTHIOCYANATE AGAINST THE CODLING MOTH

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ABSTRACT

All stages of *Cydia pomonella*, the Codling moth on apples, can be eradicated by a methyl bromide (MB) fumigation followed by a period in cold storage at 0.5 or 2°C, necessary to control the egg stages which are insensitive to MB. Depending on the chosen standard of treatment, the cold storage time varies from 40 to 60 d. This work demonstrates that fumigation with methylisothiocyanate (MITC) at a Ct product of 2 g h m^{-3} is sufficient to effectively control all three egg stages of the Codling moth. This treatment overcomes the problem of immobilising the fruit, which can be exported a short time after harvest. Quality analysis shows that an MITC fumigation at a Ct product of 2 g h m^{-3} does not alter apple quality. The desorption tests, with a detection limit of 2 mg kg^{-1}, showed that no MITC was detected 1 h after the treatment.

INTRODUCTION

The biological efficacy of methylisothiocyanate (MITC) at an application dose of 20 to 40 g m^{-3} for 24 h has been demonstrated for all stages of *Sitophilus granarius* (Ducom, 1994). Numerous tests on other species of stored-product pests has confirmed the efficacy of the fumigant. The cited application doses are generally higher than the lethal concentrations required, but this is because the high sorption of MITC handicaps its diffusion and availability.

This study on the fumigation of apples with MITC was undertaken in order to satisfy the quarantine requirements against the Codling moth, *Cydia pomonella*, laid down by certain countries. Currently, this is done with methyl bromide (MB) fumigations, either at atmospheric pressure or in a partial vacuum, followed by a period of up to 50 d cold storage to eliminate the egg stages. The objective of our study was to control these stages using MITC. Because the moth oviposits on the fruit skin, a very low dose of the fumigant was applied for a very short time, thereby avoiding a high level of sorption inside the apples.

MATERIALS AND METHODS

Analysis of the biological efficacy of MITC was carried out on three embryonic stages of the Codling moth egg: W1 (1st instar, white stage), W3 (3rd instar, red ring stage) and W5 (5th instar, blackhead stage). The most resistant stage was defined.

All insects used in this study were laboratory-reared with an annual introduction of about 2% of wild insects collected in the Avignon region in the southeast of France. Breeding techniques were based on Sender (1969, 1970); Guennelon (1976); and Guennelon *et al.* (1981). No sanitary disorder (disease or virus) affected the insects during the experiments.

Three 15-m^3 air conditioned rooms were used. One was for emergence of the adults required for apple infestation; it was held at 25 ± 1°C, 70 ± 10% r.h. and a photoperiod of 16 h day/8 h night). The second was for infestation of fruit at 25°C, 70% r.h. and the same photoperiod. The third room was for attaining the target stage and apple incubation after a cold exposure period (27°C, 70% r.h.).

The Golden Delicious variety was chosen for this test.

Batches of ten apples per stage and per dose were used, and experiments were replicated twice.

The cages (84 × 27 × 29 cm) used for infesting the apples were disinfested, before each new infestation, by fumigation with MB at 40 g m^{-3} for 24 h followed by a desorption period. The fruit, stored at 0.5°C, was taken out of the refrigerator 24 h before the infestation and kept at 25°C and 70% r.h.

The infestation was carried out at 25 ± 1°C, 70 ± 10% r.h. and a photoperiod of 16 h day/8 h night. Fifty apples were placed in each infesting cage. 100–150 unsexed adult 2–5-d-old Codling moths were introduced into each cage for a 24-h oviposition period in order to obtain approximately 20–25 eggs per apple. After 24 h, the insects were removed by aspiration.

The three embryonic egg stages were prepared as follows: 1-d eggs (W1, white stage) were kept at 27°C for 12 h after the end of the infestation and then fumigated, aged 12–36 h; 3-d eggs (W3, red ring stage) were kept at 27°C for 60 h after the end of the infestation and then fumigated, aged 60–84 h; and 5-d eggs (W5, blackhead stage) were kept at 27°C for 108 h after the end of the infestation and then fumigated, aged 108–132 h.

MITC was evaporated from a crystal block which could be cut up to obtain the required quantities of the compound.

The 43-L gastight fumigation chambers were made of "altuglass." The desired dose of MITC was placed in the chamber after introduction of the apples. The ten apples used per dose gave a loading ratio of 4%. The chambers were equipped with an exterior pump, run continuously during the fumigation, which provided a flow rate of 14 L min^{-1}. The fumigation chamber was maintained at 20°C for 24 h preceding and for the duration of the treatment, and control apples were held at the same temperature. Dosages were calculated at 0.2, 0.4, 0.6, 0.8, 1 and 2 g m^{-3}. The exposure period was 2 h.

Measurements of MITC concentrations were made with a VARIAN 3 300 gas chromatograph, and the recordings were integrated using the integration card Star Varian and

Star Varian Version 4 software. Readings were taken at 0.5, 1 and 2 h after introduction of the gas.

After the MITC fumigation, the fumigated apples and controls were incubated in the incubating room for 9 d.

Mortalities of eggs on treated and control apples were determined after 9-d incubation by examining each isolated egg under a microscope. Eggs that had hatched were recorded as live, unhatched eggs as dead.

The results were subjected to probit analysis (Finney, 1971) using SAS software (1987) and expressed as lethal dosages at the following levels: LD_{50}, LD_{90}, LD_{95} and LD_{99}.

Physical, chemical and organoleptic quality of the apples

The aim of this study was to determine the physical, chemical, visual and olfactory characteristics of Golden Delicious apples treated with MITC for 2 h at 20°C and 2 h at 10°C, followed by storage for 20 d at 20°C. To evaluate apple quality in relation to fumigant dosage, the following four doses were tested: 0.5, 1, 1.5 and 2 g m^{-3}.

Each analysis was carried out on ten weighed apples using the following equipment as specified by the French standard NF V 20-201 (AFNOR, 1981): a centrifuge, a penetrometer FACCHINI, a refractometer EUROMEX (model 0–32 Brix), a pH meter (646 KNICK) with a pH INGOLD electrode, model 0–14.

Firmness

Firmness, expressed in kg cm^{-2}, was measured using a penetrometer in two places, on the sides with the most and least colour.

Apple juice

Each apple of each batch was analysed. Two pieces, representing a fifth of the apple, were cut between the holes made by the penetrometer. The 20 pieces so obtained were centrifuged and the juice filtered through sterilised hydrophilous cotton.

Dry soluble matter (sugar content)

A few drops of juice were placed in the refractometer. The reading obtained indicated the soluble dry material, expressed in degrees Brix.

Total acidity

Twenty ml of the same juice were homogenised by a magnetic stirrer and neutralised with a sodium hydroxide solution (NaOH, 0.1 N). Once the pH reached 8.2, no further solution was added. The value obtained, expressed in ml of sodium hydroxide, indicates the total acidity level of the solution.

Method of visual analysis

Visual analysis was conducted simultaneously with the physical and chemical analyses. The interior and exterior aspects of the apples were recorded, with the overall colour,

the colour homogeneity, the different marks, and coloration and condition of the seeds all being noted.

Method of olfactory analysis

Olfactory analysis was conducted after the chemical analysis. Two apple quarters were divided and the flesh sniffed by a panel which noted the strength and characteristics of the different smells (fruity, phenolic, vinegary, terpenolic, musty, lactonic, mouldy, aminated, etc.).

RESULTS AND DISCUSSION

Biological efficacy of MITC on *Cydia pomonella* eggs

Mortality data clearly reveal that Codling moth eggs have a high sensitivity to MITC. The least sensitive stage seems to be W5. Statistical analysis shows that the LD_{50} and LD_{90} of the W5 stage are greater than those of the W1 and W3 stages (Tables 1 and 2). In fact, a Ct product of 0.39 g h m^{-3} is necessary to obtain 50% mortality of the W5 stage compared with a Ct product of 0.43 g h m^{-3} and 0.26 g h m^{-3} for the W1 and W3 stages, respectively. A similar tendency can be found at the LD_{90} level.

However, the slope of the probit line for the W3 stage (1.21) is less than those of the probit lines for the W1 stage (2.65) and W5 stage (1.54). Thus, the LD_{99} of the W3 stage is greater than that of the W1 stage and could be compared to the W5 stages (Fig. 1). A

TABLE 1

Calculated LD_{50}, LD_{90}, LD_{95} and LD_{99} values, in Ct products (g h m^{-3}) for *Cydia pomonella* eggs on mature apples, fumigated with MITC for 2 h at 20°C

Stage	Slope (ln)	LD_{50}	LD_{90}	LD_{95}	LD_{99}
W1	2.65 (±0.64)	0.43 (0.31–0.49)	0.69 (0.59–1.07)	0.80 (0.65–1.44)	1.03 (0.78–2.55)
W3	1.21 (±0.14)	0.26 (0.19–0.32)	0.76 (0.64–0.96)	1.03 (0.83–1.41)	1.81 (1.34–2.95)
W5	1.54 (±0.50)	0.39 (0.09–0.51)	0.90 (0.73–2.16)	1.14 (0.87–4.87)	1.78 (1.15–23.5)

TABLE 2

Comparison between the sensitivity of *Cydia pomonella* eggs treated with MITC and MB at 20°C

	Ct product (g h m^{-3})		
Treatment	W1	W3	W5
LD_{50}, MB atmospheric pressure fumigation	23.02	16.04	15.16
LD_{50}, MB vacuum fumigation	23.90	14.80	11.40
LD_{50}, MITC	0.43	0.26	0.39
LD_{95}, MB vacuum fumigation	100.95	32.45	23.20
LD_{95}, MITC	0.80	1.03	1.14

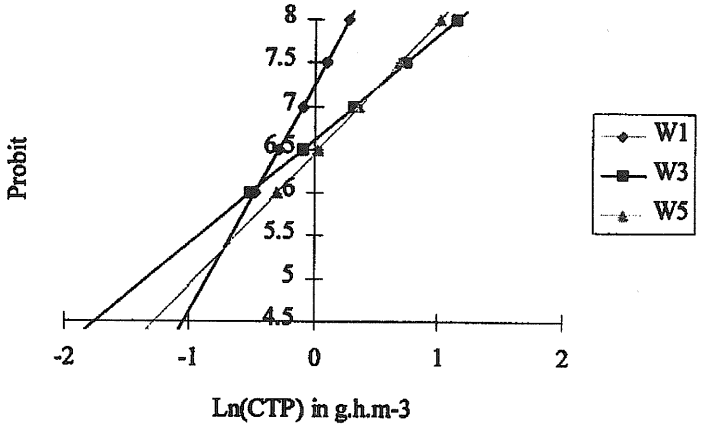


Fig. 1. Probit lines in relation to Ct product (CTP) of *Cydia pomonella* egg stages (W1, W3 and W5) exposed to MITC.

Ct product of 1.81 g h m^{-3} is necessary to obtain 99% mortality of the W3 population, as opposed to a Ct product of 1.78 g h m^{-3} to control 99% of the W5, and a Ct product of 1.03 g h m^{-3} to control 99% of the W1 population. The probit analysis of these studies therefore shows that the most resistant stage to MITC is W3.

Sensitivity comparison between Codling moth eggs treated with MITC and MB

Comparing the above results with those of Cugier (unpublished results), who used vacuum fumigation with MB, and those of Gaunce *et al.* (1980), who treated apples with MB at atmospheric pressure, reveals that, at the LD_{50} level, the MITC is 50 to 70 times more active than MB at atmospheric pressure or in partial vacuum. Comparison of vacuum fumigation with MB and fumigation with MITC shows that, at the LD_{50} level, MITC is 23 to 140 times more active than MB.

Effect of treatment on apple quality

No notable, significant difference between the control and the treated batches could be observed (Table 3). The firmness and the acidity of the apples were the same. A slightly higher refractometric index, indicating a slightly higher sugar content, was noted in the treated batches. The tasting panel did not note this difference as the fruit overall is not very sweet.

Visual analysis did not reveal any difference between the treated and untreated batches. The olfactory panel noted that the aroma of the treated apples was less marked and less enduring than that of the control.

Desorption of MITC from the apples

During a 2-h fumigation at 20°C, at an applied dose of 2 g m^{-3} and a loading of 5%, the fruit absorbed 3 ± 1% of the quantity of gas introduced into the chamber; the rest of the

TABLE 3
Quality parameters for apples treated with MITC (Ct 2 g h m^{-3}) and untreated apple batches

	Control		Treatment	
Sample	Batch 1	Batch 2	Batch 1	Batch 2
Average weight (g)	116.6	121.2	113.8	138
Refractometric index (° brix)	12.2	11.2	13.2	12.5
Free acidity (pH)	3.9	4.04	4.03	4.07
Total acidity (ml NaOH)	3.24	2.86	2.84	3.28
Firmness				
Average	3.43	3.23	3.58	3.58
SD	1.00	0.53	1.24	0.89
Minimum	2.4	2.5	2.1	2.4
Maximum	6.3	4.5	6	6.4

gas was absorbed by the chamber walls. Thus, depending on the weight of the treated apples, between 1.5 and 2.5 mg kg^{-1} of MITC remained at the end of the fumigation. After 4-h immersion in 250 ml of methanol (the method validated in the laboratory), no trace of MITC was found on the apples that had desorbed for 1 h after treatment.

CONCLUSION

In this study, 100% mortality was obtained on the three egg stages of the Codling moth with an MITC fumigation for 2 h at 20°C at an introduced dose of 2 g m^{-3}. The least sensitive stage was the the 3-d-old egg, with a LD_{99} of 1.81 g h m^{-3}. MITC fumigation of Golden Delicious apples at doses efficient against the Codling moth does not alter the quality of the fruit, and no trace of MITC was detected after 1 h of desorption at 20°C (desorption limit: 2 ppm).

MITC fumigation to control the egg stages of this pest obviated the need for about 50 d of cold treatment.

Further work is required to investigate the efficacy of this fumigant on the most MB-resistant larval stage, the 5th stage diapausing larva, before a standard method of control using a combination MB and MITC, or an MITC fumigation alone, can be envisaged.

The efficacy/quality relationship needs to be established for such standards.

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USE OF LOW-OXYGEN ATMOSPHERES IN QUARANTINE APPLICATIONS TO ERADICATE *MUS MUSCULUS*, THE HOUSE MOUSE

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ABSTRACT

The feasibility of using low-oxygen (O_2) atmospheres to eradicate infestations of mice (*Mus musculus*) in order to meet quarantine standards was investigated. Drilling equipment and transportable buildings were enclosed within standard plastic fumigation sheets both with and without floor sheets. The enclosures, 45–189 m^3, were dosed with a low-O_2 atmosphere (≈1.5% O_2) generated by a membrane nitrogen separation system. Purging was observed to follow a simple free mixing model in form. The atmospheres were created using about 20% less gas than the amount predicted on the basis of free mixing alone. Applying this model allows selection of generators of suitable size. Leakage from sheeted containers was very slow after purging (0.04% d^{-1} or less), showing that a high degree of gas retention was easily achieved.

Atmospheres containing less than 2.5% O_2 were obtained and maintained within the enclosures for a minimum of 6 h. Published literature suggests this would have caused 100% mortality of exposed mice. Work is continuing to establish the most cost-effective exposure regime.

Application of this treatment as a quarantine measure against rodents is discussed and its costs compared with conventional methods of disinfestation using methyl bromide.

INTRODUCTION

In 1994, the Stored Grain Research Laboratory was commissioned by West Australian Petroleum Pty Limited (WAPET) to find an effective replacement for methyl bromide (MB) for control of rodents in its quarantine operations. This chemical has been used for rodent control world-wide since the 1940's (Borg, 1944; Lembrez, 1966) and is still internationally recommended for this purpose (Bond, 1984).

WAPET is an oil and gas company for exploration and production, operating on two islands on the northwest shelf of Australia. Both islands, Barrow and Thevenard, are nature reserves serving as refuges for rare or endangered wildlife. Consequently, the

Company's operations are constrained by both voluntary controls and those mandated by the Environmental Protection Authority on imported material, including plants and animals.

The company, which has won industry recognition for its environmentally friendly operations, has gained two awards for its environmental policies. Even after 30 years of operation, Barrow Island is still a refuge for a number of animal species, such as the Burrowing Bettong, that have disappeared from their original habitats on the mainland. If rabbits, cats, foxes, rats, mice, snails or weeds were allowed to enter these islands, they could seriously, perhaps permanently, disturb the ecology of the native wildlife, possibly causing extinctions through predation and deprivation.

WAPET enforces a quarantine program to ensure that exotic animals, specifically mice, are not carried from the mainland to the islands. Equipment that can be infested by rodents and other vermin is subjected to MB fumigation. Items such as transportable offices, accommodation units, tool sheds, equipment used for food storage, mobile messes, etc. are commonly treated before they are shipped from the mainland to the islands. Also, because non-native mice have become established on Thevenard Island, similar restrictions apply when equipment is transported from there to Barrow Island.

In keeping with WAPET's environmental concerns, and with occupational health and safety concerns, the company desired to replace MB for this particular application. However, the company wanted any potential replacement for MB to be environmentally safe; safer for workers than the existing system; compatible with exploration and production operations for oil and gas; compatible, in terms of speed of operation, with the existing method of disinfestation; and suitable for use at remote locations.

After visiting and inspecting WAPET's on- and off-shore operations it was concluded that three potential alternatives could be considered. These were use of heat/high temperatures, high carbon dioxide (CO_2) atmospheres and low-oxygen (O_2) atmospheres.

Heat as an alternative

This was ruled out because of potential damage to the heat sensitive equipment used during the company's operations. More importantly, it posed a potential, and serious, fire hazard when used in the vicinity of gas and oil, whether wells or storage vessels.

Carbon dioxide as an alternative

Early reports of the potential use of CO_2 in the storage environment to control mice and other rodents were published by Pieniazek and Christopher (1947) and Southern (1954). It has been used to control mice both in seed and feed stores and in cold storages, for which an atmospheric concentration of 25% CO_2 was applied for periods extending up to 2 h (Hamel, 1986). It is widely used in euthanasia for killing a wide range of animals, including rats and mice (Andrews *et al.*, 1993; Hansen *et al.*, 1991; Hornett and Haynes, 1984). However, its use in this particular application was constrained by the logistics and costs involved in transporting the large quantities of CO_2 that would be required.

Low-O_2 atmospheres as an alternative

Use of nitrogen (N_2) to generate low-O_2 atmospheres was considered most appropriate because the proposed system met most of WAPET's replacement criteria. N_2 is already used in oil and gas exploration and production to remove air in tanks and pipes in order to prevent fires and explosions. A membrane generation system was chosen because such systems are reliable, small and portable, and they are already used by the industry elsewhere. Also, in our experience, low-O_2 atmospheres are more easily contained in plastic enclosures sealed with sand or sand snakes than are high CO_2 atmospheres.

N_2 is widely used for euthanasia of a wide range of animals, including rats and mice (Andrews *et al.*, 1993). Rats collapse in approximately 3 min, and stop breathing in 5–6 min, in low-O_2 atmospheres produced with N_2 flowing at a rate of 39% of chamber volume per minute, thus falling to about 3% O_2 after 5 min (Hornett and Haynes, 1984). For mink, in 100% N_2 the time to death is 134 sec, but Hansen *et al.* (1991) recommend an extended exposure period of 5 min. Dogs are killed in less than 5 min by N_2 atmospheres containing 1.5% O_2 (Herin, 1978; Quine, 1980). When a group of 72 animals consisting of cats, kittens, rabbits and dogs was placed in a N_2 atmosphere (final O_2 concentration declining to ≤4.0%), 70 collapsed within 1 min of the O_2 concentration falling to 10%; 66 suffered respiratory arrest within 2 min of collapsing; and 66 had no detectable heart beat at 6 min, or less, after the O_2 concentration fell to <10% (Quine *et al.*, 1988).

However tolerance to hypoxia, in the short exposure periods used for euthanasia, has been observed in newborn mice, rats, rabbits, kittens and puppies (Blackmore, 1993; Herin, 1978; Quine, 1980; Quine *et al.*, 1988).

Barrere (1980) reported that atmospheres containing 99% N_2 killed rodents in less than half an hour in grain storages, but he gave no indication of a specific dosage regime for these pests. Pryor *et al.* (1974) in working with mice found that deaths occurred at 7.5% O_2 in 4 h experiments. Levin *et al.* (1987) calculated LC_{50} values for rats exposed to low-O_2 atmospheres for 30 min in an atmosphere of 7.5% O_2.

This paper reports on a trial undertaken to assess the feasibility and effectiveness of using low-O_2 atmospheres to eradicate infestations of mice in equipment used by the oil and gas exploration and production industry.

MATERIALS AND METHODS

The equipment disinfected during this trial consisted of drilling pipes (in racks), a toolshed container, an equipment container and transportable offices both with and without wheels. Dimensions and volumes of the seven lots of equipment after enclosure under fumigation sheets are given in Table 1. The sheets used were made from either yellow nylon reinforced laminated PVC or black Valeron (cross laminated HDPE). All equipment was positioned on cement stabilised sand prior to being enclosed and disinfested.

To obtain an indication of gas loss downward through the ground, three of the seven treatments were carried out using a gastight floor sheet. This was laid on the ground and the equipment placed on it before being enclosed and sealed for treatment.

TABLE 1
Dimensions and volumes of equipment treated, and type of enclosure sheet used

Treatment no.	Equipment	Dimensions (m)			Volume (m^3)	Fumigation sheet	Ground sheet
		length	width	height			
1	Pipe racks 1	10.2	5	1.1	56	Valeron	–
2	Pipe racks 2	10.2	5	1.1	56	Reinforced laminated PVC	–
3	Container 1; Toolshed	6.0	3	2.5	45	Valeron	–
4	Container 2; Kumo shack	12.2	3	2.5	92	Valeron	–
5	Container 3; Transportable office on wheels	13.5	4	3.5	189	Valeron	Valeron
6	Container 4; Jan's office rooms	13.5	3	3.1	126	Reinforced laminated PVC	Valeron
7	Container 5; Transportable office on wheels	13.5	4	3.5	189	Valeron	Valeron

Four pipe racks were treated. These were enclosed as pairs under a fumigation sheet, and each pair was disinfested as a single unit. All other items were enclosed and treated as single units. Sharp edges and protrusions from the equipment were covered to prevent their penetrating and tearing the fumigation sheets. The fumigation sheets were sealed and anchored to the ground, or to the floor sheet, using sand heaped approximately 0.5 m high continuously along the perimeter of the sheeted enclosures at the base of the structures. All visible holes and tears in the fumigation sheets were patched or sealed prior to treatment. Ropes were tied around the middle of each enclosure to reduce billowing and consequent gas "pumping" in the wind.

N_2 for the treatment was supplied from a Prism (Permea Inc., St Louis, Missouri, USA) N_2 membrane system supplied by Oxair Australia, Perth, WA. It had a rated output of 18 $m^3 h^{-1}$ at 98% N_2 and consisted of an electrically powered compressor, an air storage tank, the membrane system and a N_2 receiving tank.

In this trial it was decided to obtain and hold the O_2 levels below 5.0% and maintain these levels long enough to initially ensure complete dispersal of the atmosphere throughout the equipment and thereafter to disinfest the equipment.

N_2 for the treatments was piped from the generator via a 5-cm i.d. nylon-reinforced plastic pipe to a manifold fitted with three ball valves from which it was distributed to the enclosed equipment. These valves provided full (100%) flow with a 90° turn. For the purposes of this trial it was assumed that a 30° turn of the valve delivered 33.3% of the full flow and a 45° turn delivered 50% of the full flow. Subsequent calculations confirmed this assumption.

To prevent excess ballooning of the enclosures during the purge in treatments 1–3 (Table 1), gas was allowed to vent via a small (≈5 cm i.d.) hole in the sheeting approximately 1 m from the ground at the point furthest from the gas input line. In treatments 4 and 5 (Table 1), no vent was provided.

At the end of the treatment time, if the equipment was immediately required for shipment, the fumigation sheets were removed and the containers allowed to air until O_2 levels had been restored to 20–21%. Otherwise the containers remained enclosed until required. In such cases, the opportunity was used to monitor O_2 levels to determine the rate of leakage and permeation of air through the fumigation sheets after the N_2 purge was terminated.

O_2 concentrations achieved during the treatments were measured by sampling the atmosphere inside the enclosures. The samples were drawn out through 3-mm i.d. nylon tubing from various points in the enclosures (Figs. 1–4). The sampling lines were cleared

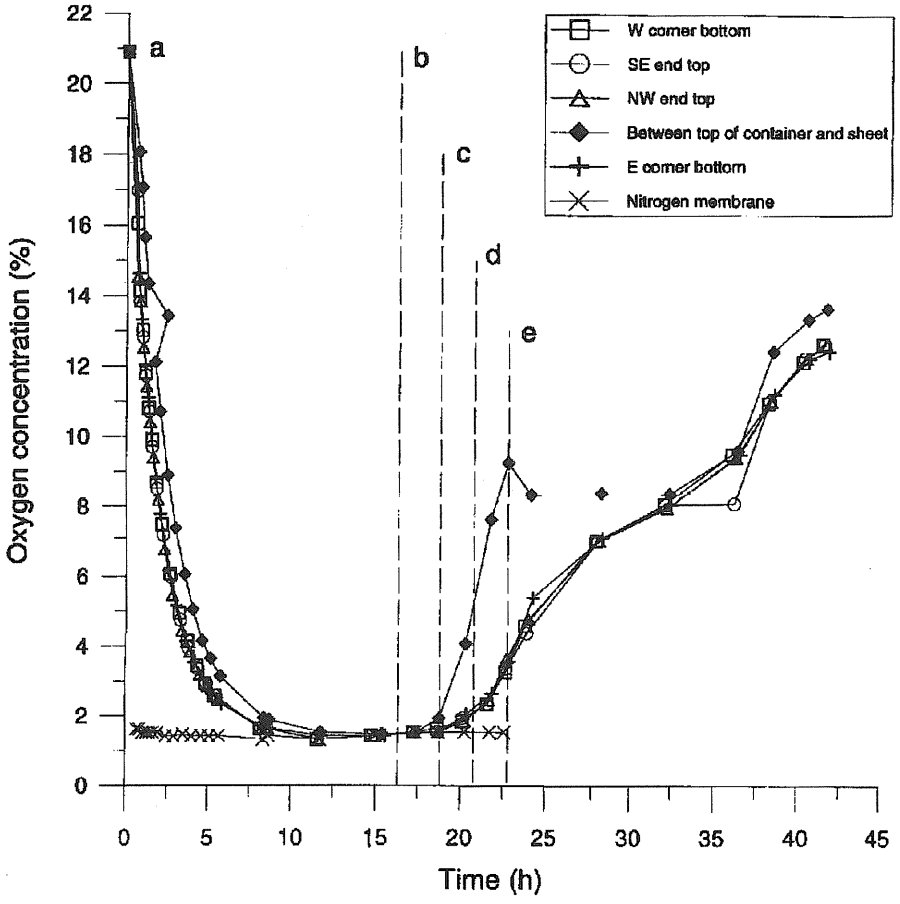


Fig. 1. O_2 concentrations during and after purge of container 1, the toolshed without floorsheet. Assumed flow rates during purge: a = 100%, b = 33%, c = flow rate reduced, d = flow rate reduced then increased and e = end of purge.

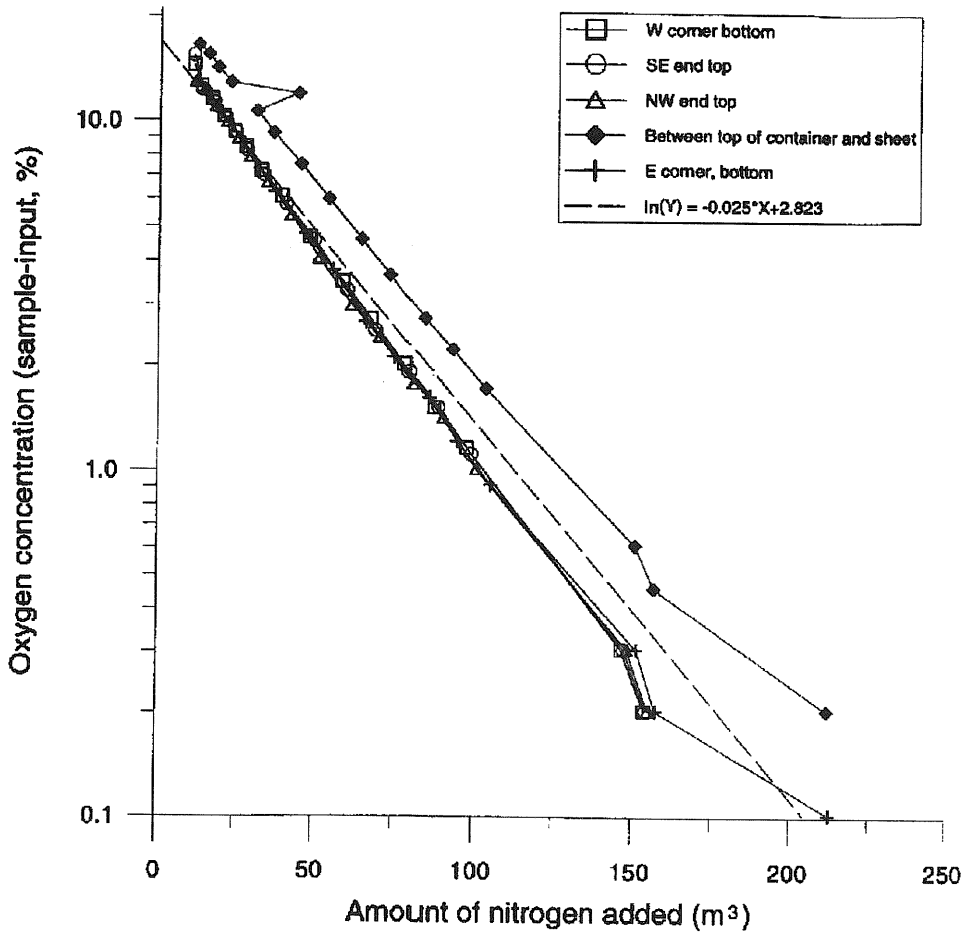


Fig. 2. Plot of O_2 concentration to show rate of O_2 decrease during purge of container 1, the toolshed without floorsheet.

by pumping for a period of 3–5 min at a rate of 150 ml min^{-1} using a Masterflex (Barnant Co, Illinois, USA) peristaltic pump until a steady O_2 concentration reading was obtained. O_2 concentrations were measured using either an Otox 90 (Neotronics, Bishops Stortford, UK) or an Oxywarn 100 (Draeger) O_2 meter. During the treatments, the calibration of these meters was checked regularly against ambient air and industrial grade N_2 (BOC, $N_2 = 99.9\%$, $O_2 < 10$ ppm).

Ambient temperature as well as temperatures attained within the enclosures were measured using T-type copper/constantan thermocouples and recorded on a data logger (Data Electronics, Datataker DT100). Temperatures within the enclosures were measured at the same points from which gas samples were withdrawn (Figs. 1–4).

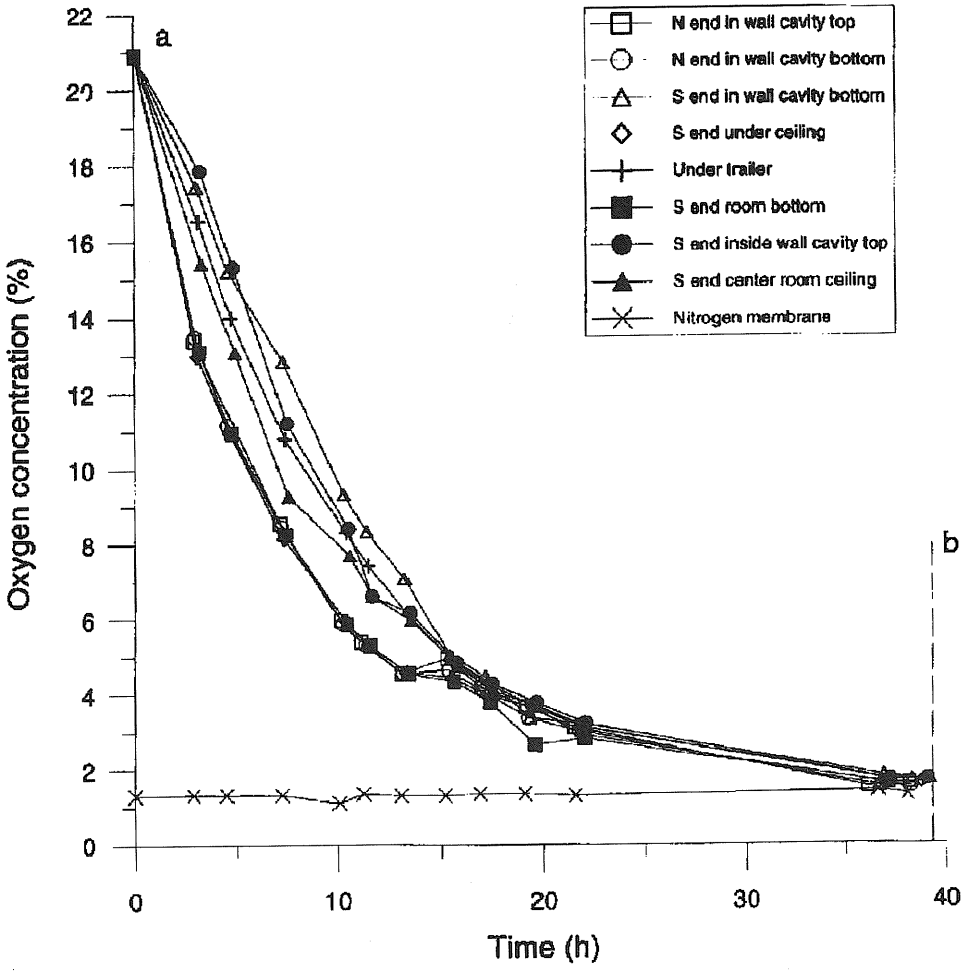


Fig. 3. O_2 concentrations during and after purge of container 5, transportable office on wheels with floorsheet. Flow rates during purge: a = 100% and b = end of purge.

RESULTS

The N_2 output from the membrane system provided an atmosphere with an O_2 concentration varying from 1.3–2.0% and averaging 1.5% O_2 (Figs. 1 and 3).

The O_2 concentrations achieved during the purges and the rate of O_2 decrease obtained are shown for container 1 (the toolshed without floor sheet) in Figs. 1–2 and for container 5 (the transportable office on wheels with floor sheet) in Figs. 3–4. The required O_2 concentrations (≤5.0%) were achieved within a 5-h treatment period for container 1 and a 16-h treatment period for container 5, the volume of the latter being approximately 4 times that of the former (Figs. 1 and 3).

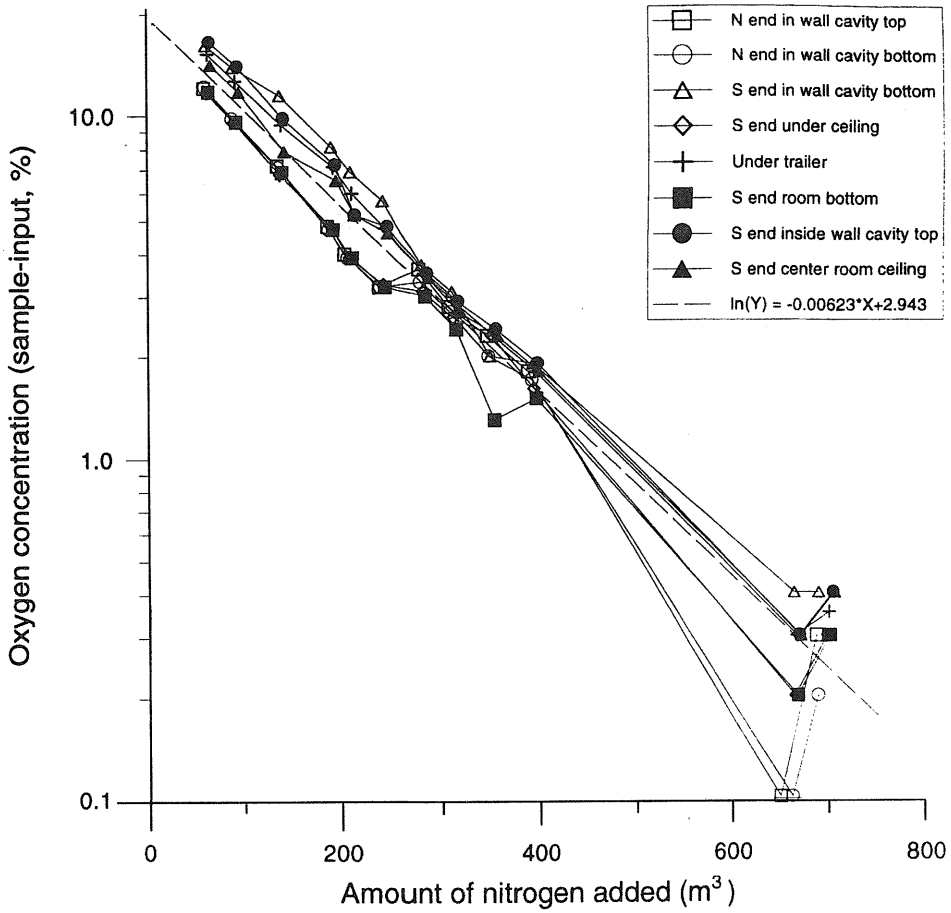


Fig. 4. Plot of O_2 concentration to show rate of O_2 decrease during purge of container 5, transportable office on wheels with floorsheet.

The amount of N_2 used during the purges was calculated from the rated output of the membrane system (18 m^3 h^{-1} at 98% N_2) and the degree of opening of the valves on the manifold regulating N_2 input into the enclosures. Table 2 compares the amount of N_2 required to reduce the O_2 level to 5% for each treatment as well as the rate and efficiency of the purge. Purging efficiency was calculated using Banks' (1979) formula, modified to take into account the fact that the purge gas, c_p, contained about 1.5% O_2. This formula gives 100% efficiency if free mixing occurs throughout the space being purged:

$$Efficiency\ (\%) = 100 \times \frac{volume\ of\ enclosure}{volume\ of\ gas\ added} \times \ln \frac{21 - c_p}{5 - c_p}$$

The ventilation rate, k, a measure of the rate of gas loss from a system, was calculated

TABLE 2
Purge data for the seven treatments

Treatment no.	Equipment	Total N_2 used (m^3)	Amount of N_2 required to reduce O_2 to 5% (m^3)	Treatment time at O_2 ≤5% (h)	Slope of pull-down line	Efficiency of purge[1] (%)
1	Pipe racks 1	235	67	13	–0.0190	129
2	Pipe racks 2	165	55	6	–0.0187	157
3	Container 1; Toolshed	329	38	20	–0.0250	183
4	Container 2; Kumo shack	394	127	11	–0.0080	112
5	Container 3; Transportable office on wheels	416	211	12	–0.0048	138
6	Container 4; Jan's office rooms	406	116	27	–0.0086	168
7	Container 5; Transportable office on wheels	707	172	30	–0.0062	170

[1]Calculated assuming 10% of the volume within enclosure filled with impervious objects.

using the formula given by Banks and Annis (1984),

$$\ln \frac{21 - c}{21 - c_0} = -kt$$

where c and c_0 are the O_2 concentrations at time t and at start of ventilation. Figure 5 shows the return of O_2 by natural leakage into the purge enclosure after the purge gas addition ceased.

The averages of the ambient temperatures and those attained within the enclosures during the treatments are given in Table 3.

DISCUSSION

The purge data for the seven treatments carried out during this trial are given in Table 2. In all cases, purging efficiencies in excess of 100%, as calculated on the basis of free mixing, were observed, suggesting there was some O_2 removal by direct displacement. Up to 50% less O_2 was needed than was expected.

Decrease in O_2 concentrations (Figs. 1, 3) during the purges was uniform at all points within the treated equipment. This included the wall and ceiling cavities of the transportable office. Even at roof height, areas which can provide nesting and breeding sites for rodents were subjected to O_2 concentrations ≤5% during the exposure periods used here.

This trial demonstrated that it is feasible to reduce O_2 concentrations within sheeted enclosures efficiently to ≤5.0% and to hold them at <2.5% for periods extending to 6 h. In the work reported here, the objective was to attain low O_2 levels and maintain them

TABLE 3
Ambient temperature, and temperatures in sheeted equipment during treatment

Treatment no.	Equipment	Ambient temperature (°C)			Temperature inside enclosure (°C)		
		Average	Average maximum	Average minimum	Average	Average maximum	Average minimum
1	Pipe racks 1	22.1	30.0	19.2	24.4	30.4	21.5
2	Pipe racks 2	22.5	30.1	19.2	23.8	29.6	21.2
3	Container 1; Toolshed	22.2	27.3	18.8	24.6	31.3	20.4
4	Container 2; Kumo shack	22.5	29.6	19.3	25.9	32.0	20.2
5	Container 3; Transportable office on wheels	21.9	25.9	20.1	25.2	29.2	18.4
6	Container 4; Jan's office rooms	20.0	27.3	17.4	20.8	29.3	18.7
7	Container 5; Transportable office on wheels	21.5	27.8	18.4	23.8	31.2	19.3

sufficiently long to kill any infestations of mice in the equipment treated. Levin *et al.* (1987) reported 100% mortality in 42 rats exposed for 30 min to atmospheres containing 7.5% O_2, while Pryor *et al.* (1974, cited in Levin *et al.*, 1987) reported that mice were killed at the same O_2 concentration in 4 h experiments. Thus, with the O_2 concentrations achieved and the exposure periods used in this trial, it can be expected that at least all adult animals would have been killed. Similarly, we believe that the prolonged exposures to the low-O_2 atmospheres attained during this work, well in excess of those used for euthanasia, would have been lethal to neonate rodents.

With respect to a dosage and exposure regime, laboratory studies will be undertaken to confirm the results obtained during this trial and establish recommendations for atmospheric O_2 concentrations and exposure periods. In the absence of detailed mortality dosage data, no recommendation is made here. However, we expect that the required exposure periods will be substantially shorter than those in these trials.

It may be important for toxicological or logistical reasons to reduce the time needed for pull-down to low O_2. This can be achieved by using a generator with a greater output than that used in this work. The modelling that was undertaken above allows for appropriate sizing of N_2 generators for a particular, specified pull-down.

Very low leakage as calculated by ventilation rates were observed in the three enclosures which were allowed to stand after purging. Two had a rate of about 0.04 per day, while the third admitted air at a rate of only only 0.008 per day (Fig. 5). The latter rate is very low, and this is perhaps attributable to the better seal afforded by the PVC and floor sheet.

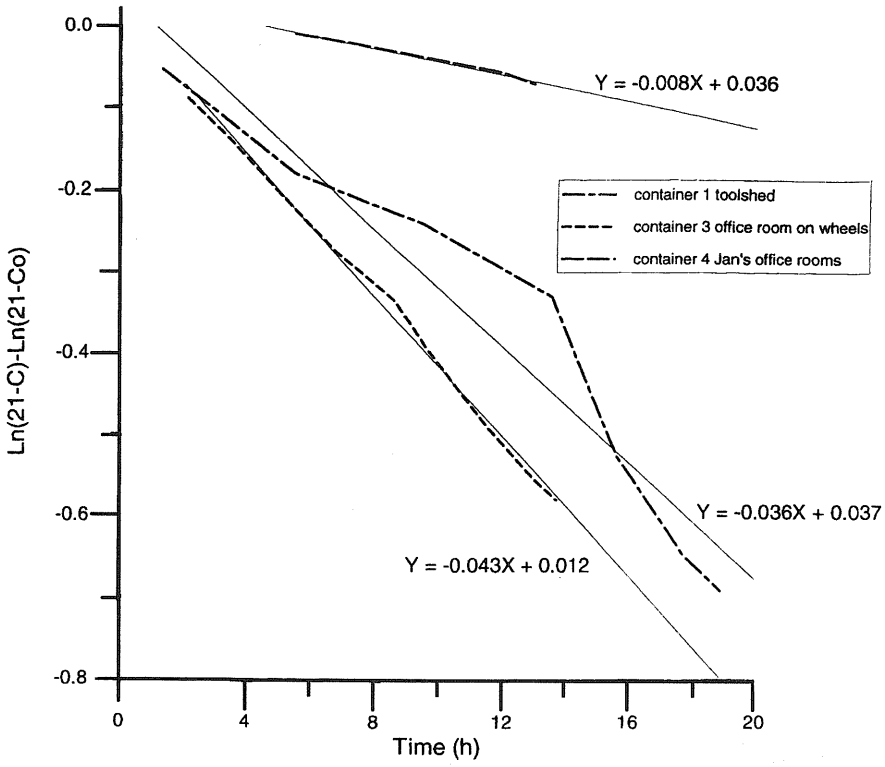


Fig. 5. Ventilation rate (O_2 decay) in enclosed equipment. Container 1 sheeted with Valeron without a floorsheet; container 3 with floorsheet; container 4 with PVC sheeting and a floorsheet.

Direct comparison of the cost of disinfestation with low-O_2 atmospheres to that of using MB is complex in this situation, where environmental and worker-safety concerns are difficult to quantify. However, the direct material costs involved in this use of low-O_2 atmospheres can be compared to those when MB is used. The cost of hiring the membrane system was AU$ 500 as compared to the material cost of AU$ 100 for MB, had it been used. Costs for electricity to run the membrane system are estimated at AU$ 0.15 per m^3 N_2. However, when MB is used, a licensed fumigator and protective equipment for the workforce engaged in this work are required. This would offset the cost difference, particularly in a remote region such as that in this trial series. Labour requirements for enclosing the equipment under gastight sheeting are the same in both cases.

MB treatments with a 4-h exposure period (Bond, 1984) followed by about 2-h airing time are typically rapid. Low-O_2 atmospheres are dependent on the capacity of the equipment available, which determines the rate of pull-down and the duration of the required exposure period. For the latter, we have not yet obtained definitive data, but we expect low-O_2 treatments to be at least as rapid as those with MB, given the appropriately sized generator and the mortality data reviewed above.

The time required for airing after treatment with low-O_2 atmospheres was shorter than for MB. The maximum time required with low-O_2 atmospheres was 0.5 h.

There are particular benefits to be gained by adopting the low-O_2 atmosphere technique in the application described here. Firstly, emissions of MB, a powerful ozone depletor (UNEP, 1992; WMO, 1995), would be reduced. Secondly, it would eliminate a worker-safety hazard from the oil and gas exploration and production workplace.

The technique may have wider application as a replacement for MB in such other situations as aircraft and other equipment already constructed to standards of gastightness. Furthermore, in situations where time constraints are critical, as with aircraft, there appears to be a case for adding CO_2 or carbon monoxide to increase the speed of action against rodents (Levin *et al.*, 1987; Pryor *et al.*, 1974).

CONCLUSION

The work described here demonstrated the feasibility of using low-O_2 atmospheres for rodent control in machinery and equipment which is under gastight fumigation sheeting. The technique using N_2 delivered from a membrane system to generate low-O_2 atmospheres is environmentally acceptable and safer than is the use of MB (Fig. 6). In operation, it is compatible with oil and gas exploration and production operations. It is also suitable for use at remote locations. The logistics of on-site gas generation provide environmental and

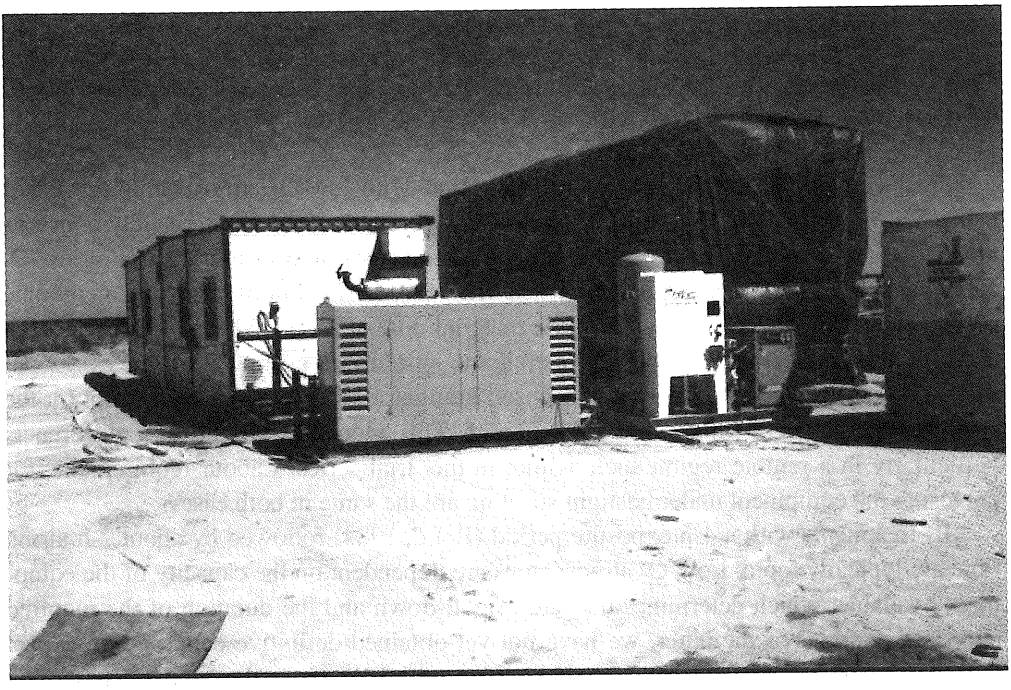


Fig. 6. View of membrane N_2 system and enclosed transportable office on wheels under treatment.

worker-safety advantages that make it an attractive alternative to MB. The technique may have wider application beyond the oil industry.

ACKNOWLEDGEMENTS

We wish to thank our colleague, Geoff Russell, for drawing our attention to the literature on animal euthanasia. The following people all helped with or undertook literature searches on our behalf: Yvonne Hawkins (SGRL), Cdr T. Dickens and Capt Armando Rosales (Armed Forces Pest Management Board, Washington DC, USA), Lt Cdr Bob Gay (Defense Personnel Support Center Pacific, Alameda CA, USA) and Dr Dan Thompson (Denver Wildlife Research Center, USA).

At Thevenard Island, Rob Ward (CBH) assisted us. Andrew McCormack (WAPET) coordinated operations during the trial. We enjoyed, and are grateful for, the hospitality, enthusiastic interest and support that we received from WAPET staff and contractors.

We also thank Peter Annis and Chris Whittle for their helpful advice and criticism of the manuscript.

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CONTROLLED ATMOSPHERE AND OTHER ALTERNATIVES FOR CURRENT USES OF METHYL BROMIDE IN THE NETHERLANDS

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ABSTRACT

Current uses of methyl bromide (MB) in The Netherlands are restricted to quarantine applications and fumigation of durables and structures. In 1991, soil sterilisation was banned. In accordance with the Montreal Protocol the policy of the Dutch government is to further reduce MB use in the future. A basic necessity for an effective reduction scheme is the availability of reliable use figures. Since 1992, notifying a central registry of fumigations with MB has been required. From the resulting database, developments in the use pattern of MB in The Netherlands have been analysed.

A major necessity for use reduction is the availability of effective alternatives. Research has been focused on identifying alternatives suitable for the Dutch situation. Controlled atmospheres seem to be a promising alternative for the two major products currently treated with MB, e.g. groundnuts and cocoa beans. Pilot tests with two different systems for creating low-oxygen atmospheres have enabled companies to amass basic knowledge in this field. Further development is still required.

Heat treatment has been tested for two consecutive years by a large commercial flour mill as a whole-site disinfestation method. The working protocol for this method is improving with every single treatment. Although not always fully effective, the company is now using heat instead of fumigation with MB as a central curative pest-control method.

Control of MB emissions at the permanent fumigation facility of the flower auction in Aalsmeer was achieved by installation of an activated charcoal filter. Chemical analyses have shown a very high removal efficiency resulting in a 99.9% reduction of MB emissions.

INTRODUCTION

Since 1991, methyl bromide (MB) has been reported to possess ozone depleting properties. Because of this potential, the production and use of MB is being controlled within the framework of the so-called Montreal Protocol. In 1994 the ozone depleting potential (ODP) of MB was finally established at 0.6, in comparison to the baseline reference ODP of 1.0 of CFC-11.

The designated parties of the Montreal Protocol have decided upon a differentiated use reduction and phase-out scheme. The current target was agreed upon during the most recent meeting in Vienna, in December 1995. For developed countries, it is a total phase-out in 2010, with intermediate reduction steps of 25% in 2001 and 50% in 2005, in comparison to the baseline use level of the year 1991. For developing countries, currently only a freeze of the use of MB at the average level of the years 1995–1998, to be achieved in 2002, has been agreed upon. In 1997 possible further reduction steps will be discussed.

The European Union has speeded up this reduction scheme by agreeing (in October 1994) to a use reduction of 25% based on the 1991 reference level, to be achieved in 1998.

The Ministry of Environment of The Netherlands has established, within the framework of a multi-year policy plan on hygiene and material protection, a specific action plan for stored-product protection and MB. This plan is designed to reduce the negative environmental consequences of the use of MB. Specific aspects of this plan include, among other actions, the reduction of MB emissions to the atmosphere and stimulation of research on alternatives to the use of MB.

Within the framework of this action plan, the Ministry of Environment has asked TNO to carry out an investigation into alternatives for MB that already are available or will shortly become available for practical implementation. The project started with an inventory which included a survey of current uses of MB in The Netherlands and listed potential alternatives that could be considered for further examination in pilot tests. The second phase of the project was focused on setting up a number of practical pilot tests with selected alternatives in order to explore their potential for future use as a replacement for MB in The Netherlands. This paper gives a concise overview of the results of both phases of the project.

OVERVIEW OF CURRENT USES OF METHYL BROMIDE

Data collection

Since the use of MB for soil sterilisation was banned in 1992, MB is currently used only for fumigation of durables and for structural and preshipment treatments. Following the Montreal Protocol these use categories are also subject to a reduction scheme.

Registration of use-figures is essential for the implementation of any effective reduction scheme. Since 1990 fumigation companies have been obliged to notify a central register of any intended fumigation. Notification time depends on the size of the fumigation. Fumigations of objects with a volume larger than 2,500 m^3 have to be announced at least 7 d in advance; small-scale fumigations up to volumes of 500 m^3 may be announced only 6 h prior to fumigation. Companies have to indicate, among other things, the following data:

1. Date, time and location of the fumigation
2. Type and amount of product being fumigated
3. Type and volume of structure intended to be fumigated
4. Quantity of MB that will be used

5. Quantity of MB used (announced after the fumigation)
6. The necessity for a fumigation

Because it is collected at a central place, this register provides a database that, after a few years of data registration, has enabled trends in the MB use pattern to be analysed.

Use pattern

Table 1 gives an overview of the total amount of MB used in the years 1990 to 1995. The first row indicates the amount of MB of which fumigation companies have given advance notice. The second row shows the actual use figures that were determined afterwards from the adminstration of fumigation companies. The total amount used decreased from 64,471 kg in 1990 to 17,038 kg in 1995. In this last year, a major use reduction of 60% was thus achieved in comparison to the reference level (43,008 kg in 1991 as per the EU reduction scheme). For comparison, the amount of MB used worldwide for applications other than soil fumigation is approximately 15,000 t, which constitutes approximately 22% of total MB consumption worldwide.

TABLE 1
Total quantities of MB (in kg) notified ahead and actually used in The Netherlands in the years 1990–1995

Category	1990	1991	1992	1993	1994	1995
Notification ahead	50,154	42,413	37,452	28,571	26,851	13,707
Actually used	64,471	43,008	38,366	29,495	27,269	17,038

In Fig. 1 the seasonal trend of total MB use (in kg) is shown. The strong reduction in 1995 can be attributed especially to lower use in the summer months. In 1992 peak levels, up to 10,000 kg per month, could be observed, whereas the summer of 1995 actually had the lowest use figures. The total number of fumigations did not decrease but in fact went up from 833 in 1992 to 1,078 in 1995.

Major products

Table 2 specifies MB use per product category over the years 1993 to 1995. For all years, the major products have been — and still are — groundnuts (peanuts) and cocoa beans; both are bagged commodities mainly stored in the ports of Rotterdam, Amsterdam and Vlissingen. However, significant reductions for both products were achieved last year.

Using the notification forms as a guide, the environmental inspection authorities have increased their activities in both 1994 and 1995, focusing on the necessity of fumigations as well as on possibilities for reduction of their size. This increase of activity has probably contributed significantly to the major use-reduction achieved in 1995.

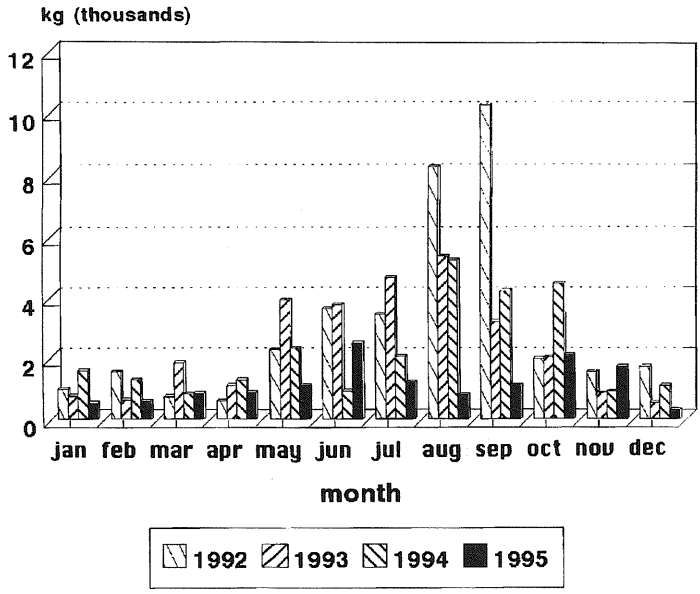


Fig. 1. Seasonal trend in MB use in The Netherlands during the years 1992 to 1995.

TABLE 2
Major products and current MB use (kg) in the years 1993–1995

	Year		
Products	1993	1994	1995
Groundnuts	10,720	9,583	2,906
Cocoa beans	4,910	5,827	2,328
Containers (*Sirex*)	–	3,616	2,774
Structures	2,666	2,507	1,991
Nuts/Seeds	691	1,363	1,303
Rice	1,430	1,575	718
Coffee	704	815	451
Pet food	1,167	505	424
Grain	3,021	40	65
Others	3,262	1,020	747
Total	28,571	26,851	13,707

Because of increased inspection, fumigation companies may have been persuaded to carry out bag-stack fumigations rather than warehouse fumigations after the necessity for this had been demonstrated. Isolation of stacks of infested produce under plastic covers eliminates a lot of empty space and thus reduces the initial dose required. The average size of a fumigation, based on quantity of MB used, was reduced from 46 kg in 1992 to 12 kg in 1995, and this indicates the shift to smaller fumigations.

Groundnuts, as well as cocoa beans, are stored in bags of approximately 50 kg piled up to 8 to 10 m high in stacks in large warehouses. During the last years there has been an increase of available cooled storage facilities in various ports. Cooled storage at temperatures below 10°C may effectively prevent insect populations from further development. An increase of the amount of produce being stored at low temperatures may also have contributed significantly to use reduction of MB. Whether this shift has indeed taken place (and can partly explain the large use reduction) is not clear.

Container fumigation is a very important use category. This is apparent from specific use figures collected in the last 2 years: more than 3,600 kg in 1994 and almost 2,800 kg in 1995 (see Table 2). These quantities were used to meet the Australian quarantine regulations with regard to possible *Sirex* woodwasp infestation in the dunnage or wooden floors of containers. The logistic process at container terminals in The Netherlands nowadays is fully adapted to a fast-acting disinfestation procedure which, until now, can only be accomplished by fumigation with MB.

Fumigation of empty structures (primarily buildings like flour mills or silo bins) is a significant use category for which, in 1995, a 20% reduction was achieved.

A striking phenomenon seen in Table 2 is the very large reduction in MB use for fumigation of grain in the years 1994 (40 kg) and 1995 (65 kg) in comparison to 1993 (over 3,000 kg). This could be due to a great reduction in grain tonnage stored in The Netherlands. Nowadays, The Netherlands no longer has any strategic stocks of grain. Replacement of MB by other pest control measures, such as phosphine (PH_3) or sprayable insecticides, might provide another explanation for the great use reduction.

In the United Kingdom the most common fumigant for use in stored grain is PH_3. Although this substance is registered in The Netherlands as well, it is not commonly used. Until March 1996 no advance notification was required for a fumigation with PH_3, so central use figures on this fumigant are not available. Thus, a possible shift from MB to PH_3 cannot be established. Similarly, a possible shift to increased use of sprayable insecticides cannot be established either.

USE REDUCTION AND ALTERNATIVES

From the environmental point of view, the best option for MB use reduction is the application of hygienic and preventive measures on a larger scale. Visits to warehouses and current application sites of MB, as well as contacts with responsible authorities, have indicated that there is room for improvement in such measures. Specific methods of improvement may vary with different types of products and different storage and processing conditions, as well as with logistic and economic factors. Cooled storage, by preventing pest populations from building up to damaging levels, can be considered a basic component of a preventive approach.

After identification of potential alternatives in the first inventory phase, the major objective of the second phase was to set up, in collaboration with interested parties, pilot

tests that would increase the level of knowledge about practical application of alternatives and might also demonstrate the feasibility of some selected alternatives. The initiatives of fumigation companies and other interested parties were stimulated and, where appropriate, experimental protocols were set up by mutual agreement.

Data from recent literature suggested four categories of alternatives that could potentially replace — at short term — part of the current use of MB: e.g. controlled atmosphere treatments, physical control methods, other fumigants and sprayable insecticides.

Among the fumigation companies there was high interest in the development of controlled atmosphere (CA) treatments as an alternative for MB. This was because CA applications are not subject to any distance requirements and registration is expected to be much easier and far less costly than that for toxic chemical methods. The use of carbon dioxide (CO_2) for pest control is already exempt from registration.

The two largest product categories (groundnuts and cocoa beans) for which MB is currently used are usually stored for periods of up to several months. Because the need for disinfestation varies, a fast-acting method is not always absolutely necessary, although currently it is still the option preferred by the interested parties, e.g. buyers, suppliers, warehouse managers and insurance companies. In principle, however, average storage periods of up to several months open opportunities for alternative treatments that require long treatment times, for instance those based on CA's.

PILOT TESTS

On the basis of both the use pattern figures and the preferences of participating companies, it was decided that pilot tests would be focused on possible alternatives for treatment of groundnuts and cocoa beans and on alternative structural treatments.

Pilot tests were carried out with CA treatment of groundnuts and cocoa beans, heat treatment for disinfestation of a flour mill, application of inert dust in grain storage and flour mills and disinfestation of rice by treatment with microwaves.

An additional test focused on emission control, determining the ability of an activated carbon filter, installed at a permanent fumigation facility, to recapture MB.

ISOGEN burner gas system

The tests using the ISOGEN burner gas system for maintaining an effective CA regime (<2% O_2, 12% CO_2, balanced by N_2) to disinfest groundnuts have demonstrated the technical feasibility of the system. Three separate tests were carried out in a 400-m^3 climatized cell. Energy and fuel consumption were determined, composition and flow of outlet gas and temperature were monitored continuously and the effects on various insect species were investigated. Effective treatment times depended on ambient temperature but were generally much longer than those of MB. Tests were conducted during a period of high average temperature (29–31°C), and effective exposure times of 7.5–8 d were found. A preliminary assessment of running costs revealed that this treatment is probably less expensive than fumigation with MB.

In Italy the system is in commercial use in large feed mills to treat a variety of products and also for treatment of stored grain in silos, indicating the economic feasibility of the technique. To enable this system to work on a larger practical scale in The Netherlands, a follow-up developmental program has recently been started.

Carbon-dioxide fumigation

Fumigation of infested commodities like groundnuts, cocoa beans, maize or rice with CO_2 is an effective technique already used in practice in developing countries like Indonesia. Effective treatment times range from 10 to 20 d, depending on the temperature. Pilot tests in The Netherlands for treatment of bags of infested cocoa beans with CO_2 at 20°C were not successful. Basically, the same technique was used, creating a high CO_2 (>40% v/v) and low O_2 (<2% v/v) atmosphere using CO_2 from cylinders.

Small bag-stacks (about 2 t) of cocoa beans were sealed in plastic enclosures with and without floor sheeting. Temperature and gas concentrations were monitored continuously. The failures could be attributed mainly to the inappropriate equipment with which the CA regime was applied. Flushing the bag-stacks with N_2 prior to CO_2 application in order to accelerate the build up of a low O_2 atmosphere did not improve the performance of the system. All sealed bag-stacks were pressure tested before starting. Despite this, a stable low O_2 atmosphere could not be maintained for a period long enough to achieve any biological effect.

Basically, however, the technique lends itself to disinfestation of bagged commodities like cocoa beans which, in The Netherlands, are usually stored for longer periods of time. Further investigations with improved equipment are required to determine the potential of this technique in The Netherlands.

Heat treatment

Pilot tests were carried out by the Meneba company in Rotterdam. Meneba has set up a very detailed working protocol listing all the activities that have to be carried out, including the cool-down period after the treatment, in relation to the implementation of the treatment. Special care must be taken to prevent insects from escaping from the heated areas to cooler areas (where they might survive and start to build up a new population) and to prevent any damage to materials and electronic equipment by heat or low humidity.

A steam boiler was used for generating heat, and hot air was circulated through the ventilation system of the building. Target air temperature was 55–60°C to be reached as quickly as possible but with a maximium temperature increase of 6°C per hour. The target temperature had to be maintained for at least 24 h. Cooling down afterwards was similarly done at a maximum rate of 6°C per hour.

During both large scale trials (in 1994 and 1995), sufficient insect mortality was in general achieved. At some particular spots, however, live insects were still found shortly after the end of the heat treatment, demonstrating the existence and location of so-called cool spots.

The costs for installation of the equipment required for effective heat treatment were quite high. Judging from the experience of Meneba, however, the energy costs for a heat treatment are reasonable and the costs for sealing and preparation of the building are less than those for a fumigation with MB. The shut-down period for a heat treatment is the same as that for a fumigation with MB.

Overall, the experience obtained so far indicates that heat treatment is a viable option when it is considered as a general disinfestation method for a building, but it is not a substitute for good housekeeping and proper sanitation and preventive measures. In the future, heat treatment may be a central curative method that, supplemented with various other control methods, can replace part of the current MB use for structural fumigation.

Inert dust

Inert dust or diatomaceous earth can be considered a physical control method due to its mode of action: it causes desiccation in insects. Its efficacy is highly dependent on ambient temperature and relative humidity. In laboratory experiments at 20°C and 65–70% r.h., the recommended dosages were found to be insufficiently active to act as a stand-alone method for the control of grain weevils and confused flour beetles.

When applied to floors in a flour mill, however, inert dust was found to have a very clear effect in retarding and sometimes preventing new populations of flour beetles from building up. As a complementary treatment to other control measures, application of inert dust may certainly contribute to improved pest control. In this respect the regulatory status of inert dust in The Netherlands should receive special attention.

Microwave treatment

Experiments with microwave treatment of rice conducted by a private company in The Netherlands have shown that, in principle, this method could be a viable option for fast and effective disinfestation of flowing bulk products. However, a substantial amount of work related to technical scale-up and assessment of economic parameters remains to be done.

Emission control

A filter installation based on activated carbon has been installed at a permanent fumigation chamber at the flower auction in Aalsmeer. A series of measurements was carried out to determine the removal efficiency and the effective lifetime of this filter during normal operation procedures. Gas concentrations were measured before and directly after passing the carbon filter during the first phase of ventialtion of the chamber. For up to approximately 75 fumigations, the filter appeared to be very effective in removing MB from a passing gas stream. Typical concentrations before and after passing the filter were 18 g/m^3 and <2 mg/m^3, respectively, resulting in a removal efficiency of more than 99.9%.

In fact, the installation was found to remain highly effective in recapturing MB from an air stream during its full time of operation. This means that more than 99.9% of the former MB emission from this facility can now be prevented. Saturated filter material is

dispatched to a high temperature incinerator in which bromine is ultimately recaptured as a salt in the flue gas scrubber.

The permanent fumigation facility in Aalsmeer is in use for quarantine treatment of a large variety of agricultural products. Expansion of similar recapturing systems to other fumigation facilities may contribute to further prevention of MB emissions.

CONCLUSIONS

The use of MB in The Netherlands for stored-product protection and structural fumigation has shown a drastic decline from 43,000 kg in 1991 (the reference year in international reduction plans) to 27,000 kg in 1994 and 17,000 kg in 1995. The reduction of 60% obtained in 1995 is far greater than the intended reduction schemes of both the Montreal Protocol (25% reduction in 2001) and the European Union (25% reduction in 1998).

Major causes for the reduction achieved are probably the implementation and improvement of an obligatory notification system, increased inspection activities of the environmental inspection authorities, a shift from warehouse fumigation to bag stack fumigation resulting in strong dosage reductions, increasing facilities for cooled storage and reduction in the amount of produce (grain) being stored in The Netherlands.

Major use categories for MB applications in The Netherlands currently are as follows: groundnuts (2,900 kg), cocoa beans (2,300 kg), structural fumigations (2,000 kg) and fumigation of containers (about 2,800 kg).

Prevention, proper sanitation and other hygienic measures may contribute significantly to use reduction of MB. Cooled storage can prevent the build up of pest populations to damaging levels. Structural integration of cooling equipment in storage facilities can contribute to further reduction of MB use in stored-product protection.

For effective treatment of commodities like groundnuts and cocoa beans, CA is a viable option. Systems using CO_2, N_2 and burner gas for generating and maintaining CA regimes are at different stages of development.

Heat treatment of buildings, although not always fully effective or applicable to every building, can partly replace the use of MB for structural fumigation. Additional treatment of so-called cold spots with contact insecticides and complementary use of inert dust at inaccessible sites or void spaces inside the building may help to suppress the build-up of new pest populations.

Currently, the registration requirements for CA applications for the purpose of pest control are not clear. In The Netherlands at present, the use of CO_2 for control of pests is exempt from registration whereas other types of CA treatments are not. Clarification of this regulatory status will help to provide a better perspective on the future role of CA treatments in the replacement of MB.

ACKNOWLEDGEMENT

This project was commissioned by the Ministry of Environment of The Netherlands under reference number 94.22.0253.

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 589–599

COMMERCIAL QUARANTINE FUMIGATION OF NARCISSUS BULBS TO CONTROL NARCISSUS FLIES

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ABSTRACT

Fumigation trials were carried using methyl bromide (MB) to control narcissus flies in bulbs destined for export. The objective was to increase fumigation efficiency by utilizing two gastight fumigation chambers (fumigation bubbles) in order to reduce the danger of phytotoxic damage by applying the minimum effective dose required to kill the insects.

It was found that for the 4 h exposure regime required for quarantine purposes, recirculation was necessary to produce initial uniform concentrations. Dosage in the 48 m^3 and 102 m^3 capacity bubbles (without inflation) had to be adjusted to take into account both the volume of free space within the bubbles and sorption by the bulbs. MB concentrations recorded by thermal conductivity (TC) monitors were influenced by the CO_2 concentrations generated from bulb metabolism during fumigation. With recirculation in the 48 m^3 bubble, an initial dosage of 25 g m^{-3} resulted in stabilization of MB concentrations after 90 min at close to 20 g m^{-3}. This concentration is close to the LD_{99} of *Merodon eques* as evaluated in laboratory studies.

INTRODUCTION

Israeli growers have been requested to fumigate narcissus bulbs in order to comply with the quarantine requirements set by importing countries, in particular the USA. This is due to the presence of at least two species of narcissus fly, belonging to the genera *Merodon* and *Eumerus*, that attack the bulbs of *Narcissus* and *Amaryllis* and are not present in some of the importing countries.

In the past the commercial fumigations were carried out in rigid fumigation chambers based on fumigation schedule N (fumigation of flower bulbs and corms) in the *Manual of Fumigation for Insect Control* (Bond, 1984) that requires a methyl bromide (MB) fumigation at a dosage of 45 g m^{-3} for 4 h at temperatures above 21°C. However, in recent years several fumigations with this schedule were found to have caused phytotoxic damage. Quality control operators discarded the damaged lots in which scorching was observed.

Although there was no clear explanation for these phenomena, certain possibilities were suggested. The cause may have been lack of uniform gas distribution within the fumigation chamber (in routine fumigations carried out under standard practice, MB concentrations were not monitored at different points within the chamber). It may have been insufficient aeration after fumigation because as the gas desorbs from the bulbs at the end of the exposure period, high concentrations are liable to accumulate and damage the bulbs unless they are removed rapidly and effectively by forced aeration. It may have been excessive dosage levels; since the amount of MB sorption by the bulbs was not known, it was not possible to calculate the concentration of free gas available to control the insects.

In order to provide better distribution, it was decided by the pest control operator to use an especially designed flexible plastic chamber (fumigation bubble) equipped with a fan driven dispenser system to facilitate distribution of MB. Because of the increased gas-tightness obtainable in these chambers, it was also planned to reduce the dosage on the assumption that the schedule N listed by Bond (1984) was based on some allowance for leakage in previously employed, less gastight, rigid structures.

To examine these possibilities and establish a fumigation schedule acceptable to both the growers and quarantine authorities, four commercial fumigations, from among a series of commercial fumigations of narcissus bulbs carried out for quarantine purposes prior to export, were monitored. The treatments were carried out at Ashdod harbor in Israel. In order to enable the results of these fumigations to be evaluated, the level of sorption of MB into bulbs was examined, and the sensitivities to MB of both the large and small narcissus flies occurring in Israel were also examined in the laboratory (Donahaye *et al.*, 1997).

The objectives of this study were to examine concentrations and application techniques at the recommended dosage levels, adjust them in order to obtain lethal concentrations over the required exposure period and, at the same time, minimise phytotoxic damage to the narcissus bulbs.

MATERIALS AND METHODS

The flexible fumigation chambers

The chambers were "Rentokill fumigation bubbles" of two sizes, consisting of flexible plastic over- and under-liners zipped together to form a sealed enclosure. The bubbles were linked to an application unit consisting of a MB dispenser and a fan employed to inflate the chamber initially, to introduce the gas into the bubble and to aerate it after fumigation.

The bulbs were packed in crates that were arranged nine layers high on wooden pallets; each layer consisted of five crates containing 20 kg of bulbs each. The pallets were then trucked to holding sheds at the port, where the fumigations were carried out. Forty-two crates were placed in the large fumigation bubble and 15 to 18 in the small one.

Dimensions and treatment capacity of the bubbles were as shown in Table 1.

TABLE 1
Dimensions and treatment capacity of the bubbles

Parameter	Large bubble	Small bubble
Length	8.5	6.0
Width	6.0	4.0
Height	2.0	2.0
Volume (not inflated, m^3)	102	48
Volume (inflated, m^3)	180	80
Number of pallets	42	15–18
Volume of each pallet with crates (m^3)	2.22	2.22
Gross volume of pallets (m^3)	93.2	33.3–40.0

Pre-fumigation procedure. The lower section of the bubble was laid on the ground, and the pallets were placed on it with a fork lift. Gas sampling points were then laid out and threaded through a gasket in the liner membrane, and the overliner was drawn over the stack and attached to the underliner by means of a tongue-and-groove zipper.

Gasing and degasing. Four fumigation trials were carried out. In the first three trials, the fumigant dosage was calculated per canister of MB and the dispenser fan was operated to blow air into the bubble. Then the canister was punctured and gas liberated into an expansion chamber in the ventilation duct from which it passed into the bubble. The fan was turned off after the bubble had been partially inflated. In the fourth trial, the MB was dispensed from a weighed cylinder and the fan was used to recirculate the gas for about 1 h after the introduction of MB after which it was turned off.

After 4 h the zipper of the bubble was opened by fumigation operators wearing gas masks. Aeration was carried out using large axial fans designed for greenhouse ventilation. Two fans were used for the large bubble and one for the small. They were located facing the pallets at a distance of about 2 m and were activated all night to remove the desorbing gas and prevent possible build-up of gas concentrations in the air spaces between the bulbs within the crates.

Monitoring and sampling procedures

Concentrations of MB. Concentrations within the bubble were measured by attaching the gas sampling tubes to a Bedfont MB gas monitor Model 415, equipped with a thermal conductivity detector which is also susceptible to carbon dioxide (CO_2) concentrations in the air. Additional laboratory tests were made to determine the rate of CO_2 release by the metabolism of the bulbs. The concentrations of CO_2 were measured separately during the trials using an infra red CO_2 analyser (Riken Model RI 550A).

In order to adjust the MB concentration readings, a set of laboratory fumigations was carried out using different combinations of MB and CO_2. A CO_2 calibration curve was

then prepared for the MB monitor, and the MB readings in these trials were corrected accordingly.

Temperature measurement. Temperatures inside and outside the fumigation bubbles were measured several times during each fumigation using t-type thermocouple cables and an electronic thermometer. Temperatures were measured at the top and bottom of the bubble.

Gas sampling. MB measurements were taken by placing sampling tubing at five positions within the bubble, points 1, 2, 3 and 5 situated at the centre of the bubble from floor level to below the upper plastic liner, point 4 next to the gas inlet site and point 6 adjacent to the wall opposite the gas inlet site, the latter two half-way up the pallets (Fig. 1).

Bulb sampling. Before fumigation, 30 bulbs previously sorted for suspected infestation were placed in the bubble at the bottom, top and center of each fumigated stack. These were removed at the end of fumigation and examined for fly mortality and visible phytotoxicity in the laboratory. Additional bulbs were taken from the gas sampling points for subsequent planting in order to examine possible phytotoxic effects.

Description of fumigations

Trial 1. The first fumigation trial was carried out using a large fumigation bubble according to the fumigation operator's normal schedule. This fumigation lasted 3 h 50 min.

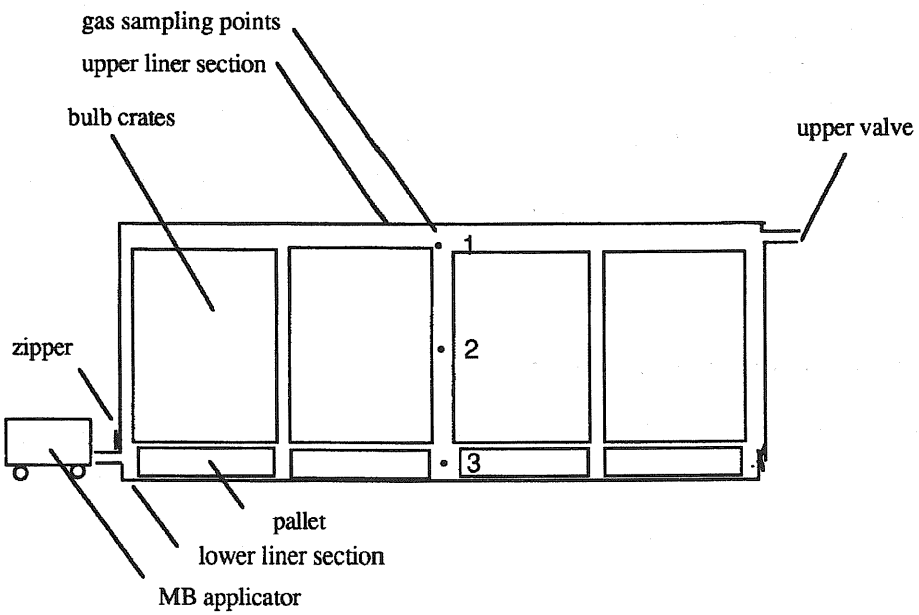


Fig. 1. Schematic view of the fumigation bubble indicating position of gas sampling points.

Dosage was with seven canisters containing 681-g MB each, calculated as equivalent to 27 g m^{-3} total space (based on a volume of 180 m^3 for the inflated bubble). Gas was introduced from the dispenser fan to the bottom of the bubble, thereby inflating it. After the gas had been dispensed and the bubble inflated, the fan was turned off.

At the end of the exposure period, the upper section of the bubble was removed and the crates were aerated with the external axial fans. Gas concentrations were measured 30 min and again 1 h after aeration was started.

Trial 2. Because of the difficulty in controlling the level of bubble inflation, and consequently in obtaining a predetermined gas concentration, the objective of this fumigation was to reduce the initial inflation and lower the dosage in order to avoid the high initial concentrations, particularly in the floor area, that were suspected of causing scorching of the bulbs. The fumigant was introduced from the bottom, as in the previous trial.

Fumigation in the large bubble was with a dosage of three canisters and minimal inflation, and in the small bubble with two canisters, to release 2,043 g and 1,362 g (equivalent to calculated dosages of 27 g m^{-3} and 30 g m^{-3}), respectively.

Trial 3. In this trial, carried out in both large and small bubbles, the fumigant was applied at the top in an attempt to decrease the problem, encountered in the previous trial, of layering at ground level. Dosage rates, monitoring and bulb sampling were all the same as in trial 2.

Trial 4. In this trial only the small bubble was used and 15 pallets were loaded instead of the 18 in previous trials. The bubble was not inflated, and the volume of the bubble was confined to the rigid volume of the pallets (including the space comprising the pathways between the pallets). The volume of this enclosure was 44 m^3. Dosage was administered from a MB canister placed on scales to enable the MB to be released by predetermined weight. The objective of this trial was to use closed-circulation fumigation so as to equalise concentrations as soon as possible after application of the dose. Recirculation using the applicator fan was carried out for 90 min. During this time the possibility of gas leakage via the aeration ducting was examined using a halogen detector, and leakages were detected at the joint flanges. An initial dose of 1,104 g of MB, equivalent to 30 g m^{-3} (non-inflated bubble), was applied by recirculation.

RESULTS

Trials

Trial 1. The results of this trial are given in Fig. 2. MB concentration readings show that although a calculated dose of 27 g m^{-3} was given, initial concentrations ranged from 36 to 81 g m^{-3} and averaged 67 g m^{-3}. At the end of the fumigation they were uniform and had stabilized at 59 g m^{-3}. Concentrations throughout the exposure period were highest at the bottom of the stack.

After 30 min of post fumigation aeration, MB concentration had fallen to between 0 and 3 g m^{-3}, and after 1 h no MB was detected. Temperatures during fumigation ranged

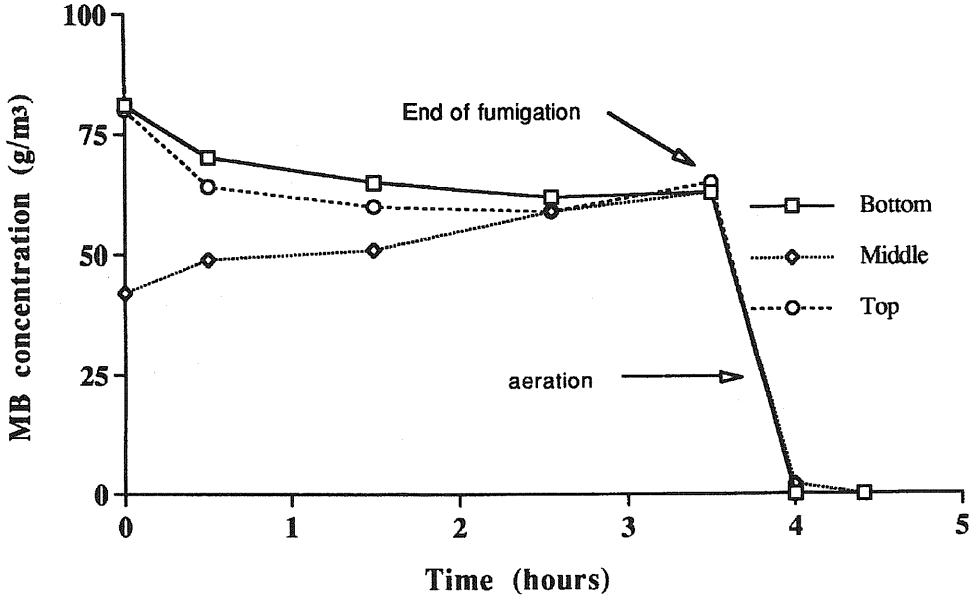
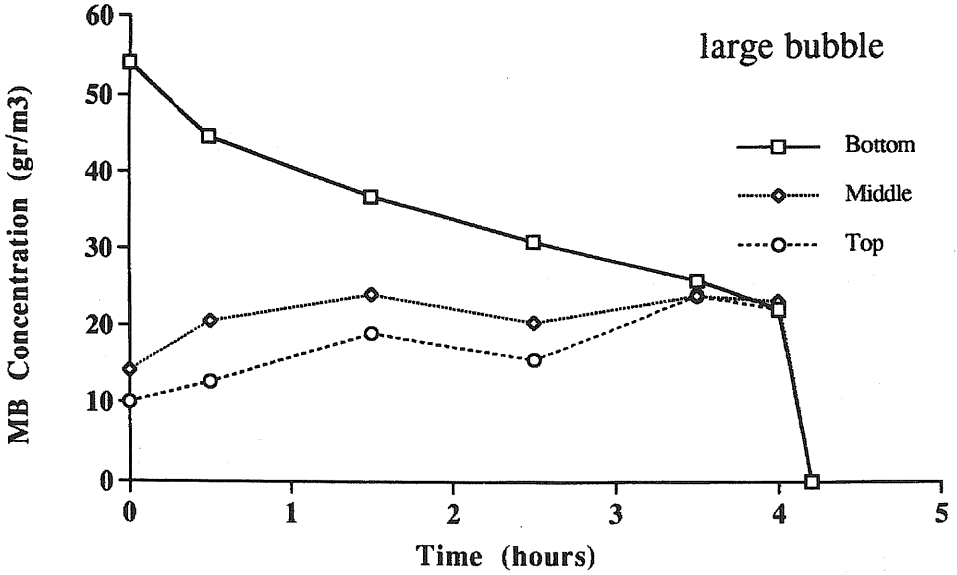


Fig. 2. Fumigation in large bubble by inflation with gas introduced from below — trial 1 (intended dose 27 g m^{-3}, actual average initial concentration 67 g m^{-3}).

between 25.6 and 30.8°C. The infested bulbs exposed to the fumigation revealed 12 dead larvae and 7 dead pupae of *Eumerus* sp.

Trial 2. In this trial, an attempt was made to reduce the number of 681-g MB cans, and the bubbles were inflated only slightly to adjust the volume approximately to the calculated concentrations. Results of the two fumigations carried out in this trial are given in Fig. 3. At the beginning of dose release, high concentrations were still recorded at the base of the fumigation bubbles, reaching a maximum of 54 g m^{-3} in the large bubble and 36 g m^{-3} in the small bubble. Only towards the end of the fumigation period were the concentrations fairly uniform at about 27 g m^{-3} in the large bubble and 30 g m^{-3} in the small one. One half hour after the bubbles had been opened and aerated, no fumigant concentrations were recorded at any of the sampling points. Fumigation temperatures were between 27.6 and 29.6°C in the large bubble and between 29.2 and 30°C in the small bubble.

Trial 3. The design of this trial was similar to trial 2, but the gas was introduced from the top of the bubbles. The results of the fumigations are given in Fig. 4. From the figure it can be seen that differences in MB concentrations in both bubbles were smaller than in the previous trials; however, there was still a tendency for the gas to sink to the bottom of the bubble, producing higher initial concentrations (35 g m^{-3}) in this region, and only towards the end of the exposure period were more uniform concentrations, averaging about 20 g m^{-3} for the large bubble and 19 g m^{-3} for the small bubble, obtained.



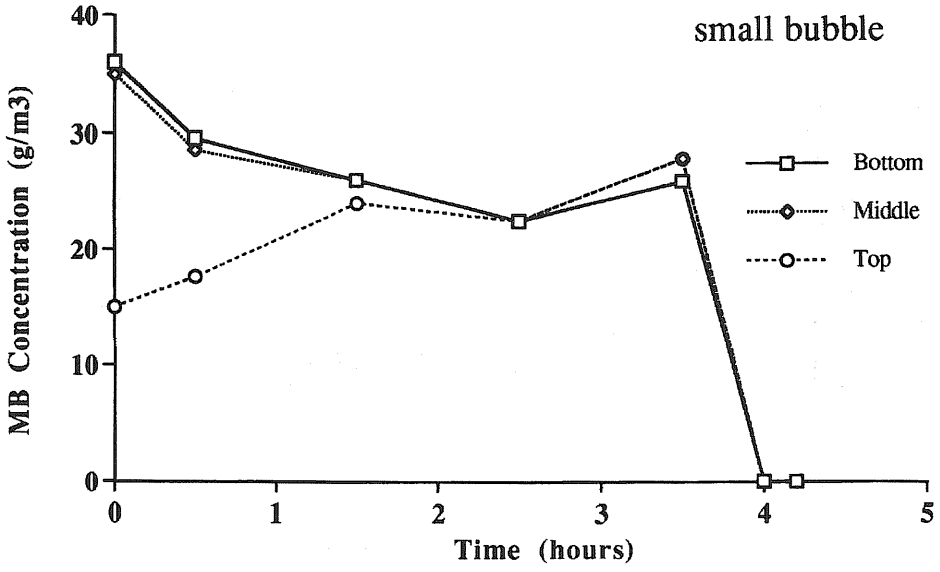


Fig. 3. Fumigation with minimum inflation and gas introduced from below — trial 2 (large bubble: intended dose 27 g m^{-3}, actual average initial concentration 26 g m^{-3}; small bubble: intended dose 30 g m^{-3}, actual average initial concentration 28 g m^{-3}).

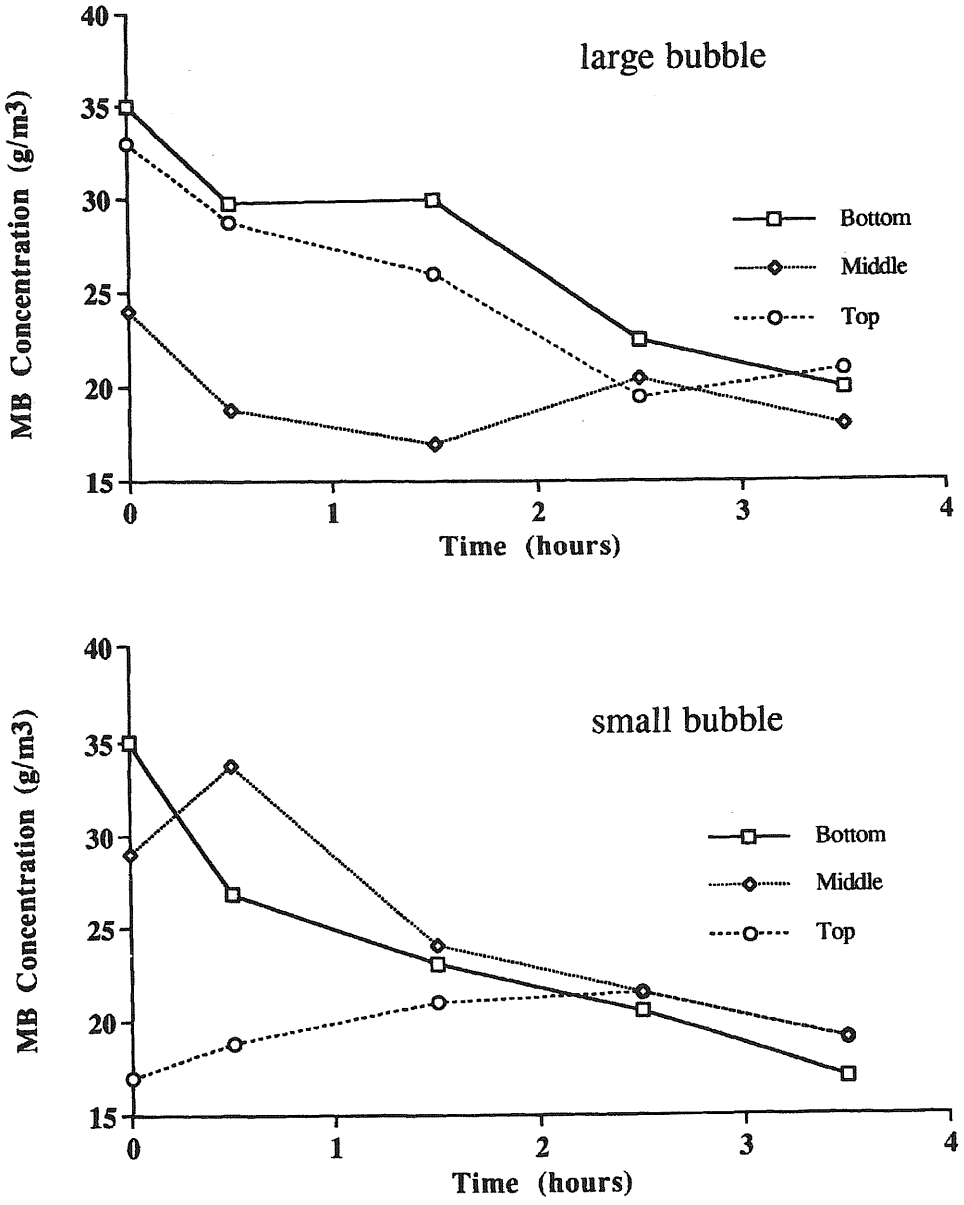


Fig. 4. Fumigation with minimum inflation and gas introduced from top — trial 3 (large bubble: intended dose 27 g m^{-3}, actual average initial concentration 31 g m^{-3}; small bubble: intended dose 30 g m^{-3}, actual average initial concentration 27 g m^{-3}).

Trial 4. This trial was designed to introduce MB without inflating the bubbles (in contrast to previous trials). Since it was very difficult to accurately calibrate the dosage using entire cans of 681 g MB each, in this trial the MB was introduced by weight. In addition, to obtain a better distribution, in this trial the fumigant was circulated. Figure 5 shows that the MB concentrations became uniformly distributed after about 1.5 h, at which time the circulation fan was turned off. Concentrations from then on remained at about 20 g m^{-3} throughout the fumigation.

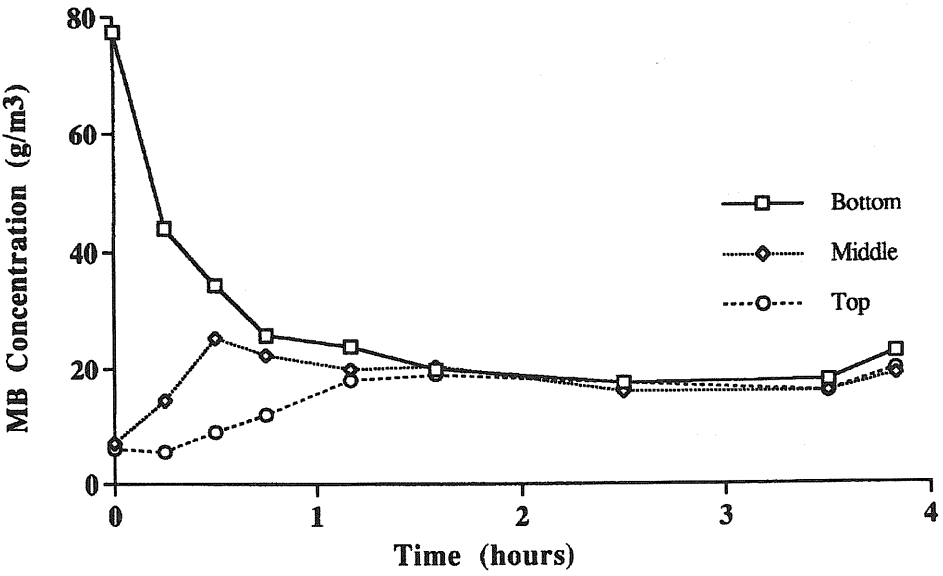


Fig. 5. Fumigation of small bubble with aisles between crates, gas introduction from top and closed recirculation — trial 4 (small bubble: intended dose 30 g m^{-3}, actual average initial concentration 30 g m^{-3}).

Plant growth

Bulbs taken from trials 1, 2 and 3 were planted, together with unfumigated bulbs as controls, in a plot at Moshav Bitzaron. Although high initial concentrations were observed in these trials, no significant differences between treated and control bulbs were observed as regards vegetative deformation, reduction in flowering heads or retardation of growth.

Typical CO_2 concentrations measured in trial 4 are shown in Fig. 6. This accumulation of CO_2, as measured within the 4 h fumigation period, shows that a considerable concentration (up to 3.8%) can evolve from the bulbs. This CO_2 concentration renders the use of instruments equipped with TC detectors unfit for monitoring changes in MB concentration. Therefore, it was decided in these trials to use an infrared detector for measuring the CO_2 accumulation within the fumigation bubbles separately in order to correct the MB readings.

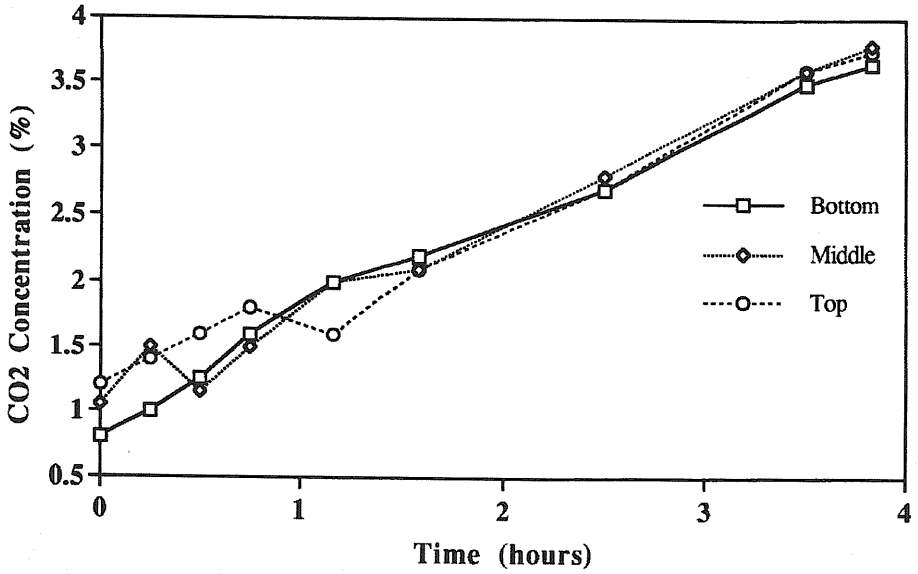


Fig. 6. Fumigation of small bubble with aisles between crates, gas introduction from top and closed recirculation — trial 4: CO_2 emission during fumigation.

DISCUSSION

Sensitivity of narcissus flies to MB has also been studied by Zumreoglu and Erakay (1978) in Turkey, though they used a 2-h exposure period and higher Ct ratios than the 180 g h m^{-3} advocated by Bond. In the present trials the Ct product was designed to be within the range of 80 to 100 g h m^{-3}. This dosage schedule was adopted following the work carried out by Donahaye *et al.* (1997).

The above series of fumigations form an initial stage in the establishment of a fumigation schedule suitable for the treatment of narcissus bulbs inside fumigation bubbles with MB. The results show that when an exposure limit of 4 h is set, the method advocated by the manufacturer, in which the gas is dispensed into the fan duct used to inflate the bubble, does not provide rapid uniform concentrations. For this to happen, the applied dosage must be recirculated and passage ways provided between the palleted crates. For bulbs, in contrast to the fumigation of less sensitive commodities, there is also a relatively narrow range of permissible fumigant concentrations that will assure complete kill of the insects yet ensure no phytotoxic effects. Therefore dosage application using canisters does not enable sufficiently fine adjustment of the required dose, and the method of weighing the dose, although less convenient, should be preferred. Further trials will be undertaken in order to optimize the fumigation method.

CONCLUSIONS

The trials revealed that the bubbles were sufficiently gastight to enable fumigation with MB for an exposure period of 4 h. For accurate calculation of the dosage, it was shown that the bubbles should not be inflated and that the considerable sorption of MB by the bulbs should be taken into account. Recirculation was needed to achieve a rapid equalization of gas concentrations. No phytotoxic effects were observed in treated bulbs grown in experimental plots, and samples of fly-infested bulbs examined after fumigation indicated complete mortality.

ACKNOWLEDGEMENTS

We wish to thank the staff of AGREXCO, the staff of Mr Eitan Amichai's Pest Control Company and the operator, Mr Avigad, for their close cooperation during the course of the fumigations. We would also like to express our particular appreciation to Mr Meir Rahat of AGREXCO for the assistance provided, Mr Arik Ben-Arie of the Flower Growers Association for coordinating the activities and Ms Ayelet Lavee of the Extension Service of the Ministry of Agriculture for undertaking the phytotoxicity trials.

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THE PRESENT USE OF METHYL BROMIDE AS A FUMIGANT FOR STORED PRODUCTS IN DEVELOPING COUNTRIES: RESULTS OF A RECENT SURVEY

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ABSTRACT

In 1992, under the terms of the Montreal Protocol Agreement, methyl bromide (MB) was listed as an ozone-depleting substance. It was expected that a programme to control its use would be decided in 1995. Some developing countries have expressed concern that any controls on MB may adversely affect trade, although uncertainties regarding the effect of future restrictions were, in part, considered likely to be due to the lack of detailed information about the continued importance of the chemical. To overcome some of these uncertainties a survey of both MB use and potential alternatives was organised by the United Nations Development Programme in three regions. The results obtained in the surveys and the implications for Latin America and Southeast Asia and the Pacific, as well as for English-speaking Africa, are described.

INTRODUCTION

Fumigation continues to play a major role in the protection of stored products against damage by insect pests. By the beginning of the 1990's only two fumigants were in regular word-wide use, phosphine (PH_3) and methyl bromide (MB); principally because of hazards to human health, almost all of the other 14 fumigants listed by Bond (1984) had been discarded. PH_3 is an extremely popular fumigant, particularly in developing countries where it may be the only fumigant used. One of its main strengths is the relative ease with which it can be applied in comparison to MB. However, a distinct disadvantage of PH_3 is the long period of exposure required; a minimum of 5 d is now being recommended. Where swift disinfestation is necessary, such as with cargoes awaiting transportation, PH_3 is often not appropriate. In such cases MB is employed because MB treatments can be completed in 24 h, reducing waiting time and minimising demurrage charges.

There is at present no ready alternative fumigant to MB for short-period treatments, and this is a significant factor in its continued importance for disinfesting stored products.

There are other continuing uses for MB in stored-products protection, and these have been dealt with comprehensively in the report of the Methyl Bromide Technical Options Committee (MBTOC, 1995). In 1992, MB was identified as an ozone-depleting substance and formally listed under the terms of the Montreal Protocol on Substances that Deplete the Ozone Layer. Of major international concern was the increase in global use of MB and this trend is illustrated in Table 1 (Watson *et al.*, 1992). Following an investigation organised through the United Nations Environment Programme into the current uses and possible alternatives to MB (MBTOC, 1995), agreement was reached that in January 1995 developed countries would introduce a freeze on consumption of the chemical at 1991 levels. The Parties to the Protocol agreed in late 1995 on a further, more detailed control programme for MB. The programme for developed countries consists of a phase-out in 2010 with stepped reductions starting in 2001. The only controls yet agreed for developing countries, consist of a freeze, starting in 2002, in use of the chemical, based upon an average of the quantities used in the period 1995 to 1998. The position with regard to developing countries will, however, be reviewed again in 1997. Under present agreements, there are exemptions from controls on MB for all countries when the fumigant is used for quarantine and pre-shipment fumigations as well as for some critical agricultural uses yet to be defined.

Although many potential alternatives to MB exist (MBTOC, 1995), some of them require considerable development and field-evaluation programmes before they can be introduced into routine disinfestation systems. These programmes are likely to be costly, and many developing countries are not well placed to carry out such evaluations without assistance. The Multilateral Fund was established to assist developing countries in phasing out ozone-depleting substances, and the Executive Committee of the Fund decided in mid-1995 that, in determining the magnitude of assistance necessary to phase out MB, a comprehensive survey of current uses for the chemical was necessary.

TABLE 1
MB sales (1990) by use (× 1000 t) (Watson *et al.*, 1992)

Year	Soil	Commodity/ Quarantine	Structural[1]	Chemical intermediates[2]	Total
1984	30.4	9.0	2.2	4.0	45.6
1985	34.0	7.5	2.3	4.5	48.3
1986	36.1	8.3	2.0	4.0	50.4
1987	41.3	8.7	2.9	2.7	55.6
1988	45.1	8.0	3.6	3.8	60.5
1989	47.5	8.9	3.6	2.5	62.5
1990	51.3	8.4	3.2	3.7	66.6

Source: MB Industry Panel/Chemical Manufacturers Association (1992).
[1]Included residential, commercial and industrial. [2]Not released into the atmosphere.

THE SURVEY PROGRAMME

The survey, organised by the UNDP Montreal Protocol Unit, New York, was conducted over a 3-month period and completed by early November 1995 for presentation in draft form to the Seventh Meeting of the Parties to the Montreal Protocol. It was co-ordinated regionally by international specialists recruited for the purpose and, in individual countries, primary responsibility for organising surveys was delegated to national ozone units. Locally-employed national survey teams undertook the tasks of gathering information in each country.

The survey was directed at three regions. English-speaking Africa comprises 19 countries (Botswana, Egypt, Ethiopia, Gambia, Ghana, Kenya, Lesoto, Malawi, Mauritius, Mozambique, Namibia, Nigeria, Seychelles, Sudan, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe). Southeast Asia and the Pacific comprise 8 countries (Brunei, Fiji, Indonesia, Malaysia, Myanmar, Philippines, Thailand and Vietnam). Latin South America comprises 10 countries (Argentina, Bolivia, Brazil, Chile, Columbia, Ecuador, Paraguay, Peru, Uruguay and Venezuela).

The main purpose of the survey, conducted with the aid of a comprehensive questionnaire, was to determine both the consumption of MB in targeted countries and the status of potential alternatives to this fumigant. Some countries, particularly the major users, were visited by international staff to verify that data-gathering was proceeding effectively. However, the exercise was somewhat constrained by the limited time available. The survey covered the uses of MB for both post-harvest protection and soil disinfestation, the latter being a particularly important use for the chemical in some developing countries.

SURVEY RESULTS — CURRENT USES OF METHYL BROMIDE

Much of the following data quoted, with sources acknowledged, is taken from the Draft Interim Report of the survey, produced by the United Nations Development Programme (UNDP, 1995).

English-speaking Africa

This region contained the largest number of countries but, partly because of both the limited survey time available and the poor communication facilities, it produced the least amount of data. Several countries failed to respond at all to repeated requests to provide information, and it was concluded that these countries used little, if any, MB for any purpose.

Although MB is widely used in Africa, there is a very wide variation in the extent of use which is often closely related to the climate and the cropping regimes of individual countries. Countries with good rainfall patterns and extensive arable farming systems usually have marketable surpluses of such staple food crops as maize. These surpluses are purchased by parastatal marketing organisations. Fumigation of this grain is an essential element of stock management and continues to be effected in several countries by means

of MB, although alternative disinfestation techniques have begun to be adopted. These countries include Kenya and Zimbabwe, and it is notable that, in both countries, there has also been a considerable increase in the use of MB for soil fumigation in recent years, due to expanding horticultural industries, increasing the consumption of this chemical. Countries where rainfall patterns make farming practices pastoral rather than arable tend to use either considerably smaller quantities of MB or none at all. The only use of the fumigant in these countries may be in connection with imports of grain or grain products where a short disinfestation period is desirable.

In Egypt, MB is used to fumigate imported foodgrains, chiefly wheat. Of the six million t of grain annually imported, it was reported that approximately 30% is disinfested on arrival. The survey indicated that for post-harvest applications, Egypt is the largest consumer of MB in Africa. Zimbabwe uses a similar quantity of the chemical annually, but there the major use is for soil disinfestation. Information gained from the survey on the quantities of MB imported by individual countries in Africa allows them to be conveniently placed in one of three groups: countries with little or no use of MB, those with moderate use and major users. These groups are shown in Tables 2, 3 and 4.

Southeast Asia and Pacific

Comprehensive data on the uses of MB were obtained from all countries surveyed except Myanmar. Of the other seven countries surveyed, only Brunei reported no regular use of the fumigant. In this region, excepting the Philippines, the major use reported for MB was commodity fumigation. The quantity imported in 1994 and the proportion used for commodity disinfestation are both shown in Table 5.

The major use for the fumigant in this region is treatment of commodities, chiefly rice, prior to export. Thailand consumes 62% of the regional MB total. This is due to Thailand's very large annual export programme.

TABLE 2
African countries with little or nil use of MB

Country	Quantity of MB used in 1994 (t)
Botswana	0.1
Gambia	?
Ghana	0
Lesoto	?
Mauritius	0.8?
Namibia	0
Nigeria	0.3
Seychelles	0
Swaziland	1.4
Uganda	0

? = indicates no information available.

TABLE 3
African countries with moderate use of MB

Country	Quantity of MB used in 1994 (t)
Ethiopia	21
Mozambique	8.3
Sudan	78.4
Tanzania	3.0
Zambia	40

TABLE 4
African countries that are major users of MB

Country	Quantity of MB used in 1994 (t)
Egypt	572 (69)
Kenya	440 (10)
Malawi	200 (10)
Zimbabwe	604 (14)

Quantities in parenthesis represent percent post-harvest/quarantine usage.

TABLE 5
MB imports and usage in 1994, in SE Asia and the Pacific

Country	Import in 1994 (t)	Use on commodities (%)
Fiji	4	98
Indonesia	255	82
Malaysia	89	90
Philippines	63	46
Thailand	590	96
Vietnam	108	99

Latin America, South

Data were obtained from all the countries included in the survey, except Ecuador, although only limited data were provided by Bolivia and Venezuela. Table 6 gives details of MB use in the region during 1994.

Except for Peru, there is only minor use of MB for fumigating durable commodities in the countries surveyed. In Peru 86% of total fumigant usage (29 t) is for quarantine treatment of imported grain. The major use of the chemical in the region is for soil fumigation and, in some countries, particularly Chile, there is considerable use of MB for fumigating perishable commodities, such as grapes, prior to export.

TABLE 6
Quantities and percent use of MB for post-harvest applications in South American countries

Country	Quarantine (t)	Post-harvest excl. quarantine (t)	% Post-harvest use	Total use (t)
Argentina	30	10	2.2	462
Brazil	0	17	2.0	850
Chile	45	0	0	201
Colombia	N/A	2.0	5.5	36
Peru	24.32	1.22	4.2	29.1
Uruguay	1.24	0.7	5.4	13.45
Venezuela	0	0	0	3.4
Total	100.56	30.92		1,595

TRENDS IN THE USE OF METHYL BROMIDE FOR DURABLE COMMODITY FUMIGATION

Table 1 indicates that, although there was a general increase in the consumption of MB between 1984 and 1990, this was principally because of increases in soil fumigation. There was little change in usage for commodity disinfestation during that period. In many grain-producing countries in Africa, MB was the principal fumigant used until the mid-1980s, and it has continued to be used for that purpose in several countries, probably due to both familiarity with the chemical and because it can provide prompt treatment over a short period when this is necessary. The short fumigation period that MB can provide continues to have particular implications for large-scale marketing organisations. For example, in Kenya grain requiring fumigation in relatively remote locations is treated by pest control teams that travel from established bases. However, in some countries, such as Malawi, where MB was previously regularly used to treat grain in central storage, the trend in recent years has been to use less MB and increase the amount of PH_3 fumigation. In the past, cocoa exported from Ghana was regularly fumigated with MB but, in the 1990s, disinfestation practices have changed entirely and PH_3 is being used instead. Exported tobacco was usually fumigated with MB in earlier years, but in both Zimbabwe and Malawi almost all tobacco is now disinfested with PH_3. This trend of gradual change from MB to PH_3 as a commodity fumigant was reported from many African countries.

Liberalisation of grain markets in Africa has affected pest control operations in several countries. In Tanzania, for example, the National Milling Corporation lost its monopoly in grain marketing under the World Bank/IMF reconstruction programme, and a steady decline in the use of MB has occurred. Where grain stocks are now fumigated by private contractors, they almost invariably use PH_3. A major need for MB arose, however, in 1992–93, in Southern Africa, in connection with the drought-relief programme. Very large quantities of grain had to be imported and, in order to ensure that grain movements

were not unduly delayed, fumigation, deemed essential to prevent movement of the larger grain borer across national borders, was primarily with MB. Grain deficits due to drought are an increasingly common occurrence, and fumigation of imported relief supplies using MB ensures that delays in transit are minimised.

In Southeast Asia, there has been little change in the consumption of MB in recent years and, because the major use is for the fumigation of commodities for export, the opportunities to use alternatives, such as PH_3, are limited. It seems likely, therefore, that little change in MB use can be expected in this region in the immediate future, and major users such as Thailand will continue to rely on the chemical for pre-shipment treatments. In Latin America, there is no obvious trend in use of MB for the treatment of durable commodities because the fumigant is used relatively little in the region for this purpose. Its only significant use on commodities was reported from Peru, where it is used for quarantine fumigation, and this use will probably continue for the foreseeable future under the current exemption agreed for this type of treatment.

CONCLUSIONS FROM THE SURVEY

MB continues to be an important fumigant for post-harvest pest control in some developing countries. Use patterns vary considerably, not only among the regions surveyed but also within regions, particularly in Africa. Of the 19 countries in Africa included in the survey, only four could be considered major users of MB, for various purposes, and only one, Egypt, employs the chemical in substantial quantities for commodity disinfestation. In Southeast Asia, of the total of 1,109 t of MB used in 1994, more than 90% was used for commodity fumigation. In contrast, in the Latin American countries surveyed, the major use for MB is for soil treatment. There is very little usage with durable commodities, although some perishable commodities are disinfested with MB prior to export.

Although there continues to be some use of MB in developing countries for routine grain disinfestation, in the last decade there has been a change to PH_3 in many countries whenever the time period available for treatment is not a constraining factor. There may still be some situations, particularly in Africa, where the use of alternatives such as PH_3 could be further extended, although this might require changes in stock management operations and also the provision of additional equipment. Where rapid disinfestation is necessary there is, at present, no ready alternative to MB, and this has been recognised in exempting fumigations conducted for pre-shipment and quarantine purposes from the agreed control programme for the chemical. With these exemptions in place, it appears unlikely that the introduction of additional controls on MB, in the future, would have significant impact on commodity disinfestion programmes in most developing countries. There are, however, exceptions to this generalisation, and further controls on MB would, for example, undoubtedly cause serious disruption to the routine use of the chemical for commodity fumigation in Egypt.

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SESSION 10

SAMPLING AND TRAPPING IN RELATION TO CONTROLLED ATMOSPHERES AND FUMIGATION

Chairpersons:
P. Cogan, *UK*
P. Trematerra, *Italy*

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 611–615

INFLUENCE OF PITFALL TRAPS AND WB PROBE II TRAPS ON THE CAPTURE OF FOUR STORED-GRAIN COLEOPTERA

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ABSTRACT

Two commercially available traps, the Pitfall Trap and the WB Probe II Trap, were used in the laboratory to monitor insects in wheat (*Triticum aestivum*) at 23–24°C and 65–70% r.h. The major coleopteran pests of cereals in Italy, *Oryzaephilus surinamensis* (L.), *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst), were added to the grain at an infestation level of 0.9 insect/kg. There were significant differences in the total number of insects collected by the two trap types. In addition, there were significant differences in the number of individuals of each species captured, depending on the trap and the trap location.

The Pitfall Trap is located on the surface and within the grain bulk 15 cm below the surface. Analysis of captures in it indicated that *T. castaneum* migrated in numbers to the surface but *O. surinamensis* remained within the bulk. The WB Probe II Trap trapped more *T. castaneum* than *O. surinamensis* specimens. Fewer *R. dominica* and *S. oryzae* specimens were trapped.

INTRODUCTION

Insect traps are effective and sensitive tools for the detection of adult beetles (Loschiavo, 1974, 1975; Barak and Harein, 1982; Lippert and Hargstrum, 1987; Cogan and Wakefield, 1987, 1990; Cogan *et al.*, 1987, 1994; Cogan *et al.*, 1990; Fargo *et al.*, 1994) and are considered superior to the standard grain sampling procedures. Various researchers have observed wide differences in trap catch, and much of this variation has been attributed to variations in trap efficiency (Hagstrum *et al.*, 1990), which are often due to environmental factors affecting insect behaviour, rather than to actual changes in the population density, and vary according to insect species, grain temperature and trapping duration (Fargo *et al.*, 1989, 1994; Vela-Coiffier *et al.*, 1996).

The specific objective of this study was to evaluate the difference in trap catch as a function of insect species, trap type and trap location.

MATERIALS AND METHODS

Laboratory cultures of the major coleopteran grain pests in Italy, the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.), the lesser grain borer *Rhyzopertha dominica* (F.), the rice weevil *Sitophilus oryzae* (L.) and the red flour beetle *Tribolium castaneum* (Herbst), were reared in glass jars containing clean, untreated wheat, *Triticum aestivum* L., at 26°C and about 14% moisture content (m.c.).

The experiment was carried out using two commercially available traps, a "Pitfall Trap" — on the surface and at a depth of 15 cm (Cogan *et al.*, 1990) — and a "WB Probe II Trap" (Burkholder, 1984).

The tests were carried out in the laboratory at 23–24°C and 65–70% r.h. They were done in plastic pipe containers, 25 cm in diameter and 25 cm in height. Each container was filled to within 8 cm of the top with 9 kg of *Triticum aestivum* wheat at 14% m.c.

The insects were introduced at the top of the grain mass and allowed to acclimate for 24 h before traps were inserted. Ten insects of each of the four species were put into separate containers (a single species per container). This resulted in a population density of about 0.9 insects per kg. The traps were placed for 72 h at the centre of each container in the infested grain. They were then removed from the grain mass and the number of insects captured was noted. Fifteen replicates were done for each species and each trap type.

RESULTS

In this study, there were significant differences in the number of insects collected by the two trap types. The number of the different species captured in each trap and treatment type was also significantly different (Fig. 1).

Pitfall Traps (average capture of 17.64%) were more efficient than WB Probe II Traps (average capture of 8%) in detecting beetles in stored wheat. Pitfall Traps at the grain surface yilded higher insect counts (18.33%) than those at a depth of 15 cm (16.94%).

Capture figures in the Pitfall Traps located at the surface and within the grain bulk indicated that *T. castaneum* migrated in numbers to the surface whereas *O. surinamensis* remained within the bulk.

The WB Probe II Trap trapped more *T. castaneum* than *O. surinamensis*; *S. oryzae* was captured in smaller numbers, and no captures of *R. dominica* were recorded.

The buried and surface Pitfall Traps (24.44% and 14.7% capture) both proved better than probe traps (10% capture) for *O. surinamensis*. Therefore, for this species the buried Pitfall Trap was the most effective.

Fewer captures of *R. dominica* were made in the buried Pitfall Trap (5.55% capture) and in the surface Pitfall Trap (3.33% capture), and none of these insects were found in the WB Probe II Trap.

The buried Pitfall Trap (11.11% capture) was better than the surface Pitfall Trap (9.33% capture) for *S. oryzae*, and only a few individuals of this species were found in the WB Probe II Trap (2.22% capture).

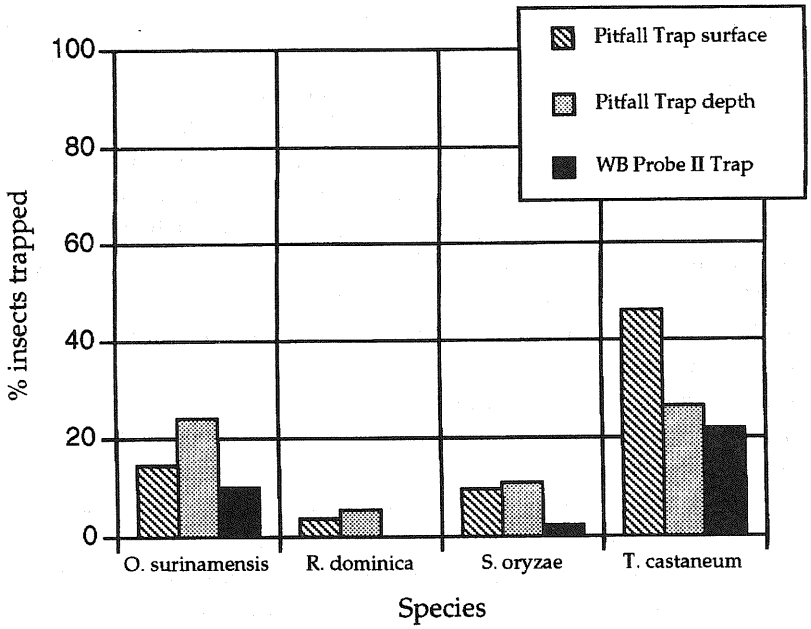


Fig. 1. Percentage of insects trapped during the experiment.

All of the traps were effective for *T. castaneum* adults, but the surface Pitfall Traps were better than the others: 46% of the adults were recorded in the surface Pitfall Traps, 26.66% in the buried Pitfall Traps and 22.22% in the WB Probe II Traps.

DISCUSSION

Since the effectiveness of all insect traps depends on insect movement, anything that influences this factor will also affect trap capture. The magnitude of this effect depends primarily on insect species, temperature, trapping duration, grain type and grain condition (Cuperus *et al.*, 1990).

Our experiment demonstrates the importance of insect species, trap type and trap placement in determining trap catch. *T. castaneum* is a very active insect; *R. dominica* does not move as much as do other species and therefore is less likely to be trapped. The same behaviour difference was observed by Subramanyam and Harein (1990).

Several researchers have found that trap catch was significantly greater at higher grain temperatures. Capture of *Cryptolestes* spp., for example, increased significantly at higher temperatures. However, between 10°C and 32°C, such species as *R. dominica*, *T. castaneum* and *S. oryzae* were not trapped in greater numbers at higher temperatures (Cuperus *et al.*, 1990).

Fargo *et al.* (1989) demonstrated that the number of insects (of any given species) captured increased significantly with trapping duration. Sampling probes are simply

inserted into and immediately withdrawn from the grain; therefore, the possibility of collecting insects, especially in lightly infested grain, is low. Response to trapping duration, however, varies with the species, and this should be considered when interpreting a trap catch. The choice of trapping duration can be complicated because of efficiency changes. The insects already trapped emit a repellent signal (an alarm pheromone) that can decrease trap attractiveness and, consequently, efficiency (Trematerra *et al.*, 1996).

It has also been shown that grain type has an impact on trap catch of known densities of insects. The amount of broken grain and fine extraneous material also influences insect movement. The condition of the grain may also affect the random movement of the insects and, thus, the number trapped. When trap samples are counted without taking these factors into account, it is impossible to evaluate the variability in trap efficiency because estimates are likely to be inaccurate.

The primary objective of insect sampling in stored grain is early detection of infestations. Detection permits control measures to be implemented before extensive damage occurs. Insect traps detect populations sooner than do grain sampling devices and they facilitate more accurate monitoring of potential insect problems.

The relationship between trap catches obtained from grain samples and actual insect densities needs to be determined. Only thus can trap information be valuable in determining an appropriate pest management strategy.

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SAMPLING TO DETERMINE TREATMENT EFFICACY: DEVELOPMENT AND VALIDATION OF SEQUENTIAL SAMPLING PLANS FOR ADULTS OF THE RUSTY GRAIN BEETLE ASSOCIATED WITH FARM-STORED WHEAT

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ABSTRACT

Sequential sampling plans, based on a variable sample size, are more cost-effective than are plans based on a fixed sample size. Sequential sampling plans can be used for estimating insect density with a fixed level of precision as well as for classifying infestation level relative to an action or economic threshold. For adults of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens), we developed sequential sampling plans based on complete counts and presence/absence of insects in 0.5-kg grain samples removed with a 1.27-m grain trier from the top 1 m of wheat stored in farm bins. Insect count data were used to develop an enumerative sampling plan for estimating *C. ferrugineus* density with a fixed level of precision. Presence/absence data were used to develop a binomial sampling plan for classifying the *C. ferrugineus* infestation level relative to an action threshold. The performance of these sampling plans was validated using independent data sets, and an IBM-PC software program was specifically designed to test the plans. These are the first sequential sampling plans reported for a stored-product insect.

INTRODUCTION

Sampling[1] is an integral component of integrated pest management (IPM) (Ruesink and Kogan, 1982). Cost-effective IPM decisions require methods to both accurately estimate insect density and determine if the density or infestation level has exceeded a threshold beyond which control action is necessary. Cost-effective sampling plans for both

[1]For definitions, concepts and statistics pertinent to stored-product insect sampling, see Subramanyam and Hagstrum (1995).

estimating insect density and classifying the infestation level relative to an action or economic threshold can be developed using sequential methods. Sequential methods utilize a flexible sample size and are therefore generally less expensive than are methods based on a fixed sample size (Waters, 1955). In general, sequential sampling plans require about 40–60% fewer sample units than do fixed-sample size plans to attain the same precision or accuracy of classification (Sterling, 1975).

Sequential sampling plans have not been previously developed for stored-product insects (Subramanyam and Hagstrum, 1995). Sequential sampling plans for estimating insect density (Hutchison, 1994) or for classifying infestation level relative to a threshold (Binns, 1994) can both be developed based on either complete counts of insects in sample units (enumerative sampling) or the presence/absence of insects in sample units (binomial sampling). Development of enumerative and binomial sampling plans for pest insects can reduce both sampling effort and sample processing costs as well as preventing unnecessary pest management actions and permitting the evaluation of pest management tactics.

Once the sampling plans are developed, they should be validated using independent data sets or through computer simulation (Binns and Nyrop, 1992). The final step is the implementation of the sampling plans under field conditions by the end users — farmers, pest managers and scouts. Therefore, sampling plans should be simplified for these end users. Furthermore, the plans should be flexible and should also perform well in the field despite errors introduced during implementation.

The rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) is a common and abundant species infesting farm-stored grain in the United States and Canada (Sinha and Watters, 1985). For *C. ferrugineus* adults infesting farm-stored wheat, we developed both an enumerative sequential sampling plan to estimate density and a binomial sequential sampling plan to classify infestation level relative to an action threshold. These sampling plans were validated using a resampling approach (Naranjo and Hutchison, 1996). The resampling approach utilizes an IBM-PC computer program (Resampling for Validation of Sample Plans or RVSP) specifically designed to validate sequential sampling plans.

FEATURES OF THE RVSP SOFTWARE

The RVSP software evaluates two enumerative sampling plans (Kuno, 1969; Green, 1970) for estimating insect density with a fixed level of precision and two binomial sampling plans (Wald, 1947; Naranjo *et al.*, 1996) for classifying infestation level relative to a threshold. The software runs on an IBM-PC computer in the DOS environment. To use RVSP, insect counts in each sample unit from a single sampling occasion are entered in a vertical column in a single file. Data from different sampling occasions are entered as separate files (with different file names). All file names are stored in a batch file for program execution. The RVSP program simulates actual field sampling by resampling observations from these files. Therefore, resampling analyses are based on actual distributions of the insect population. A random number generator selects observations from each

field data file, and this resampling is repeated 500 times (iterations). Individual observations in a data file can be sampled once (resampling without replacement) or multiple times (resampling with replacement). We chose the latter because the program failed to resample certain files when we chose the former option. We set the minimum sample size as 5. For enumerative and binomial sampling, RVSP requires two data sets, one to fit a statistical model of spatial dispersion to data and the other to evaluate model performance (see Naranjo and Hutchison, 1996). The output containing information from all 500 iterations can be saved and used with other software applications. Summary statistics based on the 500 iterations are also printed and stored by RVSP. This public-domain software is available upon request from S.E. Naranjo or W.D. Hutchison. It can also be downloaded via the internet from the following World-Wide-Web sites: http://gears.tucson.ars.ag.gov/wcrl/; http://www.mes.umn.edu/~vegipm/.

COLLECTION OF *C. FERRUGINEUS* SAMPLING DATA

Farm-stored wheat in Kansas was sampled during 1983 and 1984 to collect data on *C. ferrugineus* and other insect species (Hagstrum *et al.*, 1985; Hagstrum, 1987). Hagstrum *et al.* (1985) sampled wheat from four bins (5.8 or 6.4 m diameter) of 82- or 122-t capacity during 22 October 1983 to 6 January 1984. Wheat was sampled with a 1.27-m grain trier in the top 1 m of the bins. Each grain trier sample removed 0.5 kg of the wheat. On each of three separate occasions, 18 locations in three bin strata were sampled twice for a total of 36 observations/sampling occasion/bin. Therefore, from Hagstrum *et al.* (1985) a total of 12 data sets were available. During July to December 1984, Hagstrum (1987) sampled two bins (4.3 or 6.4 m diameter) holding newly-harvested wheat (27- or 82-t capacity). On each sampling occasion, 11 sites in the top 1 m of each bin were probed twice with the grain trier to obtain a total of 22 0.5-kg samples. In the 27- and 82-t bins, sampling was done on 11 different occasions. Therefore, from Hagstrum (1987), a total of 22 data sets were available.

The presence and number of *C. ferrugineus* in each 0.5-kg sample (sample unit) were determined. A sample unit with one or more *C. ferrugineus* adults was scored as '1' and a unit without any adult was scored as '0'. From this information, the proportion of sample units with insects ($P(I)$) was calculated. For each bin and sampling occasion, counts of insects in all 22 or 36 sample units were used to calculate sample mean (m) and sample variance (s^2). These sample statistics can be calculated manually (see Subramanyam and Hagstrum, 1995) or by a statistical software program (SAS Institute, 1988). For the 22 data sets, m ranged from 0.09 to 7.91 insects/sample unit, s^2 from 0.185 to 121.661 and $P(I)$ from 0.045 to 0.909. For the 12 data sets, m, s^2 and $P(I)$ ranged from 0.03 to 8.36 insects/sample unit, 0.029 to 338.56 and 0.028 to 0.944, respectively.

During bin sampling (Hagstrum, 1987), wheat moisture ranged from 10.2 to 13.0% and grain temperature from >1 to 32°C. The grain samples were collected from harvest until the onset of cold temperatures; therefore, the insect densities reported here are representative of those occurring in bins under a range of environmental conditions.

SAMPLING PLAN FOR ESTIMATING *C. FERRUGINEUS* DENSITY WITH A FIXED LEVEL OF PRECISION

Fixed precision level sequential sampling plan

When m is unknown, the number of sample units needed to estimate density at a fixed level of precision can be determined sequentially. Green (1970) proposed a sequential sampling approach in which sampling is terminated when a defined level of precision is achieved. This fixed precision stop line is calculated as

$$\ln(T_n) = \{[\ln(D^2/A)]/[b-2]\} + [(b-1)/(b-2)]\ln(n) \quad (1)$$

where T_n = the cumulative number of insects in sample units, D is the precision expressed as a ratio of the standard error of the mean (*SEM*) and m (*SEM*/m) and A and b are estimated by regressing $\ln(s^2)$ against $\ln(m)$ (Taylor, 1961). A is a scaling factor related to the sample unit, and b is an indicator of the degree of insect aggregation. A is calculated as e^a, where e = 2.71828 and a = y-intercept; b is the regression slope. After choosing a suitable D, Equation (1) is solved for different n values. A reasonable D is usually between 0.20 and 0.35 (Southwood, 1978; Hutchison *et al.*, 1988) because the observed precision is not fixed but stochastic. A plot of $\ln(T_n)$ against $\ln(n)$ gives a straight line (Green, 1970). A plot of T_n against n is nonlinear.

The linear regression of $\ln(s^2)$ against $\ln(m)$ based on the 22 data sets (Hagstrum, 1987) gave a y-intercept ($a \pm$ SE) of 1.117 ± 0.105, and a slope ($b \pm$ SE) of 1.461 ± 0.084. The y-intercept and slope were significantly greater ($P < 0.01$) than 0 and 1, respectively. This indicated that *C. ferrugineus* adults were distributed in an aggregated fashion in the top 1 m of wheat stored in the bins. The scaling factor, A, was 3.056. Similar estimates were obtained for *C. ferrugineus* adults sampled with perforated probe traps placed just below the surface of farm-stored shelled corn (Subramanyam and Hagstrum, 1995, p. 156). To generate Green's (1970) fixed precision stop lines, Equation (1) was solved using a D of 0.25 or 0.35 and Taylor's estimates of A = 3.056 and b = 1.461.

Fixed precision level sequential sampling plan validation

Figure 1 shows the fixed precision stop lines generated using Equation (1). The number of sample units required to cross the fixed precision stop lines decreased with an increase in insect density. In practice, to evaluate performance of the sampling plan, independent sample units are taken sequentially and the number of insects in them enumerated. The cumulative number of insects in sample units is plotted against the cumulative number of sample units with reference to the stop line. Counting stops when the fixed precision stop line is crossed. T_n/n at this point estimates m. The number of insects in each of the n sample units required to reach the stop line is used to calculate *SEM*. D is calculated as a ratio of *SEM* and m. This calculated D can then be compared with the fixed D used in Equation (1).

The performance of the fixed precision sampling plan was validated using the RVSP software. The 12 independent data sets (Hagstrum *et al.*, 1985), with a density range of 0.03 to 8.36 insects/sample unit, were resampled by RVSP for this analysis. Performance was evaluated by examining the actual n and D obtained from sequentially estimating

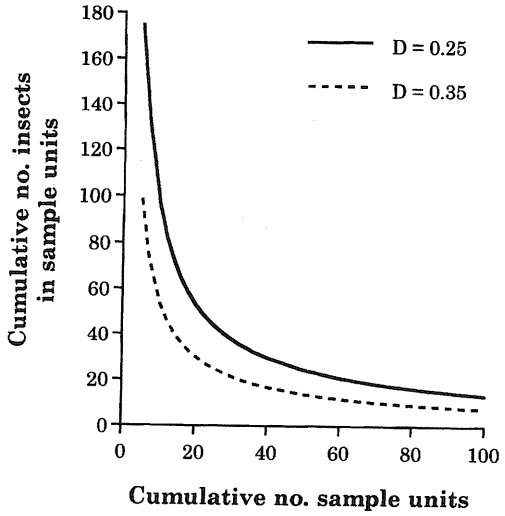


Fig. 1. Fixed-precision stop lines for sequential estimation of rusty grain beetle density. The precision (D) is expressed as a ratio of standard error of the mean and mean.

C. ferrugineus densities (0.03 to 8.36 insects/sample unit) at specified precision levels of 0.25 and 0.35. To estimate 0.03 to 8.36 insects/sample unit at $D = 0.25$, 341 to 18 samples were required (Fig. 2). To estimate the same density range at $D = 0.35$, 181 to 10 samples

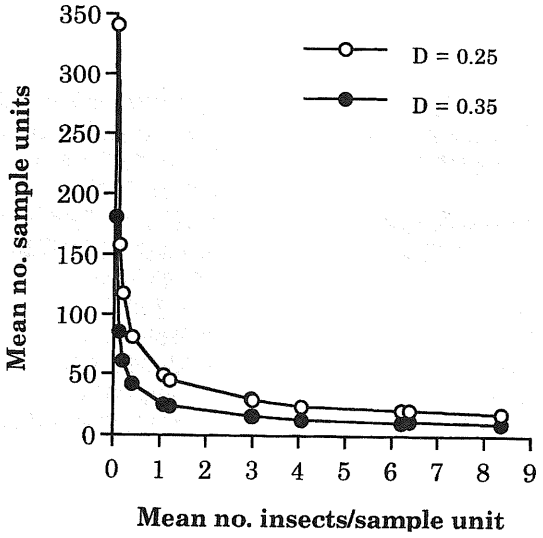


Fig. 2. The observed mean number of sample units (0.5-kg samples of wheat) required to estimate rusty grain beetle density between 0.03 and 8.36 insects/0.5 kg of wheat (sample unit). The RVSP program resampled each of the 12 independent data sets 500 times to determine the observed mean number of sample units. Therefore, each observed mean is the average of 500 simulations.

were required. About twice as many samples were required to estimate insect density at $D = 0.25$ than at $D = 0.35$. The observed mean precision levels were worse than expected when estimating densities ≤0.03 or ≥6.22 (Fig. 3). The observed mean precision was better than expected when estimating densities between 0.11 and 1.22 insects/sample unit. For example, at $D = 0.25$ and $D = 0.35$, the observed mean precision when estimating 0.11 to 1.22 insects/sample unit ranged from 0.22 to 0.18 and from 0.31 to 0.24, respectively. The observed precision when estimating densities of 2.97 and 3 insects/sample unit was worse than the expected precision. However, when estimating a density of 4.06 insects/sample unit, the observed precision was better than the expected precision. The precision was worse than expected (see Fig. 3) only when the variance predicted by Taylor's Power Law underestimated the actual (observed) variance (Table 1).

According to the United States Federal Grain Inspection Service (FGIS) standards, grain is classified as "infested" if a representative 1-kg grain sample contains a minimum of two live adults (Hagstrum and Flinn, 1992). Our sample unit was based on 0.5 kg of grain. Therefore, we chose one *C. ferrugineus* adult/sample unit as the economic threshold.

Based on Green's (1970) plan, a density of one insect/sample unit (1.08 insects/sample unit) can be estimated with 49 samples at $D = 0.25$, and with 25 samples at $D = 0.35$ (Fig. 2). We recommend a fixed precision of 0.35 for estimating the economic threshold density of *C. ferrugineus* because the observed mean precision was 0.24 (Fig. 3). Except for a density of 4.06 insects/sample unit, the precision in estimating densities ≤0.11 or

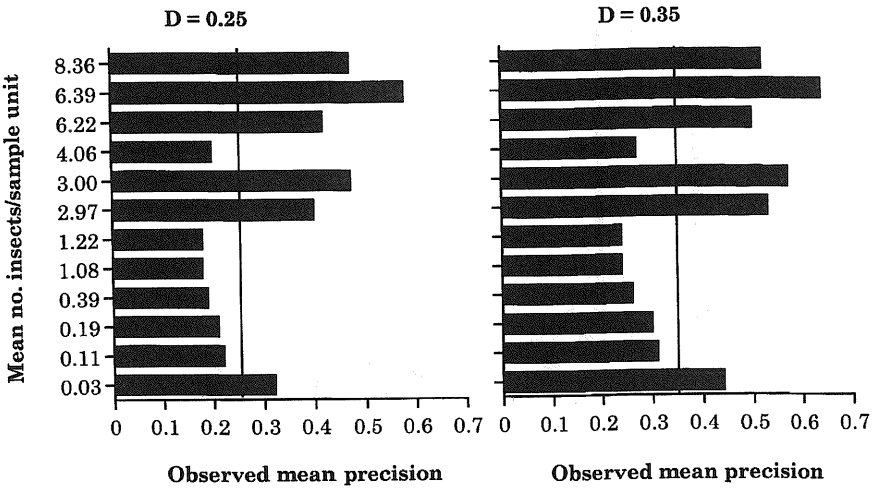


Fig. 3. The observed (actual) mean precision during estimation of rusty grain beetle density between 0.03 and 8.36 insects/0.5 kg of wheat (sample unit) at fixed precision levels (D) of 0.25 and 0.35. The RVSP program resampled each of the 12 independent data sets 500 times to determine the observed mean precision. Therefore, each observed mean is the average of 500 simulations.

TABLE 1
Observed sample mean (m) and variance (s^2) for the 12 independent data sets and the variance predicted by Taylor's Power Law ($A = 3.056$ and $b = 1.461$)

Data set no.	m*	s^2	Predicted s^2**
1	0.03	0.029	0.018
2	0.11	0.102	0.122
3	0.19	0.221	0.270
4	0.39	0.476	0.772
5	1.08	1.850	3.420
6	1.22	2.132	4.086
7	2.97	44.489	14.992
8	3.00	71.234	15.213
9	4.06	7.556	23.671
10	6.22	161.798	44.145
11	6.39	338.560	45.919
12	8.36	302.412	67.998

*Expressed as mean number of live rusty grain beetles/sample unit (0.5 kg of wheat). Each mean is based on 36 observations.
**The predicted variance was calculated as $3.056\ (m)^{1.461}$, where m is the observed sample mean.

≥1.22 insects/sample unit often exceeded that desired. Furthermore, for estimating densities below 1.08 insects/sample unit, an unrealistically large number of samples was required. Our results suggest that the fixed precision sequential sampling plan is useful for estimating *C. ferrugineus* density at or near the economic threshold.

SAMPLING PLAN FOR CLASSIFYING *C. FERRUGINEUS* INFESTATION LEVEL RELATIVE TO AN ACTION THRESHOLD

Presence/absence or binomial sampling is a viable alternative when counting insects in sample units would be cumbersome or expensive (Binns and Nyrop, 1992). We developed a binomial sampling plan based on the sequential probability ratio test (SPRT) (Wald, 1947) to classify *C. ferrugineus* infestation levels relative to a threshold. To develop a binomial SPRT sampling plan, the relationship between the proportion of sample units with one or more insects (P(I)) and the mean density (m) should generally be established (Jones, 1994). Establishing this relationship is important for determining P(I) at the threshold density of one insect/sample unit.

Relationship between proportion of infested sample units and mean density

For the 12 data sets (Hagstrum *et al.*, 1985), the relationship between P(I) and m was established by regression techniques using TableCurve 2D (Anonymous, 1994). Figure 4 shows the nonlinear relationship between P(I) and m. The regression model fit the data well ($R^2 = 0.907$). At the economic threshold density of one insect/sample unit, the P(I)

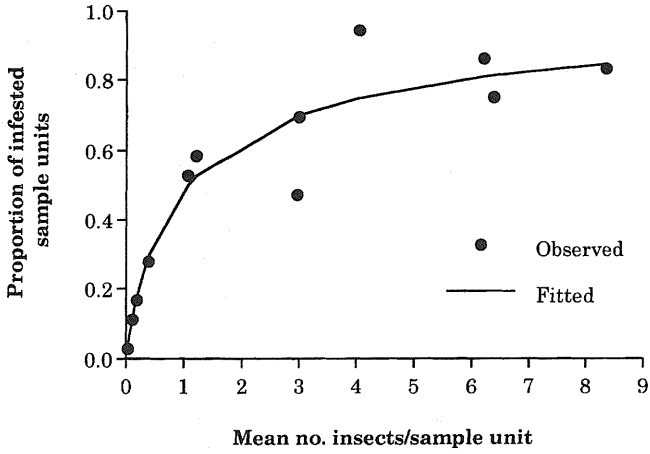


Fig. 4. Nonlinear relationship between proportion of sample units with one or more rusty grain beetles and mean density. The regression curve was fitted using TableCurve 2D, and the regression equation is described by: $Y = 1.093(1 - 1/(1 + 2(1.093)^2\ 0.935X)^{0.5})$; $n = 12$; $R^2 = 0.907$. At the United States Federal Grain Inspection Service standard for "infested grain" of 2 insects/kg of grain or 1 insect/0.5-kg of grain (sample unit), about 0.48 or 48% of the sample units were infested.

was 0.485. For the 22 data sets (Hagstrum *et al.*, 1985), the $P(I)$ against m regression gave similar results. Therefore, we used the 12 data sets for establishing the relationship between $P(I)$ and m, and the 22 data sets for binomial SPRT sampling plan validation. Because of the variation in the empirical relationship between $P(I)$ and m, and to take control action before the infestation level exceeds the economic threshold, we chose a $P(I)$ of 0.43 or 43% as the action threshold (AT).

Binomial sequential sampling plan

To develop a sampling plan based on the binomial SPRT, information on four parameters is needed (Binns, 1994): p_1 = the upper threshold, p_0 = the lower threshold (usually a certain fraction of p_1), α = the probability of wrongly rejecting the null hypothesis that $P(I) \leq p_0$ and β = the probability of wrongly rejecting the null hypothesis that $P(I) \geq p_1$ (Jones, 1994).

We set p_1 5% above the AT and p_0 5% below the AT. Thus, $p_1 = 0.48$ (equivalent to one insect/sample unit) and $p_0 = 0.38$. We set $\alpha = \beta = 0.2$. The error rates may be unequal (Waters, 1955) so the values used should be based on the acceptable risk of incorrect classification. For example, if $\alpha = \beta = 0.2$, then the probability of correctly classifying $P(I)$ as being at or below the lower threshold is at least 0.8, and the probability of classifying $P(I)$ as being at or below the lower threshold when it is at or above the upper threshold is at most 0.2. Based on the above thresholds and error rates, the upper (T_U) and lower (T_L) stop lines were generated using Equations (2) through (5):

$$T_U = (\text{intercept}) \ln((1 - \beta)/\alpha) + n\ (\text{slope}) \quad (2)$$

$$T_L = (\text{intercept}) \ln(\beta/(1 - \alpha)) + n\ (\text{slope}) \quad (3)$$

The intercept was calculated as:

$$1/[\ln\{(p_1q_0)/(p_0q_1)\}] \quad (4)$$

where $p_0 = 0.38$ and $p_1 = 0.48$; $q_0 = 1 - p_0$ and $q_1 = 1 - p_1$.

The slope was calculated as:

$$\ln(q_0/q_1)/[\ln\{(p_1q_0)/(p_0q_1)\}] \quad (5)$$

The upper and lower stop lines were generated by plotting T_U and T_L against n (Fig. 5). Data from independent samples, compared to these stop lines, could be used to determine if sampling should stop or proceed (Subramanyam and Hagstrum, 1995). Should data from the cumulative number of sample units with insects against the cumulative number of sample units cross either the upper or lower stop lines, sampling should be stopped. If the data falls within the stop lines, sampling should continue until the upper or lower stop line is crossed.

The two most important properties of the binomial SPRT sampling plan are the operating characteristic (OC) and the average sample number (ASN) functions. The procedures for calculating OC and ASN functions for binomial SPRT were given by Fowler and Lynch (1987). The OC function predicts the chance of correct classification; the ASN function tells us the average number of sample units required in the long run to reach a decision at each infestation level. The OC curve shows the probability of "not to inter-

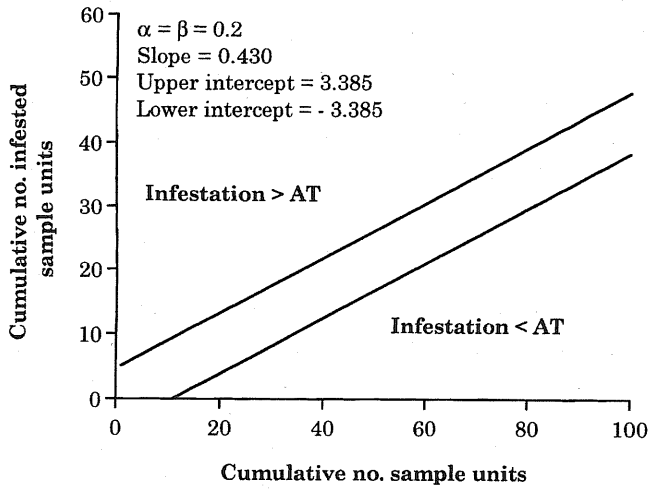


Fig. 5. Binomial sequential probability ratio test stop lines for classifying sample units infested with one or more rusty grain beetles with respect to the action threshold (AT) (see text for details).

vene" as a function of the infestation level. Typically, the OC function is near 1 when $P(I) < p_0$, and near 0 when $P(I) > p_1$. The OC function is 0.5 when $P(I) = AT$ (for $\alpha = \beta$). The ASN function increases if the lower and upper thresholds are closer together and if α and β are set lower (Waters, 1955; Binns, 1994).

Binomial sequential sampling plan validation

The performance of the sampling plan was evaluated by examining the actual OC and ASN functions for a range of *C. ferrugineus* infestation levels based on the 22 independent data sets. For each infestation level ($P(I)$), the RVSP gave OC and ASN values, each ASN value an average of 500 iterations and each OC value the proportion of 500 resampling iterations terminating with a decision of not to treat (Naranjo and Hutchison, 1996). The OC or ASN values were plotted against corresponding $P(I)$, and a curve (Fig. 6) was fitted to the data points using TableCurve 2D (Anonymous, 1994).

At $\alpha = \beta = 0.2$, the OC function was near 1 when $P(I)$ was ≤ 0.3. At a $P(I)$ of 0.38 (p_0), the OC function was 0.851. At a $P(I)$ of 0.48 (p_1), the OC function was 0.189. At a $P(I) > 0.48$, the OC function decreased from 0.189 to 0. At a $P(I)$ of 0.43 (AT), the OC function was 0.55. The OC curve at $\alpha = \beta = 0.2$ indicated that the binomial SPRT sampling plan performed well in correctly classifying the *C. ferrugineus* infestation level with respect to the action threshold. Although the nominal errors were set at 0.2, the actual error rates generated by RVSP may be different. Actual error rates are determined from the OC curve (Fig. 6). 1 – OC value at the lower threshold gives the actual α; OC value at the upper threshold gives the actual β. For the 22 data sets, actual α and β were 0.149 and 0.189, respectively.

The ASN curve indicated that to classify $P(I)$ between 0.045 and 0.909, an average of 7 to 51 sample units needed to be examined (Fig. 6). As expected, the ASN function was greatest near the action threshold. More sample units needed to be examined to reach a decision at $P(I)$ between p_0 and p_1 than at $P(I) < p_0$ or at $P(I) > p_1$.

The risks associated with using $\alpha = \beta = 0.2$ are minimal, because the action threshold was set 5% below the economic threshold of 48% infested sample units (1 insect/sample unit). Furthermore, *C. ferrugineus* cannot feed on sound, dry grain; it can only develop on the germ of cracked or damaged grain (Sinha and Watters, 1985; White and Bell, 1990).

The ASN curve indicated that fewer sample units (~10) may be sufficient to classify very high and very low *C. ferrugineus* infestation levels. However, collection of sample units from a storage bin involves climbing up the bin, hauling sampling equipment into the bin, determining sampling locations, probing grain with the trier, bagging the sampled grain and climbing down the bin. Furthermore, because of inadequate bin head space, it is difficult, and sometimes impractical, to process sample units within the bin. In addition, the bin head space may get very hot during a warm day. Therefore, to avoid unnecessary sampling costs, we recommend collecting and bagging a large number of sample units from a bin. These sample units can be processed sequentially in the laboratory within 24 to 48 h of collection to separate live *C. ferrugineus* adults from the grain.

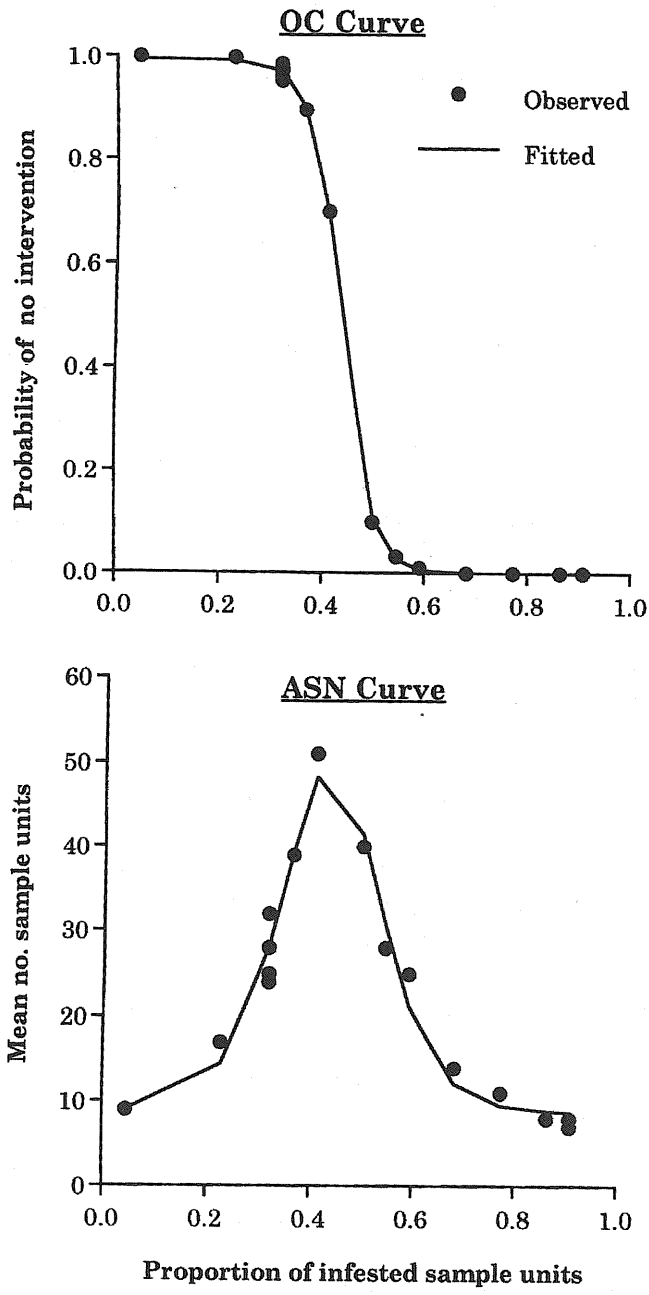


Fig. 6. Operating characteristic (OC) and average sample number (ASN) curves at $\alpha = \beta = 0.2$ for the binomial sequential probability ratio test sampling plan. The RVSP program resampled each of the 22 independent data sets 500 times to determine the OC and ASN values. Therefore, each OC or ASN value is based on 500 iterations. The curves were fitted to data points using TableCurve 2D.

In Fig. 5, the lower stop line intersects the x-axis at 12 sample units. Therefore, the minimum sample size should be ≥12 sample units. The ASN curve indicated that a maximum of 51 sample units needed to be examined to reach a decision near the action threshold. Taking 51 sample units on a given day would be cumbersome. To reduce sampling costs, sampler fatigue and the risk of failing to find insects with ≤12 sample units, we suggest that at least 22 sample units be collected from a bin on a given day. The 22 0.5-kg wheat samples (sample units) should be taken with a 1.27-m grain trier from various locations in the top 1 m of wheat stored in a bin, following the sampling scheme outlined in Hagstrum *et al.* (1985) or Hagstrum (1987). The sample units should be processed sequentially in the laboratory, and information on the proportion of sample units with live *C. ferrugineus* adults compared to Fig. 5. If the proportion of sample units with one or more *C. ferrugineus* adults crosses the upper or lower stop line, sample processing should be stopped. If the upper stop line is crossed, indicating that infestation has exceed the action threshold, *C. ferrugineus* populations should be managed with a pesticide or a pesticide alternative. After a management action, the wheat should be resampled periodically (weekly or bimonthly) to determine the degree and duration of suppression of *C. ferrugineus* populations. If the lower stop line is crossed, indicating that infestation is below the action threshold, the wheat in bins should be resampled after 2 to 4 weeks depending on the grain temperature and moisture and the *C. ferrugineus* population growth rate at the existing environmental conditions. With 22 or fewer sample units, infestation levels of ≤0.26 or ≥0.6 can be classified with respect to the action threshold (see ASN curve in Fig. 6). Thus, to classify infestation levels between 0.26 and 0.60, more than 22 sample units are required. If a decision is not reached after processing all 22 sample units, it is apparent that the infestation level is between 0.26 and 0.60. If more than 22 sample units are required, they should be collected after a few days or a week. These additional 22 sample units should be collected and processed and the sampling information then interpreted as outlined above.

The development and validation of sequential sampling plans for estimating density and classifying infestation level of *C. ferrugineus* adults in 0.5-kg samples of wheat have been illustrated here for the first time. The methods proposed here are useful for accurately estimating *C. ferrugineus* insect density and infestation level, which are important for evaluating the effectiveness of a control measure.

ACKNOWLEDGEMENT

We wish to thank M. Jagannadham and Mike Tufte for assistance in entering sampling data into the computer. S. Srinivas helped in typing portions of this paper. Thanks are also due to Gerrit Cuperus, Alan Dowdy and David Weaver for reviewing an early draft of this paper. Research reported here was supported by funds from the USDA/CSREES, Washington, D.C., to the Minnesota Pesticide Impact Assessment Program.

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COMPARISON OF TWO METHODS OF DETECTING INSECTS IN GRAIN

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ABSTRACT

Detecting insects in grain is fundamental in establishing the need for a fumigation, in post-fumigation assessment and in comparing the effectiveness of alternative treatments. Despite the importance of pest detection, there are few comparative studies of the various methods. This work compares the Berlese funnel method, commonly used by the Canadian Grain Commission (CGC), and the newly developed INSECTOMAT, a mechanised sieving mechanism capable of handling large samples of grain.

Samples of grain with a natural infestation of *Oryzaephilus surinamensis*, *Sitophilus granarius* and *Cryptolestes* spp. were used. The INSECTOMAT assessed 5-kg batches, and the funnels assessed 1-kg batches. Taking the sample sizes into account, the INSECTOMAT was generally better than the funnel at extracting all three species of insect. The INSECTOMAT allowed sample assessment to be completed in about 5 min as compared to more than 6 h for the Berlese funnel.

The size of the sample used by each system would appear to be the key to detection. The work indicates the potential dangers of using small samples taken from larger bulks in order to estimate insect populations and to determine the effectiveness of treatments.

INTRODUCTION

Insects are unacceptable in grain, both because of their potential to cause damage and because they are unhygienic contaminants. The grain industry devotes much effort to preventing infestation by use of good storage practice, but even their best efforts cannot prevent some infestation. Therefore, detection and control are also important aspects of storage, particularly when grain is being supplied to a market with a nil tolerance for pests.

Insects in grain destined for export are always regarded as a serious problem because of both quarantine restrictions and the potential for rapid population increases while the

grain is in transit. To combat this risk, governments in several countries, including the USA and Canada, set and enforce standards for levels of infestation in grain as it is loaded for export. In the case of Canada, this standard is zero, and inspectors of the Canadian Grain Commission, using Berlese funnels, test samples of all grain destined for export. This involves heating a 1-kg sample of grain for a minimum of 6 h to drive out any live, free-living adults or larvae. The method is widely recognised as being sensitive and effective, and Smith (1977) obtained recoveries of better than 80% for adult and large larval *Cryptolestes ferrugineus*. However, it is not easy to integrate this technique into the normal working of a commercial silo. During intake and outloading, silo staff must make decisions about the quality of grain in minutes, so the 6 h necessary for the Berlese funnel test is often impractical.

Research pioneered by scientists of Agriculture Canada has shown that using static traps is an excellent approach to pest detection in bulk grain (Loschiavo and Atkinson, 1967, 1973). Following these early developments, trapping is now an accepted approach to pest detection in commercial grain stores in many parts of the world. However, it is not a suitable method for assessing the quality of grain during transit. There also remain some concerns about the lack of data relating to how well the system works in deep silos with limited surface access and also about the exact commercial implications of the number of insects caught in traps.

The standard approach to detecting insects in grain during transit is to collect and examine samples. Unfortunately, the irregular distribution of insects in a bulk of grain adds to problems of pest detection via sampling and means that the chance of detection is directly related to the size of sample that is examined (Wilkin and Fleurat-Lessard, 1991).

Many new approaches to the detection of insects in samples of grain do not address the problem of looking at large amounts of grain. An exception is the inclined plane sieve developed by Ashman (1966) and refined by White (1983). Samplex Ltd. adapted the principle into their commercial prototype, the INSECTOMAT, which has the ability to remove insects from 10-kg samples in less than 2 min. Initial testing showed better than 90% recovery of two species of insects from 5- or 10-kg samples of wheat or barley (Wilkin, *et al*, 1994). The purpose of the investigation reported in this paper was to compare the efficacy of the INSECTOMAT with the standard Berlese funnel method in use by the CGC.

MATERIAL AND METHODS

Equipment and materials

The INSECTOMAT used in all tests was the latest production version with a 2.5-mm, round-hole sieve plate. The machine was operated at the standard reciprocation speed and sieve inclination which gave a throughput of 10 kg wheat in about 105 sec.

Berlese funnels, supplied by the CGC, were used in conjunction with locally manufactured screens (2.5 × 12 mm slots). Either 60-W, 240-V or 100-W, 240-V lamps were used to heat the wheat.

The test wheat (14% m.c.) was supplied as animal feed grain in 25-kg sacks by a local merchant.

Methods

Preliminary tests. A series of initial tests were undertaken to confirm that the INSECTOMAT would remove insects from grain. Between two and five adult *Sitophilus granarius* or *C. ferrugineus* were added to 10-kg batches of wheat which were then passed through the INSECTOMAT. An illuminated magnifying lens (10× magnification) was used to examine the sievings collected in the undertray for insects.

During the course of the initial tests it became apparent that the grain had a natural infestation of *S. granarius*, *Oryzaephilus surinamensis* and *Cryptolestes* spp. Therefore this grain without the addition of other insects was used for most of the subsequent tests.

Comparison between the INSECTOMAT and the Berlese funnel. Tests were done by dividing 10 kg of the naturally infested wheat into two equal parts using a mechanical sample divider. One lot of about 5 kg was passed through the INSECTOMAT and the insects were removed, counted and identified. This grain was then put through the INSECTOMAT a second time to check for the extraction of more insects. The second 5-kg sample was further divided to give 1-kg sub-samples, and three of these were set up in Berlese funnels while the remaining 2 kg was discarded. Each funnel was heated for 6 h and any insects driven out were collected in water traps beneath the funnels. The temperature of the grain at the centre of the funnel at the start and end of the heating process was measured with a thermistor probe.

Four 10-kg batches were tested. The tests were then repeated with the lamps changed from 60 to 100 W to increase the amount of heat applied to the samples in the Berlese funnels. Exceptionally, after the division of one of the 10-kg batches, five *C. ferrugineus* larvae were added to each of the two resulting 5-kg lots. One of these was passed through the INSECTOMAT and the other was further divided to give 1-kg samples that were assessed in the funnels.

After heating in the funnels, the three 1-kg samples from each test were re-combined and passed through the INSECTOMAT to check for the recovery of any further insects.

Recovery of C. ferrugineus larvae from grain by the INSECTOMAT. A series of tests was done to assess both the efficacy of the INSECTOMAT in extracting *C. ferrugineus* larvae and the ease with which they could be sighted amongst the sieved material. Two adult and two larval *C. ferrugineus* were added to each of six 5-kg batches of insect-free wheat which were then passed through the INSECTOMAT. The screenings were inspected and the number of adults and larvae found were recorded.

A second, similar test was done with a further three 5-kg batches of grain, except that five larvae and no adults were added to each batch before assessment. The grain was passed through the INSECTOMAT and the time taken to find the first larva was noted.

RESULTS

Preliminary tests

The initial tests showed that all insects added to the 10-kg samples of grain were recovered by a single pass through the INSECTOMAT. These tests also showed that the wheat was naturally infested with *S. granarius*, *O. surinamensis* and *Cryptolestes* spp. at a density of 1–4 adults per kg.

Comparison between the INSECTOMAT and the Berlese funnels

Table 1 compares the number of insects extracted from samples by the Berlese funnels heated with 60-W lamps and by the INSECTOMAT. The results obtained when 100-W lamps were used are given in Table 2. During the 6-h exposure, the temperature of the grain at the centre of the sample rose from about 15°C to about 28°C with the 60-W lamps and about 36°C with the 100-W lamps.

Various species of mites, psocids and lepidopterous larvae were also seen in the screenings produced by the INSECTOMAT, but these were not recorded. All second passes through the INSECTOMAT were negative for insect findings. However, when the samples previously extracted using the funnel were passed through the INSECTOMAT, in some cases a few further live insects were recovered.

TABLE 1
Numbers and species of insects removed from grain sample by the INSECTOMAT and the Berlese funnel (heated with 60-W lamps)

Test	Method	*S. granarius*	*O. surinamensis*	*C. ferrugineus*
A	INSECTOMAT	6	0	0
	Funnel A1	2	0	0
	Funnel A2	1	0	1
	Funnel A3	0	0	0
B	INSECTOMAT	7	5	0
	Funnel B1	1	0	0
	Funnel B2	2	0	0
	Funnel B3	0	0	0
C	INSECTOMAT	18	3	1
	Funnel C1	4	0	0
	Funnel C2	3	0	0
	Funnel C3	1	0	0
D	INSECTOMAT	23	6	7
	Funnel D1	5	0	0[1]
	Funnel D2	2	0	0
	Funnel D3	1	0	0

All insects were adults unless otherwise stated. [1]One small, unidentified larva.

TABLE 2
Numbers and species of insects removed from grain sample by the INSECTOMAT and the Berlese funnel (heated by 100-W lamps)

Test	Method	*S. granarius*	*O. surinamensis*	*C. ferrugineus*
A	INSECTOMAT	8	1	2
	Funnel A1	1	1	0
	Funnel A2	1	1	1
	Funnel A3	2	0	0
B	INSECTOMAT	11	5	0
	Funnel B1	0	1	0
	Funnel B2	2	0	0 (1 larva)
	Funnel B3	3	0	0 (1 larva)
C	INSECTOMAT	8	0	0
	Funnel C1	0	0	0
	Funnel C2	2	0	0 (1 larva)
	Funnel C3	0	1	0
D[1]	INSECTOMAT	5	1	0 (4 larvae)
	Funnel D1	2	0	1 (1 larva)
	Funnel D2	1	0	0 (1 larva)
	Funnel D3	2	0	1 (1 larva)

All insects were adults unless otherwise stated.
[1]Larvae added to this replicate.

Recovery of *C. ferrugineus* larvae from grain

The results of passing seeded samples through the INSECTOMAT are given in Table 3. Small larvae were not easy to locate in the screenings, but the first larvae were always found within 75 sec of the start of the extraction process.

In the second assessment of the recovery of larvae, in which five larvae were added to 5 kg of wheat, the total number of larvae recovered from each replicate was: four (replicate 1), three (replicate 2) and four (replicate 3). The time taken to detect the first larva was as shown above.

DISCUSSION AND CONCLUSIONS

The INSECTOMAT proved to be simple to operate, and it was able to process the samples of grain rapidly. All adult insects appeared to be removed by a single pass through the machine. There was no evidence of any insects "hanging up" within the machine and then escaping to contaminate later samples.

The key objective of this work was to complete a practical comparison between the INSECTOMAT and the Berlese funnel method. Therefore, the results obtained by the

TABLE 3
Number of adult and larval *C. ferrugineus* removed by the INSECTOMAT from 5-kg samples of wheat spiked with two adults, two larvae and additional screenings

	C. ferrugineus	
Replicate No.	Adults	Larvae
1	3	2[1]
2	2[1]	0[2]
3	3	1
4	2	2
5	2	2
6	2	2[1]

[1]1 dead, [2]1 recovered on second pass through INSECTOMAT.

INSECTOMAT should be compared to those obtained from a single funnel extraction rather than to the mean of all three replicates as, in practise, only 1 kg of grain was examined. Viewed in this light, the INSECTOMAT was somewhat more successful in detecting insects than the Berlese funnel. In one case (Test B) a single *Cryptolestes* sp. was found in one funnel sample when none were found by the INSECTOMAT. Conversely, insects were found by the INSECTOMAT on five occasions when none were found by the funnel method.

The larvae added to the grain used in the second test were recovered as effectively by the INSECTOMAT as by the Berlese funnel, although those removed by the funnel method were much easier to see. Careful searching was necessary to ensure that an occasional larva was not missed in the INSECTOMAT screenings. By contrast, live adults were detected readily in the screenings.

The results show that the INSECTOMAT will reliably remove insects from samples of wheat and that it is then easy to detect the live adult insects that are removed. It will also remove free-living larvae from wheat, but these are more difficult to detect in the screenings. The efficacy of the method in detecting early instar larvae is dependent upon the skill of the operator. During these tests, adults and larvae extracted by the Berlese funnel were easy to spot as there were no visual distractions. Smith (1977), however, showed that the funnel was not effective in the removal of early instar larvae. Therefore, it may be that both the INSECTOMAT and the Berlese funnel lack sufficient sensitivity for the detection of smaller, early instar larvae. In addition, neither method is suitable for detecting insects which are developing within the grains.

The effects of sample size on the detection of insect populations at densities below 3/kg was apparent during the experiments. On occasion no insects were found in 1-kg samples used with the funnel, whereas several were found in corresponding 5-kg samples passed through the INSECTOMAT. This difference would probably have been more

pronounced if the recommended sample size of 10 kg had been used with the INSECTOMAT.

The ability of the INSECTOMAT to detect free-living insects in grain was at least as good as that of the Berlese funnel and appeared to be better for free-living adults. Therefore, it could, in some circumstances, be used as an alternative or supplement to the Berlese funnel method. It is hoped that a further series of comparative tests will be carried out under practical conditions at a Canadian grain elevator.

ACKNOWLEDGEMENTS

These trials were sponsored by the Rural Development Commission, UK, and the Canadian Grain Commission. The equipment and facilities were provided by Samplex Ltd and the INSECTOMAT concept was developed with the aid of a grant from the Home-Grown Cereals Authority.

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ON THE CAPTURE OF *TRIBOLIUM CASTANEUM* (HERBST): EFFECTS OF ALIVE AND DEAD INSECTS IN A TRAP

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ABSTRACT

The effectiveness of traps, whether baited or not baited with 4,8-dimethyldecanal (the pheromone of the red flour beetle *Tribolium castaneum* (Herbst)), is influenced by the presence of accumulated trapped specimens, living or dead.

The results of this study suggest that the presence of live insects in traps attracts other free specimens; however, under conditions of overcrowding quinone secreted by the insects regulates population density by counteracting the effect of the aggregation pheromone (4,8-dimethyldecanal). The quinone causes the insects to disperse or suppresses aggregation behaviour. The presence of dead insects in the traps, whether or not baited with 4,8-dimethyldecanal, can repel other free specimens of *T. castaneum*. This is probably due to the presence of a residual alarm pheromone produced by the insects before death.

INTRODUCTION

The aggregation pheromone of *Tribolium castaneum* (Herbst) is produced by the males at a continuous rate under all conditions. Quinones are secreted by both sexes but only under certain conditions, such as crowding, excitement, agitation and partial narcosis (Engelhardt *et al.*, 1965; Ogden, 1969; Irwin *et al.*, 1972). The aggregation pheromone and the quinones interact; the effects of this interaction depend on the number and sex of the beetles present in the infested commodity and on the duration of the infestation (Mondal, 1983, 1985, 1993; Mondal and Port, 1994). The quinones regulate the population density of *Tribolium* by counteracting the effect of the aggregation pheromone. Under conditions of overcrowding and lack of food, the secretion of aggregation pheromone on the male femora is inhibited, causing the insects to disperse or suppressing the aggregation behaviour (Faustini and Burkholder, 1987).

Use of the aggregation pheromone has been proposed in the monitoring and control of *Tribolium* spp. Where traps of various designs are used for monitoring this pest, pheromone traps appear to be more effective in the detection of low infestations (Burkholder and Ma, 1985; Mullen, 1992). The effectiveness of *Tribolium* pheromone-baited traps depends on many factors, including trap location, trapping duration, grain type and

condition, grain temperature, movement and dispersion of the insects and pheromone release (Fargo *et al.*, 1989; Barak *et al.*, 1990; Obeng-Ofori and Coaker, 1990a, b; Pinniger, 1990; Trematerra and Daolio, 1990; Trematerra, 1992; Trematerra *et al.*, 1996). An understanding of the environmental and physiological factors affecting insect response to pheromones is required for their successful employment in influencing insect behaviour. For this purpose a series of experiments was designed to measure the response of *T. castaneum* under different conditions. The main aim of the experiments was to determine if trap catches, whether or not the traps were baited with synthetic pheromones, were affected by the presence of accumulated living and dead insects.

MATERIALS AND METHODS

T. castaneum were cultured in a constant-environment room at 25 ± 2°C and 70% r.h. using wheat flour as the food medium.

The beetles were released and recaptured in two "Flit-Trak M" traps in a covered plastic cage (43 × 21 × 10 cm). One trap (A) was baited with cracked maize and *T. castaneum* adults at densities of 2, 5, 10, 20 and 40 live adults/trap, and the other trap (D) was baited with cracked maize and 10 dead insects. Fifty adult beetles of mixed sex and age were released into the cage. The experiment was carried out at 24 ± 2°C.

The numbers of trapped *T. castaneum* were counted after 0.5, 1, 2 and 3 h, and again after 19 h, and the percentage frequency of detection was calculated. A set of all the counts under each of the experimental conditions was termed a 'watching run', and each 'watching run' was replicated four times.

A similar experiment using the same densities of *Tribolium* was carried out to determine the effect of the pheromone, 4,8-dimethyldecanal, on beetle-trap counts.

Insects found in traps baited with live insects were designated as "first choice," while insects found in traps baited with dead insects were termed "second choice." There was no guarantee that all the specimens attracted were trapped. Therefore, the number of specimens in the trap did not necessarily correspond to the number of specimens actually attracted during the watching time. Nevertheless, the specimens in each trap must be assumed to have chosen between the two available possibilities.

The presence of specimens under and over the traps can be attributed to partial failures in trap performance. Therefore the larger number of specimens in a given trap, reflecting the quantity of specimens which chose that trap, must indicate that the related data are reliable. According to such an assumption, the level of trap performance must be considered as a statistical weight.

On this basis, the efficiency or performance level of the first-choice trap is given by the ratio between the number of specimens actually captured in the trap and the number of specimens which chose it, i.e. specimens in, under and over the trap.

We assumed that the performance levels of the two traps were uncorrelated. We also assumed that the number of specimens was large enough so their independent response to the trap was a reliable indicator. On these bases, we define a mean efficiency of the

overall capturing performance as the arithmetical mean of the two levels of performance. We define this as overall capturing performance.

Three main possibilities (first choice, second choice or no choice) have to be accounted for for each given time interval and each experimental run. Of course the time required for the number of specimens to choose (or not to choose) a trap must also be analyzed.

As the experimental conditions change, the response of the group of specimens under examination also changes.

In principle, one could use the mean of these data. Unfortunately, this would not take into account the peculiarities, such as the differing reliability of the traps during the different runs, characteristic of each run.

Another point to take into account is the number of specimens which choose a trap in comparison with the overall number of specimens. Actually these two quantities are different and, in principle, the closer their ratio is to unity, the more reliable the trial is. Thus, the ratio between these two quantities can indicate the overall run reliability.

An interesting point is the time during which the specimens choose a trap (first or second choice) or do not choose. The statistical question is what percentage of specimens makes a choice during the time interval of the experimental run.

Thus the data in which one is interested emerge from the means of the rough experimental data weighted by overall capturing performance and overall run reliability. This is indicated by e_i (where i indicates time). It is of course also possible to do the same for non-choice, where the final data is indicated by e_i^*.

In principle one expects that $e_i + e_i^* = 1$, which is not the case since the data have been transformed. Therefore, the final data must be normalised in order to obtain the unity from the sum of these two quantities. Therefore the data we are looking for are:

$$e_i^{\text{norm}} = \frac{e_i}{e_i + e_i^*}; \quad e_i^{*\text{norm}} = \frac{e_i^*}{e_i + e_i^*}$$

Figures 1 and 2 show the issues for the variables $e_i^{*\text{norm}}$ and e_i^{norm} for the case with and without the pheromone for the two different experimental conditions, without pheromone in Fig. 1 and with pheromone in Fig. 2.

Another point to discuss is the distribution of live specimens between the first choice and the second choice. The rough data for analysis must be weighted by both the overall run reliability and the trap reliability. In this way, one obtains the quantities q^{first} and q^{second}. Once again, the data must be normalised to set the sum of the probabilities to unity. Therefore we get:

$$q^{\text{first (norm)}} = \frac{q^{\text{first}}}{q^{\text{first}} + q^{\text{first*}}} \quad \text{and} \quad q^{\text{second (norm)}} = \frac{q^{\text{second}}}{q^{\text{second}} + q^{\text{second *}}}$$

In Figs. 3 and 4 we report the behaviour of the quantities $q_i^{\text{A norm}}$ and $q_i^{\text{B norm}}$ as a function of i (time) under the two experimental conditions, with pheromone in Fig. 4 and without pheromone in Fig. 3.

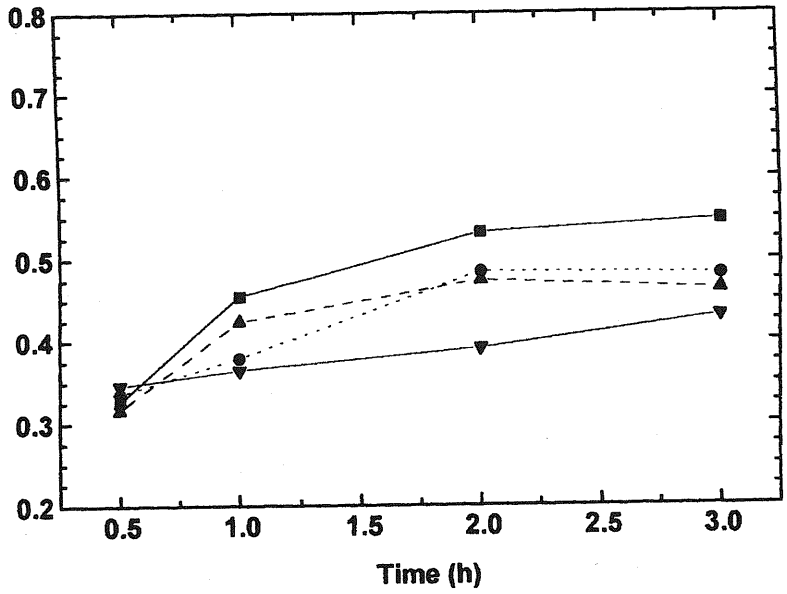


Fig. 1. Evolution in time of the variable e_i^{norm} (on the ordinate) when no pheromone is added. Squares: A = 0, D = 10; circles (dotted line): A = 5, D = 10; up triangles (dashed line): A = 10, D = 10; down triangles: A = 40, D = 10.

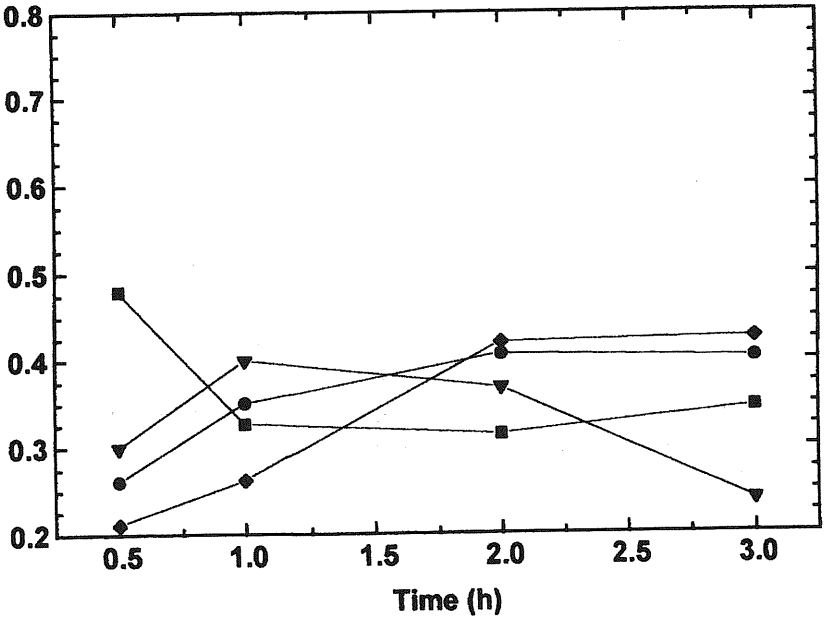


Fig. 2. Evolution in time of the variable e_i^{norm} (on the ordinate) when pheromone is added. Squares: A = 0, D = 10; circles: A = 5, D = 10; diamonds: A = 20, D = 10; down triangles: A = 40, D = 10.

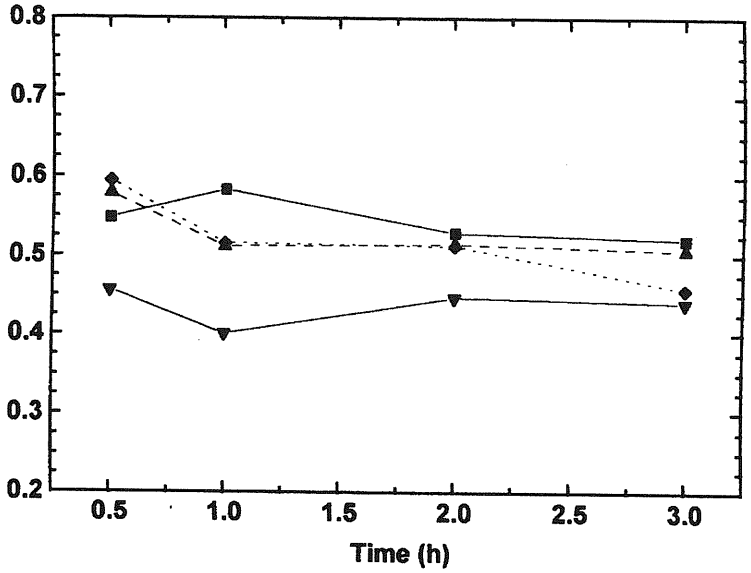


Fig. 3. Evolution in time of the variable $q_i^{A\ norm}$ (on the ordinate) when no pheromone is added. Squares: A = 0, D = 10; up triangles (dashed line): A = 10, D = 10; diamonds (dotted line): A = 20, D = 10; down triangles: A = 40, D = 10.

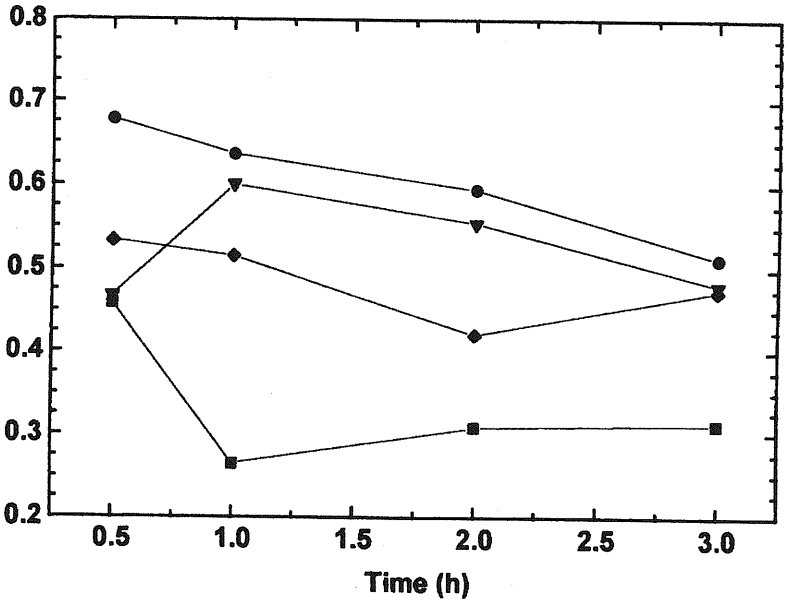


Fig. 4. Evolution in time of the variable $q_i^{A\ norm}$ (on the ordinate) when pheromone is added. Squares: A = 0, D = 10; circles: A = 5, D = 10; diamonds: A = 20, D = 10; down triangles: A = 40, D = 10.

RESULTS

The experiments reported here suggest that the presence and accumulation of living or dead insects in traps, whether baited with aggregation pheromone or not, interferes with the behaviour of other free specimens of red flour beetle. This could be due to the chemical substances produced by live *T. castaneum*.

According to the experimental data, the overwhelming majority of specimens which make a choice do so within the first 3 h after the starting time. As a consequence we confined the analyses to this time limit.

Figure 1 shows the time dependence of the probability of making a choice, whether A or D, where A stands for alive and D for dead in the case in which no pheromone was added to the trap, for four starting ratios of living/dead specimens (0/10, 5/10, 10/10 and 40/10).

It seems evident that specimens prefer to make a choice when either the trap is empty or it holds 10 dead specimens. When 40 living specimens are present in the trap, a large percentage of specimens prefer to remain outside both traps. Middle-range responses correlate with middle-range starting conditions. In Fig. 2 the experimental data using the same number of insects — but with pheromone added to the traps — are shown. In this case the situation is confused. No clear trend or intrinsic difference appears among the specimens regarding the traps. After 3 h, in the most favourable conditions, only 42% of the specimens had made a choice. In the previous case this represented the lowest limit.

Figure 3 shows the time dependence of the two choices (A or D) with no pheromone added. As a general rule, no definite preference for one trap over the other appears. Nevertheless, a statistically meaningful difference does appear between A and D. Between the choice of no living specimens in the trap and 10 dead specimens in the trap, a slight preference for choice A appears after 3 h (52% to 48%).

With the choice between 40 living specimens in the trap and no dead specimens, a slight preference for choice D appears after 3 h (44% to 56%).

The result is quite different when pheromone is added (Fig. 4). With a choice between no living and 10 dead specimens in the trap, the free specimens largely prefer D, with 10 dead specimens (30% to 70%).

Otherwise, no statistically meaningful preference appears between A and D where living specimens are placed in the trap, no matter their number (5, 20 or 40). To demonstrate to what extent one choice predominates over the other one, in Figs. 5 and 6 we show the time dependence of the normalised data corrected by the weighting factors previously introduced.

Even though a clear preference of A over D (for none and 10) or of D over A (for 40 and 10) appears, due to the statistical spread of the data only a weak statistical significance can be given to these results. On the other hand, when pheromone is added to the traps, a statistically meaningful preference appears: D is preferred to A, 0 and 10. No clear preference appears with 40 and 10.

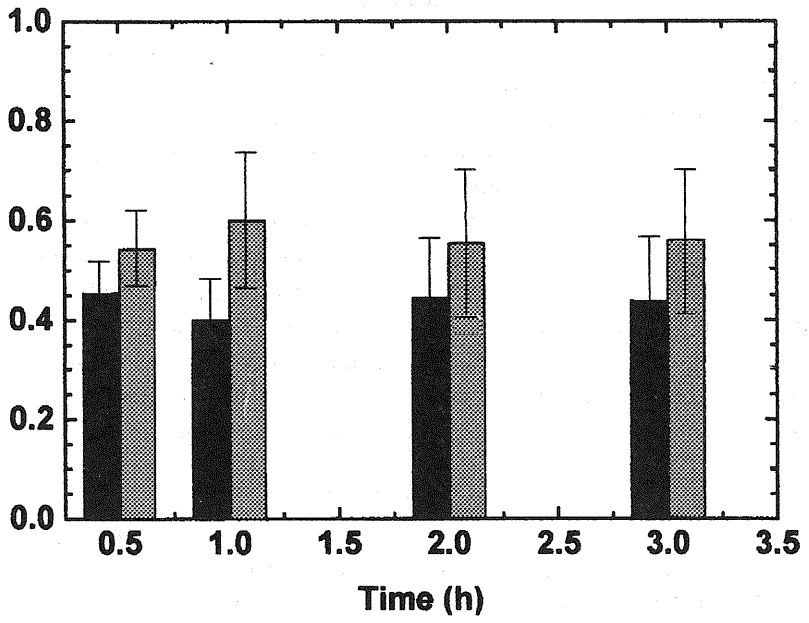


Fig. 5. Comparison of the evolution of the variables $q_i^{A\ norm}$ (black columns) and $q_i^{B\ norm}$ in time for the case A = 40, D = 10 without pheromone. Error bars related to each set of data are SD's.

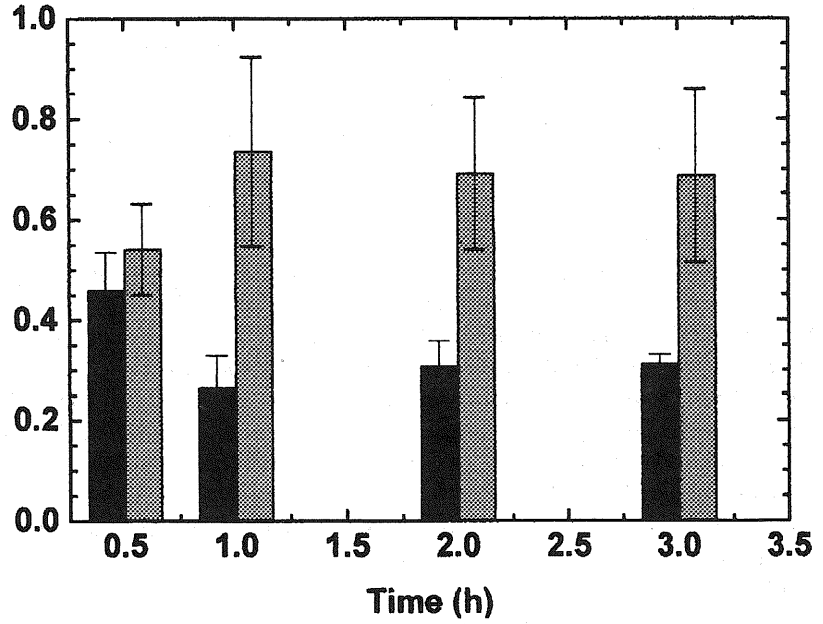


Fig. 6. Comparison of the evolution of the variables $q_i^{A\ norm}$ (black columns) and $q_i^{B\ norm}$ in time and for the case A = 0, D = 10 with pheromone. Error bars related to each set of data are SD's.

CONCLUSIONS

In trapping *T. castaneum*, the efficacy of traps (whether or not baited with aggregation pheromone) is influenced by the presence in them of accumulated specimens. This fact has been observed both with traps containing living insects and those with dead specimens.

The interactions of the different substances produced by *T. castaneum* adults for chemical communication are involved in this behaviour.

The results obtained in our trials suggest that living *T. castaneum* in traps attract other specimens, but under conditions of overcrowding the quinone they produce regulates population density by counteracting the effects of the aggregation pheromone, either causing the insects to disperse or suppressing aggregation behaviour.

The presence of dead insects in the traps, whether or not baited with 4,8-dimethyldecanal, can repel other free specimens of *T. castaneum*. This is probably related to the presence of a residual alarm pheromone produced by the specimens before death.

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THE USE OF TRAPS TO MONITOR INSECT INFESTATIONS IN COCOA CONTAINER SHIPMENTS

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ABSTRACT
(Full paper not available)

Cocoa beans are routinely fumigated with phosphine before being shipped in containers to the UK. If, upon arrival, the containers are found to be infested, they are re-fumigated. During 1993 an increase in the infestation rate in containers originating from West Africa was observed. To explore the potential of insect pheromone traps as a monitoring tool for infestations in such shipments, as well as to provide information on the effectiveness of the fumigations, traps were placed in containers prior to shipment and examined upon arrival. Initially, eight trap locations were used in each of 39 containers to evaluate three trap types: window trap and locator trap (both from AgriSense-BCS) and a prototype floor trap from CSL. The results indicated both the relative effectiveness of the trap types and the most effective placement of the traps. The results also demonstrated that the infestations were the result of a failure in the West African fumigations rather than of post-fumigation re-infestation. Recommendations are made concerning the number, placement and type of trap to use for monitoring infestations of moths and beetles within cocoa containers. The traps demonstrated their value to the cocoa trade, not only in pin-pointing fumigation problems but also as an inexpensive, essential monitoring tool.

POSTERS SESSION

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 653–658

A NEW MODEL FOR ANALYSIS OF DEVELOPMENT RATES OF THE RICE WEEVIL *SITOPHILUS ORYZAE* (L.)

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ABSTRACT

A way to analyse the experimental data on the comparative developmental rates (at a fixed temperature and relative humidity) of *Sitophilus oryzae* (L.) on five cultivars of wheat (*Triticum aestivum*, *T. dicoccum*, *T. durum*, *T. monoccoccum* and *T. spelta*) is proposed.

A simple exponential model is proposed for the cumulative curves of the development rates. The model accounts for the evolution of a given system from a stable state (no insects at all) to another stable state (complete adult emergence). The main advantage of our approach is that no fitting parameters are required; the only parameters required can be taken directly from the experimental data. Although the model used is the same, different behaviour of *S. oryzae* is manifested according to the food supplied. This corresponds to different survival strategies depending on the food available.

In all the cases examined, the reliability of the simulated data was above the 95% confidence level.

INTRODUCTION

The time required for insect development is influenced mainly by temperature, relative humidity (r.h.), diet and crowding (or population density). For stored-product insects, extensive data on the effects of temperature on developmental times are available. This facilitates predictions of fluctuations in insect life cycles and in the population trends of insect species (Dobie, 1977; Wagner *et al.*, 1984; Hagstrum and Milliken, 1988; Hagstrum and Throne, 1989; Subramanyam and Hagstrum, 1991, 1993; Beckett *et al.*, 1994). In the present study, a way is proposed to analyse and compare experimental data on the development of *Sitophilus oryzae* (L.), at a fixed temperature and r.h., for five different wheat cultivars (*Triticum aestivum*, *T. dicoccum*, *T. durum*, *T. monoccoccum* and *T. spelta*).

MATERIALS AND METHODS

Insect rearing

S. oryzae adults, obtained from the wild, were reared on wheat in the laboratory at 25 ± 1°C and 65 ± 5% r.h.; the photoperiod was natural (approximately 14 h light and 10 h dark). The cereal grains used in the experiments were *Triticum aestivum*, *T. dicoccum*, *T. durum*, *T. monococcum* and *T. spelta*, all obtained from local production in the Molise region of central Italy.

Fifty *S. oryzae* adults of mixed sex and age were released in a 1,700-ml glass jar containing 250 g of grain. They were removed 15 d later. The experiment was conducted at 25 ± 1°C and 65 ± 5% r.h. The number of next-generation adults obtained from the cereal cultivars was recorded every 2–3 d.

Analytical models

Previous models. There is no doubt about the importance of a mathematical/statistical model that permits both description and simulation of individual behaviour under different experimental conditions. As a first step, the data concerning adult emergence must be analysed under firmly established environmental conditions. It is widely acknowledged in the current literature that one must start by carrying out experiments in which all the parameters except time are fixed.

Several attempts have been made during the years to describe experimental data by simple models, using either the cumulative emergence or the daily emergence curves. Both of the most statistically reliable models proposed so far are based on the combination of the exponential function with a suitable choice of fitting parameters and constants (Howe, 1966; Sharpe and De Michele, 1977; Wagner *et al.*, 1984).

Preliminary considerations. Before introducing a new model, some fundamental preliminary considerations should be clarified. Whatever model is presented, it must have as few fitting parameters as possible to ensure that the fitting curves are statistically reliable. The model and its parameters must both have a clear biological interpretation in order to avoid misleading considerations of the behaviour of the individuals described. They must not only be fittable; it must be possible to estimable these parameters, in a straightforward way, on the basis of the data sets. The introduction of dependence on the environmental parameters must be straightforward and must not change the essence of the model.

It is clear from the literature that using the daily emergence data is much more difficult and statistically less reliable than using the cumulative emergence data. Because sampling times influence the data, experimental errors more directly affect both the daily data and the estimation of the fitting parameters. Thus, it is best to restrict the analysis to the cumulative data, leaving the comparison of the model outcomes with the daily or derivated data until a later time.

The simplest way to compare the data for different species or environmental conditions is to deal only with normalised, cumulative data. The underlying assumption is that one need not consider the actual number of adults emerging during each experimental run.

Instead, one should explore the general dynamic of the biological system made up of the species being studied and the fixed environmental conditions in which it is being studied.

The number of adults emerging must in itself be considered a parameter. It is a measure of the efficiency of the interrelationships between the species under examination and the environment in which it is living.

The function $y(t)$, modeling cumulative normalised adult emergence, is characterised by two obvious boundary conditions. As time approaches zero, it becomes zero. As time increases indefinitely, it tends to unity. In simple words, a time ($t = 0$) exists before which no emergence occurs, and a time ($t = \infty$) exists at which the biological cycle of the group of insects has been completed and the maximum possible number of adults has emerged.

When comparing different experimental runs, their synchronisation deserves attention. In order to ensure the clarity of the analysis of the results, the point of first adult emergence must be the same in all experiments. Therefore, during the first days of the experimental run special attention must be paid to carefully recording the first emergence, thus obtaining the zero point of the emergence curve.

The proposed new model. The model here proposed is based on the considerations developed in the previous sections. As far as the cumulative data themselves are concerned, one function describing them is the so called "survival curve" or Boltzmann function:

$$y(t) = -\frac{1}{1 + \exp[(t - t_0)/dt]}$$

This is a well-known formula often used when there is a complex system running from one stable state (in our case, no adult emergence) to another stable state (in our case, complete adult emergence). The daily emergence curve can be obtained as the derivative of the above formula.

Two parameters appear. One (dt) represents the half-width time at the half-height time in the daily emergence curve, while the other (t_0) represents the time at which the daily emergence curve reaches its maximum. Thus, both parameters can be estimated directly from the experimental data. As a consequence, the above expressions are more than fitting curves; they are models with "no free parameters" (Trematerra *et al.*, 1996).

RESULTS

The emergence of *S. oryzae* developing on five different wheat cultivars was analysed in order to validate the above model. Daily emergence (obtained from the experimental data collected as per the test procedure described above) was processed to give cumulative emergences. In all the cases analysed the chi^2 test indicated that the confidence level of the model was over 95%.

The curves obtained from the different sets of data are compared in Fig. 1 to show the actual differences in cumulative emergence for the different culture media. It can be seen

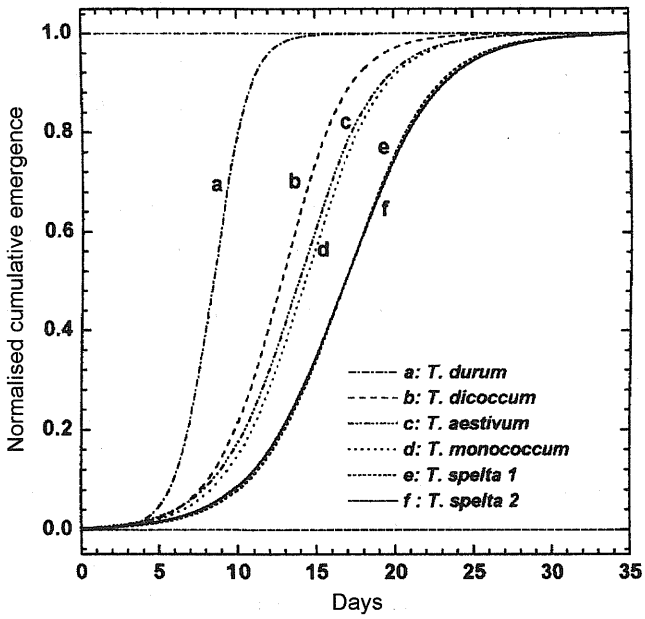


Fig. 1. The normalised cumulative emergence curves for all the experimental cultivars.

that the population dynamics of *S. oryzae* developing on the five wheat cultivars is described by only two parameters.

In some cases, maximum emergence of adults occurs within only a few days, whereas in other cases a long time period is required. In Fig. 2 the actual data (the non-normalised curves) are shown. These curves indicate both the rate and the number of adult progeny emerging during the experiment. Analysis of these curves provides information about the developmental efficiency of the given species for each of the different cereals used.

DISCUSSION

Even though the model presented is quite simple and restricted in the number of parameters, two main features deserve special attention. The model parameters can be estimated by observation of the experimental daily emergence curve because they represent the abscissa at which the maximum occurs and the half-width of the bell-shaped curve itself. Despite the reduced number of parameters, the data from all the culture conditions are fitted at the same level of statistical accuracy, revealing significant differences in development of the *S. oryzae* cultures depending upon the cereal used.

In Fig. 2 it is possible to compare the actual, non-normalised data. This shows that *T. monococcum* and *T. spelta* are the most favourable and *T. durum* and *T. dicoccum* the least favourable hosts in terms of total daily emergence.

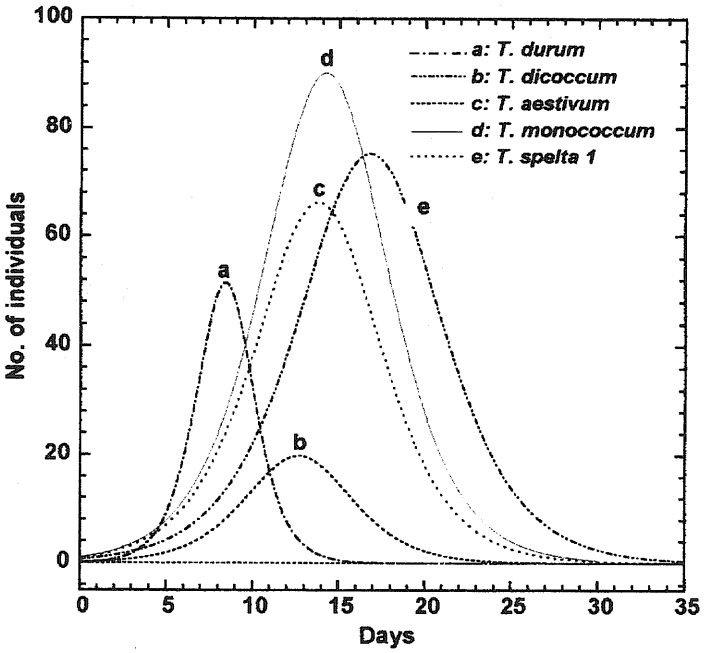


Fig. 2. The actual daily emergence curves for all the experimental cultivars.

The model is of interest in that it illustrates in a simple way, and using only two simple parameters, the inter-dependence between daily emergence of the insect pest and the kind of cereal. Accordingly, it should be possible to analyse the dependence of emergence curve on such other parameters as temperature and humidity.

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AN INNOVATIVE APPROACH TO MONITORING INSECT PESTS IN SILOS

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ABSTRACT

A new trap for monitoring pests in stored grain and other commodities was designed. A conical metal device is attached to a strong string which enables it to be used in silo bins holding deep grain masses. Usable as either a trap or a probe, this sampler enables simple, rapid and regular monitoring of stored grain to be done. The tool is also suitable for field evaluation of fumigation efficiency. Laboratory trapping data on this device's efficiency with four stored-product beetles are here presented.

INTRODUCTION

Early, precise detection of pests in bulk grain is a prerequisite for either successful fumigation or other protective measures. Traps are considered more sensitive than conventional sampling methods for detecting infestation. Insect pests can enter bins through openings above the headspace (Hagstrum *et al.*, 1994). Condensation at the surface layers of stored grain can encourage the build-up of infestations (Mills, 1990). Various surface traps, such as the grain probe trap, the PC trap, the cup trap and the pitfall trap, have been developed for monitoring insect pests in stored grains. These can all be used easily on the surface of bulk grain. However, their use in deep silos/bins is more problematic. Hagstrum *et al.* (1994) used sticky traps placed in the bin headspace. Wright (1991) used cardboard traps hung on strings to sample insect pests in empty silos. In the Czech Republic, 50% of the annual cereal production is stored by district agricultural trading companies (ZZN) in large silos and bins. No traps of any type have so far been used. Monitoring of pests (if done at all) is done by taking grain samples at the silo outlet since mobile vacuum samplers are considered by most storekeepers too time consuming for use in regular monitoring.

A new conical metal trap and sampler was developed in the Czech Republic to improve the quality of stored cereals. Initial testing has been carried out at the Research Institute of Crop Production in Prague. An evaluation of the efficiency of this trap in comparison with that of the "PC trap" (Cogan *et al.*, 1991) is here presented.

MATERIALS AND METHODS

Description and use

The metal conical trap/sampler is fastened to a long strong string. Its heavy tip permits it to be dropped from a height onto a remote grain surface. The basic set can be used by storekeepers in several configurations.

The hollow metal cone can be covered by a screw ring holding sets of removable wire-mesh lids with apertures of various sizes. This mode allows insect entry but retains grain (Fig. 1a). The metal cone can serve as a container for the "PC trap", thus giving results comparable with those obtained by that device (Fig. 1b). The final price for this mode, however, is higher than that of the preceding mode.

The plain cone can be fastened to the string (without the cover top, screw ring and wire sieve or PC trap). In this mode it can serve as a surface scoop for the quick and easy grain sampling required for periodic laboratory evaluation of moisture content, biochemical composition and hidden or mite infestation. This tool can also be used for field evaluation of fumigation results. Laboratory infested kernels (eggs/larvae/adults) enclosed in the trap chamber with the appropriate mesh for insect retention can be placed in the silo. After fumigation the samples can either be checked for insect mortality or incubated under favourable conditions until adult emergence.

Laboratory experiments

The laboratory experiments were carried out in complete darkness to avoid phototaxis in a controlled climate chamber (air, 26°C and wheat, 24°C with 50–60% r.h.). We

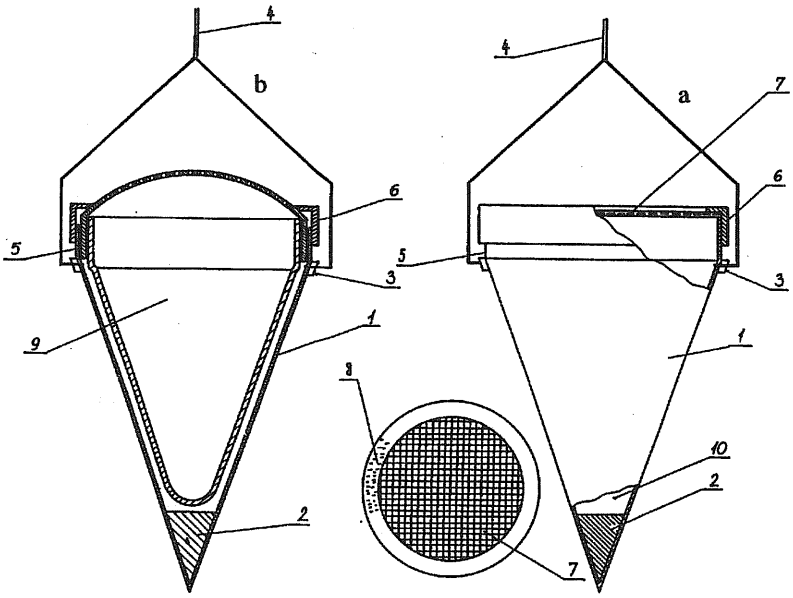


Fig. 1. Metal conical trap/sampler (a), with the PC trap (b) (1 = metal cone, 2 = heavy tip, 3 = ledge, 4 = string, 5 = thread, 6 = ring, 7 = mesh lid, 8 = rough surface, 9 = PC trap, 10 = chamber).

evaluated the responses of four species of stored-product beetles: *Tribolium confusum* J. duVal, *Sitophilus granarius* (L.), *Oryzaephilus surinamensis* (L.) and *Cryptolestes pusillus* (Schonherr). The strains were all insecticide-susceptible, having been mantained in the laboratory for many generations. A metal conical trap (in the first mode) was compared with a plastic PC trap (F. & B. Eng., Slough, UK). Five kg of sterilised wheat in plastic bags were placed in 22-cm diameter cardboard bins. At the start of each experiment, 50 adults of one of the above species were released and mixed into each bin so that the average infestation density reached 10 beetles/kg wheat. After 3 d of conditioning, either one "PC" or one "metal cone" trap was inserted into each bin. Each experiment consisted of three replicates. The traps were checked daily except on weekends.

RESULTS

Figure 2 shows the capture rates for the four beetle species in the two tested traps. The trapping curves were very similar for *T. castaneum* and *S. granarius*, but differed considerably for *C. pusillus* and *O. surinamensis*. Figure 3 shows the difference in capture rates between the traps after 30 d during which there was a significant difference only for

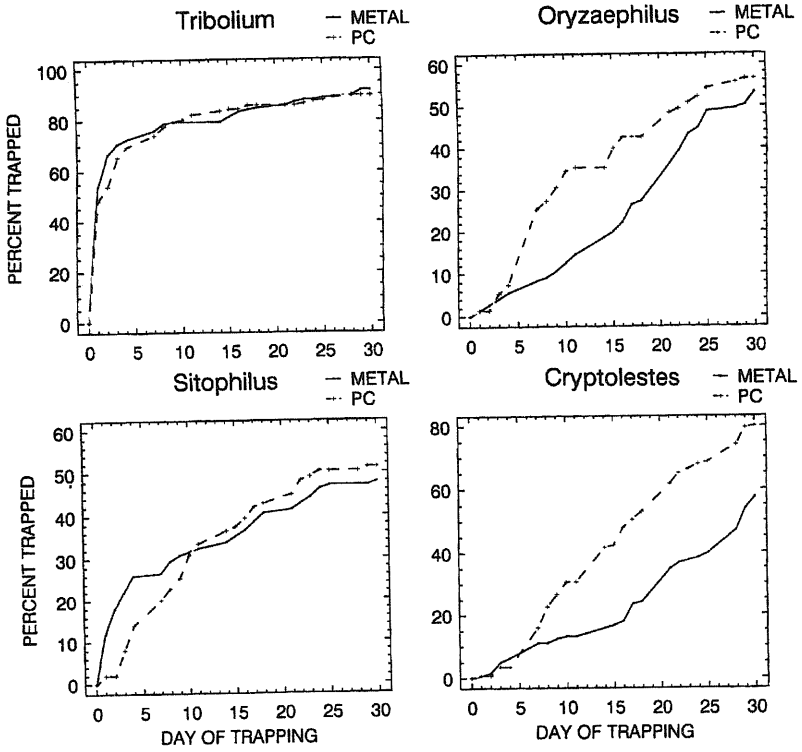


Fig. 2. Capture rate of *Tribolium confusum, Sitophilus granarius, Oryzaephilus surinamensis* and *Cryptolestes pusillus* in tested traps.

C. pusillus. After 30 d, the catch of the tested beetle species in both traps was, in descending order, *T. castaneum*, *C. pusillus*, *O. surinamensis* and *S. granarius*. However, as is clear from the curves in Fig. 4, because trapping response differed with time and species the trapability order was also time-variable.

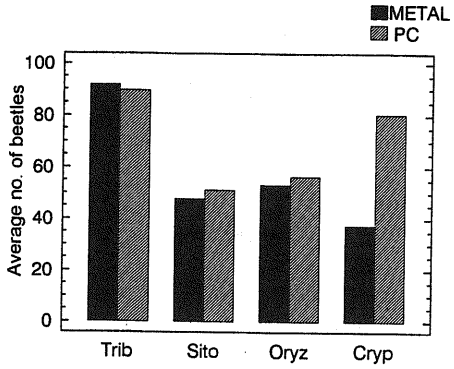


Fig. 3. Number of trapped insects after 30-d trapping period.

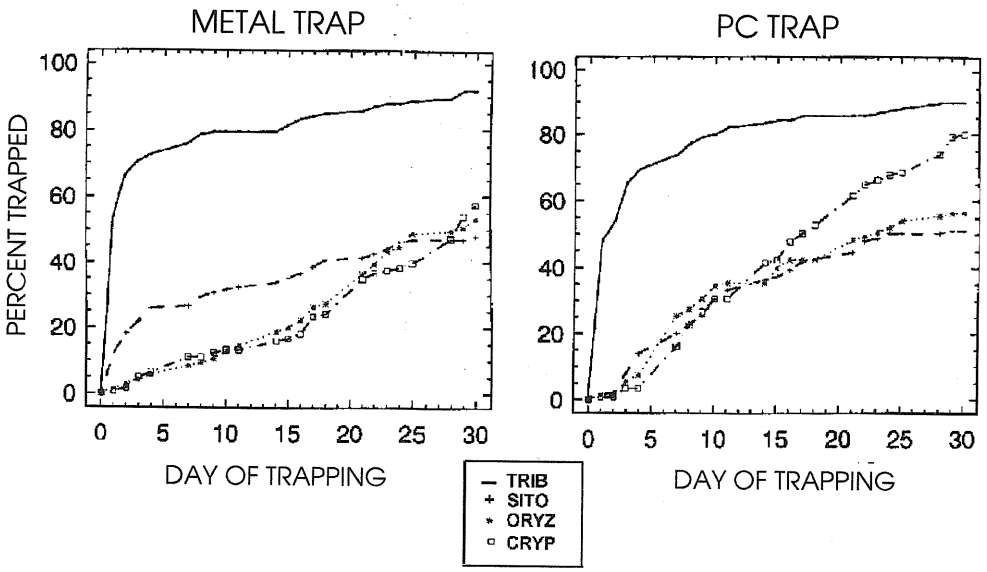


Fig. 4. Capture rate of four tested stored-product beetles in the PC trap and metal conical trap.

DISCUSSION

An increasing variety of insect traps with poor or no efficiency documentation has appeared on the Czech market (Stejskal, 1993). Nevertheless, there seems to be a widespread

tendency to demand accurate interpretation of trapping results in terms of population density (Wilkin, 1990). It is known that trap efficiency and catch interpretation depend on trap type as well as other parameters (Barak *et al.*, 1990) including the environmental conditions for each pest species (Cuperus *et al.*, 1990; Stejskal, 1995). The increasing product proliferation exacerbates the number of unknown variables (Wright and Cogan, 1995). In order to avoid adding to this trend, we compared our trap to the PC trap data of Cogan and Wakefield (1994). The first laboratory results indicate that the metal cone trap/sampler is similar in efficiency to the PC trap for all the tested species of stored-product beetles except *C. pusillus*.

Barak *et al.* (1990) stressed that traps must be both easy to use and cost effective. The metal cone trap enables easy sampling of inaccessible grain surfaces in silos. It can facilitate examination for hidden larval infestations at low temperatures by monitoring of commodities or grain samples. This increases its effectiveness. Acoustical detection devices may become a more promising monitoring method for large silos and bins (Fleurat-Lessard *et al.*, 1994) because they provide population density estimates without disturbing the grain bulk. Nevertheless, in many cases traps will continue to be the cheapest and most easily used monitoring alternative.

ACKNOWLEDGEMENT

I wish to thank J. Zuska for reviewing the manuscript. This work was partially funded by a grant (A 5533) provided by the Czech Ministry of Agriculture.

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EFFICACY OF OZONE TO CONTROL INSECTS, MOLDS AND MYCOTOXINS

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ABSTRACT

With the reduced availability of traditional post-harvest storage pesticides, safe alternatives are desperately needed. The long range goal of this research is to find new technologies for controlling insects and mold growth in stored grain and decreasing the likelihood of mycotoxin contamination. With this as the goal, this research project focused on the use of ozone technology in post-harvest grain storage. The specific objectives were to determine the effect of ozone on the survival of insects (*Tribolium confusum* and *Oryzaephilus surinamensis*) and mold (*Aspergillus flavus* and *Fusarium moniliforme*), and the production of mycotoxin in stored corn. Ozone atmospheres (5 ppm) were compared to air environments (controls) in their respective effects on insect mortality, mold radial growth, sporulation and aflatoxin production. For *T. confusum*, 100% mortality was found in 5 d, and for *O. surinamensis* in 3 d. Radial growth of both *A. flavus* and *F. moniliforme* was inhibited for the first 2 d, but after 3 d in the ozone atmosphere growth paralleled that in the control. Sporulation and hyphal growth above the surface of the agar were completely inhibited by the ozone. Aflatoxin production was also reduced by over 99% in the *A. flavus* cultures exposed to ozone.

INTRODUCTION

Insects, molds and vertebrates cause numerous quality problems in stored grains and processed food and feeds. More than 15 billion bushels of grain are stored every year in the United States, and total annual storage losses are estimated at more than $1 billion. Estimated losses due to stored-grain insects exceed $12 million annually in Indiana alone. In the grain storage industry it is essential to have effective pest management programs which protect against economic loss; insect, mold and mycotoxin contamination; and disease due to pest-contaminated foods. Insect pests in storage are currently managed by the application of chemical pesticides. Only two fumigants are still permitted, one of which is methyl bromide (MB). Because of environmental concerns, the US government has dictated that

MB will be eliminated from use by the year 2001. There is no direct substitute for MB that is equally effective and fast-acting, nor is the development of such a corrective tool expected. Additionally, no chemicals except organic acids such as proprionic acid are available for controlling mold growth in stored grain. Unfortunately, in many situations these acids are not suitable. Therefore, new pest management practices are needed in stored grain. The principal investigator (PI), in cooperation with other members of the post-harvest research team, is currently investigating existing residual pesticides (Actellic and Reldan) and alternative pest control strategies (aeration, temperature control and modified atmospheres using carbon dioxide (CO_2)). Ozone (O_3) technology will complement our existing research program.

Ozone is one of the strongest oxidizing agents known. O_3 technology is currently being used in the industrial and medical industries as a disinfectant of microorganisms and viruses and for reducing odor and removing taste, color and environmental pollutants. O_3 is an attractive alternative to other chemicals primarily because it has a short half-life and with it there is an absence of residuals. With a half-life of about 20–50 min in the atmosphere and 1–10 min in water, O_3 rapidly decomposes to diatomic oxygen (a natural component of the atmosphere). O_3 is also attractive because it can be generated on site, eliminating the need for storage.

In recent years, the manufacturers of O_3-generating devices have addressed the possibility of its use in agriculture. Potential applications include deodorizing poultry and swine-waste lagoons and sterilizing the water used to wash chicken carcasses in poultry packaging operations. Published research suggests that the application of O_3 to a stored-grain facility may be a feasible alternative to the fumigant MB. The possibility of controlling mold growth and mycotoxin formation in storage would be additional benefits.

Because of the worldwide use of O_3 for water purification, most of the published research documents its effects on microorganisms in water. Except for a few studies done in the 1960's on its effects on fungal plant pathogens, essentially nothing is known about how O_3 affects fungi (Rich and Tomlinson, 1968). Some effort has been made to determine if ozonation is an effective means of eliminating aflatoxin from peanut and cottonseed meals (Dollear *et al.*, 1968; Maeba *et al.*, 1988), and this research indicated that it can destroy aflatoxin.

There are a few studies suggesting that O_3 has potential for controlling insects. Research on the flour beetles, *Tribolium confusum* and *T. castaneum*, has shown that these insects are sensitive to O_3 (Erdman, 1980). It was found to be lethal to all stages of the insects' life-cycle with the larval and pupal stages being most sensitive.

Both the published literature and our preliminary studies suggest that O_3 can be used for grain pest management. The technology for generating O_3 concentrations capable of fumigating grain storage bins exists today. Our discussions with those in the industry indicate that the generators, already in use for water and air purification, can be adapted for delivering whatever concentration of O_3 is needed. However, some questions need to be answered before this technology can be used. Can O_3 effectively prevent insect and mold growth on stored grain? What concentration, and duration, of O_3 exposure is needed

to control these organisms? Are the benefits of using O_3 great enough to reduce the current use of pesticides in stored grain? The current study is the first step in answering these questions.

RESEARCH OBJECTIVE

The long-range goal of our research is to find new technologies that control insects and mold growth in stored grain and decrease the likelihood of mycotoxin contamination. In accordance with this goal, the research project here reported focused on the use of O_3 technology as a means of controlling insects, molds and mycotoxins in post-harvest grain storage. The specific objectives were to determine the effect of O_3 on insect (*T. confusum* and *Oryzaephilus surinamensis*) and mold (*Aspergillus flavus* and *Fusarium moniliforme*) survival and on mycotoxin production in stored corn.

METHODS

Ozone detection

O_3 levels were measured using SENSIDYNE® detector tubes (4–400 ppm range) in a Gastec Multi-Stroke Gas Sampling Pump. The concentration of O_3 around the insects and petri plates was maintained at 5 ± 1 ppm. Concentrations were confirmed at the beginning and once again either during or at the end of each trial.

Insect mortality

Insects were obtained from a laboratory colony maintained at 21°C, 14:10 L:D photophase. Fifty unsexed adult insects (less than 1 week old) of each species were placed singly in 1.7-ml micro-centrifuge tubes that contained approximately 1 ml flour/cornmeal. The snap-cap lid was replaced with fine mesh fabric. The micro-centrifuge tubes were then placed in a cardboard tray with holes cut to support each tube vertically. The tray was then placed in a 8.3 L (24 × 34 × 12 cm) RUBBERMAID® container. O_3 was then allowed to flow into the container, around the tubes, and out of the container. Mortality counts were determined every 24 h. Control insects were held in tubes under similar temperature and photophase conditions. *T. confusum* trials were replicated four times (200 insects total) and *O. surinamensis* three times (150 insects total).

Mold radial growth, sporulation and aflatoxin production

Petri dishes containing potato dextrose agar (PDA) or coconut agar (CA) medium were inoculated in the center with 5 µl of conidial suspensions of *A. flavus* and *F. moniliforme*. The plates were incubated at 21°C in an atmosphere of either O_3 or air (control). Radial growth was determined daily by measuring the colony diameter. After 5–6 d growth, the PDA-grown cultures plates were flooded with 5 ml of water, and the conidia concentration was determined using a hemacytometer. Simultaneously, aflatoxin production was determined by thin-layer chromatography analysis of extracts taken from the CA culture plates.

RESULTS AND DISCUSSION

Insect mortality

O_3 caused significant mortality in both species examined. On day 3 *T. confusum* mortality was significantly higher than the control, and it continued to increase for the next 2 d (Fig. 1). One hundred percent mortality was achieved by day 5. Only a few of the 200 control insects died during the 5-d test period. O_3 had a much more rapid influence on *O. surinamensis* mortality. Significantly more adults died on day 1 when compared to the control (Fig. 2). One hundred percent mortality was achieved by day 3. These data indicate that O_3 at 5 ppm has a significant influence on adult mortality of *T. confusum* and *O. surinamensis.*

Differential mortality between species has been found in other studies (Erdman, 1980), in which O_3 toxicity ontogenies for *T. confusum* and *T. castaneum* at 45 ppm O_3 were compared. In his study, exposure times were less than 6.5 h and mortality was measured 1 month post-eclosion. He found that *T. castaneum* life stages were more sensitive than those of *T. confusum*; however, both species were equally sensitive in the pupal stage. Our data indicate that O_3 at much lower levels (5 ppm) can cause significant mortality to the adult stage when exposure times are increased (24 h minimum). Experiments underway indicate that even shorter exposures are needed for 100% mortality of other life stages.

Mold radial growth, sporulation and aflatoxin production

At 5 ppm O_3, the radial growth of both *A. flavus* and *F. moniliforme* was inhibited for the first 2 d (Figs. 3 and 4). After 3 d in the O_3 atmosphere, the growth paralleled that of

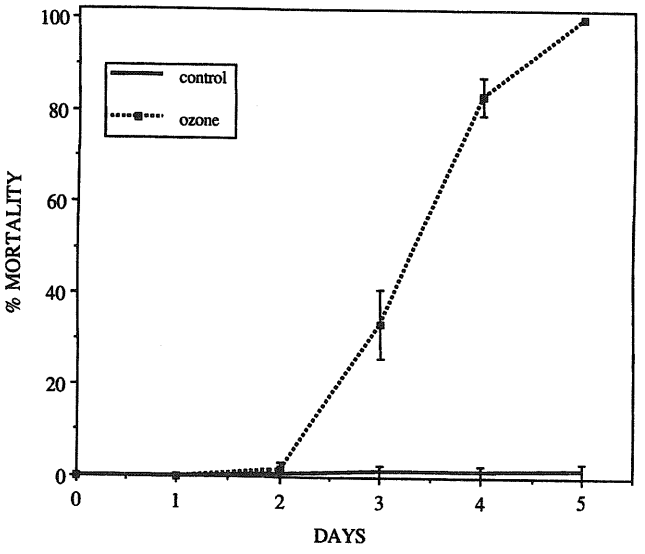


Fig. 1. The effect of ozone on the mortality of *Tribolium confusum* (Bars represent mean ± SE).

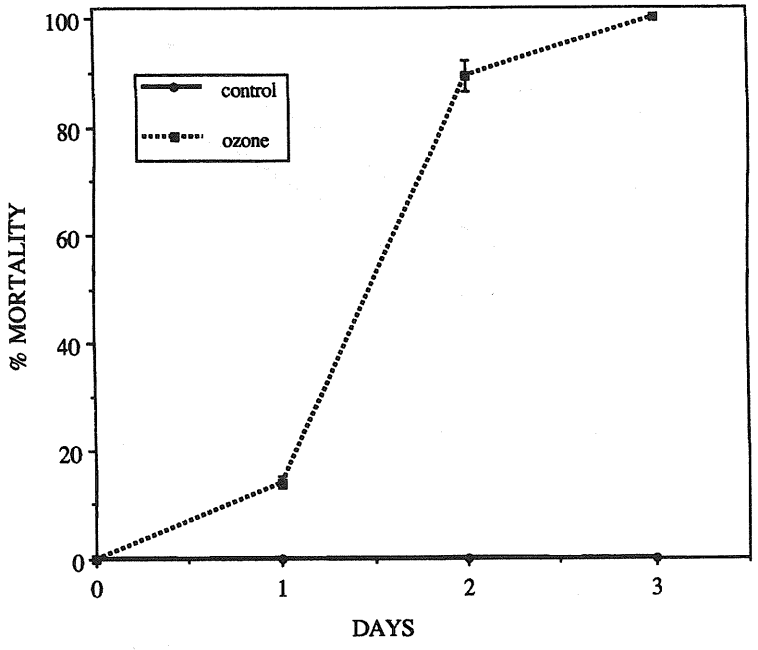


Fig. 2. The effect of ozone on the mortality of *Oryzaephilus surinamensis* (Bars represent mean ± SE).

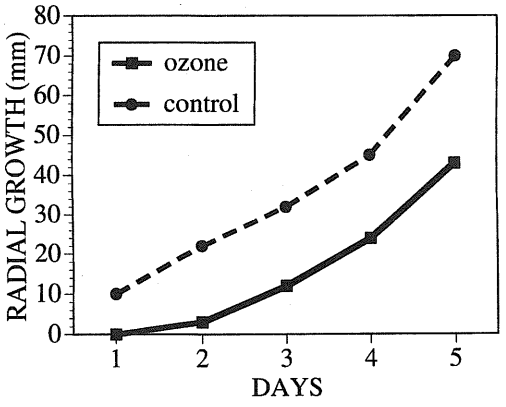


Fig. 3. The effect of ozone on the radial growth of *Fusarium moniliforme*.

the control. Sporulation and hyphal growth above the surface of the agar were completely inhibited by O_3 (Table 1). In contrast, in the control atmosphere there were both profuse sporulation and surface mycelium. These data indicate that O_3 has a direct inhibitory effect on the growth of these fungi and suggest that the gas does not penetrate the surface of the agar medium. Aflatoxin production was also reduced by over 97% in the *A. flavus* cultures exposed to O_3 (Table 1).

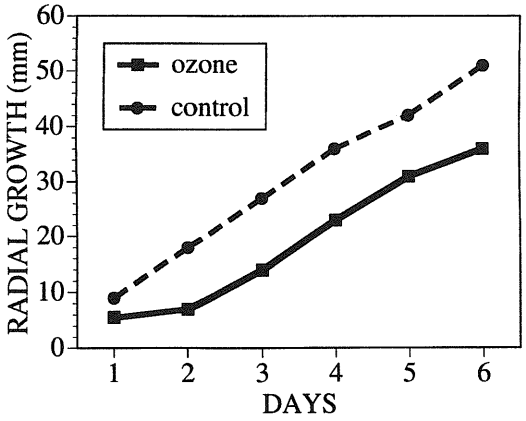


Fig. 4. The effect of ozone on the radial growth of *Aspergillus flavus*.

TABLE 1
The effect of ozone on the sporulation and aflatoxin production by *Aspergillus flavus* and *Fusarium moniliforme*

Mold	Ozone-treated	Control
Aspergillus flavus		
Sporulation	0 conidia/plate	1.0×10^9 conidia/plate
Aflatoxin	32 µg/plate	1,000 µg/plate
Fusarium moniliforme		
Sporulation	0 conidia/plate	1.0×10^8 conidia/plate

SUMMARY

Studies are currently under way to examine additional insect life stages, insect species and O_3 concentrations. In addition, diffusion models are being developed for O_3 within a grain mass. It is hoped that full scale trials will begin later this year.

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RAPPORTEURS' REPORTS

(The editors regret that the Rapporteurs' Reports of Sessions 6 and 9 could not be included in this Proceedings volume)

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 673–687

SESSION 1: BIOLOGICAL RESPONSES OF ARTHROPODS TO TREATMENTS WITH CA AND/OR FUMIGATION

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Rapporteur's Report

In session 1 a total of ten papers were presented covering in general the results of research carried out in different countries on arthropods' biological responses to treatment with CA's and/or fumigation. The research work presented at the session covered several subjects.

The first paper was "The use of mixed-age cultures in the measurement of response to phosphine." This work was conducted in Australia by E.A. Hyne and R.W. Winks, who also presented the results. They found that the use of mixed-age cultures in laboratory assey tests is an effective approach, and the results of such tests are of high value for field applications. The main advantage of this method is that it is unnecessary to know which is the most tolerant stage of the test insects because all developmental stages are exposed to the toxicant at the same time. For the application of this method it is necessary that all stages, and especially the most tolerant ones, be available in adequate numbers. Dosage rates and exposure periods can be adjusted accordingly until full control of the most tolerant stage is obtained.

The second paper, "On the efficacy of sulfuryl fluoride against stored-product pest moths and beetles," was presented by M. Schöller from Germany. The results of the experimental work on the effectiveness of this chemical against eight species of stored-product pests were presented, and the methodology followed was described. It was concluded that in practice the use of sulfuryl fluoride for stored-product insect control in the future is rather limited due to the high dosage rates required for complete control of the most tolerant stages of insects, the high cost of the material, its limited efficacy and its potential for high levels of residues.

The next paper, "The sensitivity of narcissus flies to methyl bromide," was presented by J. Donahaye. The work was conducted in Israel by the speaker in cooperation with S. Navarro and others. The objective was to find the most suitable dosage rates of methyl bromide (MB) for complete control of both species of narcissus flies without causing any phytotoxic effects on treated narcissus and amaryllis bulbs. The characteristics of both

narcissus flies, the methodology used and the results obtained were presented. It was established from the results that a higher dosage of MB is required to achieve 99% mortality of the large narcissus fly than that required for the small narcissus fly.

The next paper, on the effect of temperature on the response of susceptible and resistant strains of stored-product beetles to phosphine (PH_3), was presented by E.A. Hyne. The work was carried out in Australia by the speaker and R.G. Winks. It was found that although in general insects are more tolerant to PH_3 at low temperatures, this model does not always hold when resistant strains are examined. In their trials, populations of test insects were exposed to constant PH_3 concentrations at temperatures ranging from 15 to 35°C, and the times required for population extinction of susceptible and resistant populations were established. At all concentrations tested, the higher temperatures reduced the exposure period required for complete mortality of the susceptible strains of *Sitophilus* spp. and *Rhyzopertha dominica* and the resistant strains of *S. oryzae*. In contrast, the exposure period for complete control of resistant *R. dominica* was longer at 35°C than at 25°C at all except one of the concentrations tested. It was concluded that insect response to PH_3 at any temperature depends on species resistance to the fumigant, the concentration and the time of exposure.

The next paper, "The toxicity of methylphosphine to susceptible and phosphine-resistant strains of four species of stored-product insects," was presented by M.Q. Chaudhry. The experiment was carried out in the UK by the speaker in cooperation with two other researchers. The objective was to study the insecticidal value of methylphosphine, a close analog of PH_3, against PH_3-resistant strains. The mode of action, the uptake rate by susceptible and resistant strains and the mechanisms of resistance to PH_3 were discussed. Higher mortalities were obtained with exposure of four species of PH_3-resistant strains to methylphosphine than were obtained with susceptible strains. It was noted that it is likely that the methyl group in the fumigant prevents exclusion of the fumigant by the resistance mechanism. It is also possible that the PH_3-resistance mechanism in resistant strains metabolizes methylphosphine to toxic products.

The next study, "Effects of carbonyl sulfide on *S. granarius*, *Fusarium avanaceum* and *F. culmorum* and with regard to possible corrosion of copper," was presented by Rudy Plarre, USA, in collaboration with C. Reichmuth, Germany. All the life stages of *S. granarius* and of both *Fusarium* species were tested for their susceptibility to different exposure times and concentrations of this fumigant at 20°C and 70% r.h. The dosage rates and exposure periods for complete kill of *S. granarius* were established. Eggs of this beetle were the most tolerant stage to this fumigant, followed by pupae and adults. Larvae were the most susceptible stage. It was also found that dosages lethal to *S. granarius* caused inhibition of *Fusarium* growth during the fumigation period only. In the presence of high relative humidity (r.h.), this fumigant caused copper corrosion. The possibility of future practical use of this fumigant as an alternative to MB was discussed.

The next paper, "The effect of modified atmospheres on the juvenile stages of six grain beetles," was presented by Chris Bell. The study was conducted by the speaker and S.T. Conyers at Slough, UK. The effect of three modified atmospheres on adult

emergence after exposure of juvenile stages to these atmospheres at 15°C and 70% r.h. was assessed. The methodology used and the results obtained were presented. It was found that internal grain feeders like *R. dominica*, *S. granarius* and *S. oryzae* were able to emerge after lengthy exposures to modified atmospheres. Similarly, *T. castaneum* was the most tolerant species. Carbon dioxide (CO_2) was the most effective of the MA's tested, followed by simulated burner gas and nitrogen (N_2).

The paper on the control of the dermestid beetle, *Dermestes maculatus*, was presented by C. Adler, who worked in cooperation with C. Reichmuth and Ana Sa-Fischer from Germany. The objective was to evaluate various controlled atmospheres, under laboratory conditions, against all stages of this very serious dermestid beetle. Atmospheres of pure N_2 or pure CO_2 provided complete control of all stages of the test insect within 48 h. Similar control levels were obtained by using an atmosphere consisting of 98% N_2 and 2% oxygen (O_2). Other combinations and concentrations of CO_2, O_2 and N_2 were also evaluated, and exposure periods required for the control of all stages of the pest were established.

The next paper, on rapid disinfestation through the combination of controlled atmospheres and heat, was presented by C. Adler. He reported that in experiments with *S. granarius,* treatments with CO_2 (60% or 90%) in air, and with 98% N_2 and 2% O_2, took 46 and 72 d, respectively, for complete control of the test insect at 10°C, although the exposure period can be reduced to 8 d at 35°C, or even to 2 d at 40°C. Therefore, controlled atmospheres can be used very effectively at high temperatures for disinfestation of valuable crops or stored products in warm climates.

The last paper dealt with a comparative study of PH_3 resistance levels in stored-grain beetles collected from sealed and unsealed farm storages in Western Australia (WA). The paper, presented by Robert Emery, was an analysis of a large survey carried out in 1991 to evaluate the frequency of resistance of *T. castaneum*, *S. oryzae*, *S. granarius*, *R. dominica* and *O. surinamensis* in storage sites in WA. His findings showed that there is no significant difference between sealed and unsealed storages with respect to frequency of resistant populations although whether they give rise to significantly different levels of resistance remains to be determined.

SESSION 2: BIOLOGICAL RESPONSES OF MICROFLORA TO TREATMENT WITH CA AND/OR FUMIGANTS

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Rapporteur's Report

Fungi remain important causes of deterioration of stored grain that has been insufficiently dried, especially in humid, tropical climates. They cause grain to discolor, heat and lose dry matter, and they attract insects and mites. Some species may also produce toxic secondary metabolites (mycotoxins) that can cause diseases in man and animals through toxic, immunotoxic and carcinogenic action on specific organs. Fungal growth is completely prevented only if grain is dried to water activities (a_w) under 0.65–0.70 a_w, equivalent to water contents of 12–13% in cereal grains or 6–7% in oilseeds. Mycotoxin production may be prevented within narrower limits. The six papers presented in this session illustrated many of the problems of doing, and interpreting the results of, laboratory and field experiments.

Bagged paddy with 14% water content generally stored well at the center of Volcani cubes, constructed of gastight, heavy-duty plastic liners. However, *Eurotium chevalieri* increased in number, even though an atmosphere containing up to 15% CO_2 and down to 3–4% O_2 was generated. Numbers of fungi generally increased at the periphery of the bulk. With 16% water content in a plastic silo, *Aspergillus flavus* increased as other species declined, even though CO_2 increased to 18% and O_2 became undetectable (Caliboso *et al.*). It remains questionable whether the modified atmosphere or water activity had the greater effect on colonisation.

E. chevalieri also increased in bagged maize treated with PH_3 but declined in similar maize enclosed in plastic sheeting and treated with CO_2. However, *A. flavus* continued to grow at up to 80% CO_2, and aflatoxin was produced in significant amounts at up to 60% CO_2. *Aspergillus permicilloides* increased in similarly treated rice. Although CO_2, PH_3 and MB decreased fungal growth, no treatment eliminated it (Dharmaputra *et al.*).

In general, attempts to prevent moulding and mycotoxin contamination of stored grain have utilised only one strategy. The utilisation of synergistic and additive effects from applying different methods of control in an integrated strategy has seldom been considered. The use of biological, chemical and physical control methods in a single control strategy could inhibit moulding without having to use extreme levels of any one factor. Thus storage periods for damp grain have been extended by the combined use of 0.2% propionic acid, 2–4 kGy gamma irradiation and a 60% CO_2 modified atmosphere although no single treatment would alone have prevented moulding. Natural products inhibiting mould growth or mycotoxin production could be substituted for propionic acid, while partial drying could increase the effects of other treatments (Paster).

The tolerance of fungi for low O_2 atmospheres has been largely underestimated. Although generally considered aerobic, fungi can be efficient scavengers of O_2 and grow with <5% O_2. A combination of high CO_2 and low O_2 concentrations is most effective in preventing fungal growth, but results of experiments in culture sometimes contrast with those using a grain substrate. Increasing O_2 concentration from 0.5% to 20% O_2 with 80% CO_2 could sometimes negate the inhibitory effect of the larger CO_2 concentration. Mycotoxin production could be found with up to 60% CO_2 + 0.5% O_2. Although 20% CO_2 + 0.5% O_2 allowed only slight growth and no aflatoxin production by *A. flavus* on agar, aflatoxin concentrations in cheese were sufficient to be of concern with 20 or 40% CO_2 and 1 or 5% O_2 (Hocking and Taniwaki).

Elevated CO_2 concentrations in stored grain often result from respiration of the grain and its associated microflora. Continuous measurement of respiration has previously been difficult, but a new electrolytic respirometer has made possible the measurement of O_2 production in response to CO_2 absorption into alkali. This has allowed input of data into models of ambient-air drying and could be used in modelling CO_2 production during damp-grain storage. The contribution of fungi to total grain respiration remains controversial, and different species differ widely in their respiration rates. It seems likely that respiration at low a_w (where germinability remains high) is primarily attributable to the grain, while that at high a_w (where germinability has been lost) is mainly due to microorganisms (Lacey *et al.*).

To draw a single conclusion from the data presented is not possible, given the diverse nature of the papers. Considerably more work is required before the tolerances of fungi for controlled atmospheres and fumigants are fully understood. This will require fully replicated experiments with adequate statistical control and an attention to detail that is not always evident in published reports. Methods must be described in such a way that work can be interpreted and repeated. Water activity must be determined in different parts of a bulk of stored grain, both near the centre and at the periphery, especially if this is covered by an impermeable membrane. Actual concentrations of CO_2, O_2 and fumigants attained during treatment, and their persistence, also need to be measured, while the experimental design needs to be adequate to distinguish between the effects of controlled atmosphere or fumigant and those of water activity. The measurement of fungal growth in grain continues to exercise microbiologists. Neither plate counts of colony-forming units nor direct plating of grain (to determine the percentage infected), estimates fungal biomass. Mycelial growth and sporulation may respond differently to treatment. Ergosterol allows a measure of total biomass, but it is subject to limitations. Another method that responds equally to all fungi is urgently required.

SESSION 3: INFLUENCE OF CA AND/ OR FUMIGATION ON QUALITY PRESERVATION OF STORED PRODUCTS

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Rapporteur's Report

Although nine papers were offered in this session, it was disappointing that only four of these were presented at the conference.

The first paper, presented by Shlomo Navarro, described collaborative investigations conducted by the Department of Stored Products in Israel and the National Post Harvest Institute for Research and Extension in the Philippines. The main project objective was to design an affordable, user-friendly system, which did not rely on the use of pesticides, for the farm storage of paddy in the Philippines. The Volcani Cube, a flexible, gastight structure previously developed for use in Israel, was evaluated, using 10- and 20-t structures, for suitability for storing paddy under tropical conditions in the Philippines. It was concluded that paddy spoilage during storage, caused by wetness due to condensation, could be prevented by using an insulating layer of rice hulls to reduce temperature gradients. Under Philippine conditions, bulk or bagged paddy could be stored for 4 months without reduction in seed viability or in milling recovery, and yellowing was not affected. The cost of the structure was not addressed during the presentation but was subsequently questioned. There was an indication that this was likely to be substantial, and cost could be a significant factor in the potential for adoption of the structure by farmers in the Philippines.

The second paper, given by Boris Yakobson of the Kimron Veterinary Institute in Israel, dealt with a topic rather different from those usually presented at the conference. B. Yakobson presented the results of an investigation into control of the greater wax moth *Galleria mellonella*, which damages beeswax and combs, using CO_2 as a replacement for the chemical control methods formerly employed. It was found that effective control of all developmental stages of the moth could be achieved using a 60% concentration of CO_2 maintained for 100 h. It was reported that this method of control has now been in use for 5 years and is considered to have many advantages, including its being environmentally friendly and safe to apply and its enabling honey to be produced without chemical residues.

Some attempt to use the hermetic principle of grain storage had been traditionally practiced in Cyprus for many years, and in the third session paper a description of four methods formerly employed was jointly presented by the Ministry of Education and Culture, Nicosia, and the Cyprus Grain Commission. Eleni Papedemetriou described four traditional grain storage methods, one an underground structure and the others, including an oven and a clay pot, for storing grain above ground. The use of fire before loading

grain in the underground store and in the oven was an attempt to both control any residual insect infestation and reduce the r.h. One other grain-storage technique employed a thick-walled bag that was buried under a layer of straw. Andreas Varnava, in providing some scientific explanations for the use of these traditional storage techniques, concluded that the underground store and the oven had probably provided some degree of airtightness, but clay pots, because they were opened very frequently, offered little if any opportunity for hermetic control of insects. Although the storage of grain within straw resulted in some modification of the surrounding air, there was insufficient evidence to suggest that the method provided effective control of insects. It was concluded that none of the traditional methods described provided truly hermetic conditions for grain storage.

The final paper of the session described investigations, conducted in Cyprus over 7 years, into the storage of barley under PVC liners. The programme was carried out by the Cyprus Grain Commission and, in presenting the results of investigations, A. Varnava acknowledged the collaboration of the Israel Department of Stored Products. Trials evaluated the storage of bulk barley loaded on concrete platforms and covered with PVC sheeting. The effect of using a polythene base sheet, on which the grain was loaded, was investigated. In some trials, the platform storage technique was evaluated over a 3-year period. Although the use of a base sheet was found to be beneficial in influencing CO_2/O_2 concentrations, it did not affect overall grain losses. Storage of barley using the platform technique resulted in germination being retained at levels in excess of 94% and, although grain that was visibly damaged by mould growth contained mycotoxins, sound grain remained toxin free. Costs using the platform technique were shown to be reduced when the period of storage increased. Substantial losses of grain were sustained in Cyprus during the period 1992–1995 because of rain damage, and it was clear that, had the Cyprus Grain Commission been using the platform storage technique routinely during this period, grain losses would have been much reduced. It was concluded from investigations into platform storage that, for dry barley under Cyprus conditions, the technique was very cost-effective and also gave good protection against rain, birds and rodents. Conference delegates had the opportunity to learn more about the platform storage method and to see it in operation during a field excursion in Nicosia.

The general conclusion from the session presentations was that techniques employing CO_2 and controlled atmospheres were being widely researched and developed to a degree where, in some instances, they were suitable for introduction into routine storage practice. The benefits to be derived from such introductions were effective techniques for pest control purposes which were both user and environmentally friendly.

SESSION 4: PHYSICAL AND CHEMICAL PROCESSES IN THE APPLICATION OF CA AND/OR FUMIGATION

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Rapporteur's Report

This session dealt with the effects of physical and chemical processes on fumigation and/or controlled atmosphere treatments of commodities. From the sorption of CO_2 by wheat (to cause ingress of the outside atmosphere) to the sorption of MB on activated carbon (to prevent atmospheric emissions), this session focused on our understanding of the sorption process and its effects on successful uses of CA's and fumigation. We heard about the role of sorption within a treatment in which there is ingress of air from the outside due to the negative pressure resulting from the sorption process. We also heard about the effects of sorption on flowers and how it may help determine the most efficacious treatment.

In terms of effectiveness and predictability, we heard about the advantages and results of modelling CA systems so that losses and sorption of CO_2 can be predicted. Certainly one advantage of mathematical models is the saving of research effort. Once a model has been validated, the necessity to test each CA variable or variable combination is removed. Attempting to construct these models led to the conclusions that our present limited knowledge is getting better. Models in the future will better predict the results actually seen in CA treatments.

We heard how sealing, tarpaulin condition, rate of fumigant sorption and fumigant loss all affect the final concentration profile during the fumigation of milled rice. Deficiencies in our knowledge of the entire fumigation process could lead to the creation of models that do not accurately predict for all cases what actually happens in practice. It was stressed that, because our knowledge is not complete, it is best to monitor fumigation concentrations so that we have a maximum rate of success in avoiding control failures.

By comparing laboratory systems which are tightly sealed with those having known controlled leaks, we may be able to predict more accurately the results of actual fumigations. Factors such as temperature, sorption and leakage were investigated; the results were applied to actual fumigations of bins at farm sites. It was found that in farm situations temperatures in the range of 25–30°C might provide the best chance of successfully controlling susceptible and resistant insects with PH_3.

Finally, we heard about the sorption of MB on activated carbon to eliminate emissions to the atmosphere. Studies have shown that it is feasible to sorb MB in carbon, and these studies have shown the effects of temperature, humidity, flow rate and concentration on the sorption characteristics of three different activated carbons. With the reduction and/or

control of MB pending, this method of eliminating emissions to the atmosphere may provide a way to continue to use this fumigant, which is extremely important to many areas of post-harvest commodity treatment.

Clearly, we must continue our endeavors to better understand the physical and chemical processes that affect CA/fumigant systems. Results of these endeavors will provide us with better predictive tools to understand the dynamic process of such treatments and allow us to increase the success rate of any gaseous treatment which we might devise for commodities.

SESSION 5: APPLICATION METHODOLOGY OF CA AND/OR FUMIGATION, INCLUDING THE USE OF CARBON DIOXIDE UNDER INCREASED PRESSURE

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Rapporteur's Report

The application methodology session was well supported, with 12 papers presented. These epitomized the mood of the conference, emphasis being on alternatives to methyl bromide (MB). However, the papers varied considerably in content; they covered the following areas: controlled atmospheres (CA's) generated using nitrogen (N_2), CA's generated using carbon dioxide (CO_2), CO_2 under high pressure and phosphine (PH_3) (a, fumigation techniques; b, manufacture; c, disposal).

There were no papers covering MB, which appears to have a limited future. On-site production of N_2 for stored-product disinfestation was the subject of a paper presented by Cornel Adler. He outlined work undertaken to establish environmentally friendly, residue-free, worker-safe insect control methods. He indicated in his conclusion that, once the high capital cost of the equipment and the cost of supervision could be reduced or justified, membrane and PSA systems could develop into viable alternatives for fumigation in gastight bins, particularly in remote areas.

Chris Bell also reported on on-site generation of low-oxygen (O_2) atmospheres. He reported on the evaluation of the performance of a propane burner used to generate low-O_2 atmospheres in sealed bins. The system had some potential for future application, but a number of operational challenges remained to be addressed. Variations in bin sealing techniques, bin dimensions, effect of wind, effect of temperature and cost were discussed.

Jonathan Banks in his presentation discussed the accuracy of models used to describe the gas-distribution characteristics involved in purging grain bulks with N_2. He referred to the plug-flow and mixing processes observed under field conditions. Results obtained from the models came within 90% of the field data in which these phenomena actually occurred. He indicated that the models had been derived from data collected over 20 years.

A new product for short term fumigations was introduced by Lothar Benzing. He described a new metal phosphide product. This formulation in bags provides a 20-min delay before decomposition and generation of any PH_3. He reported that once decomposition commences, this product breaks down more rapidly than other existing ones. This development will, to some extent, address the safety concerns associated with use of metal phosphide preparations, and it will be a real bonus to those who carry out fumigations.

The need for fumigations to be active rather than static was highlighted by Bob Winks in his presentation. He contended that the many variables that may influence the success of a fumigation, such as wind and the chimney effect, could be minimised or eliminated by the use of active fumigation systems. SIROFLO® (the subject of an exhibit during a field trip) and SIROCIRC™ were discussed as examples of active systems that provide improved efficacy, better economy and, most importantly, far greater ability to comply with environmental standards. He reported that active fumigation has been widely adopted in Australia.

"Gaseous phosphine: a revitalised fumigant" was the subject of a paper presented by Bob Ryan, who described the BOC product PHOSFUME™. The potential for this product as a replacement for MB was discussed.

Disposal of metallic phosphide formulations by burial was the subject of Jan van Graver's presentation. He indicated that emissions of PH_3 from the burial site during the first 10 days after burial can exceed the TLV. Thereafter PH_3 emissions remained below 0.3 ppm. He suggested that burial of unused (unspent) formulations results in lower concentrations of PH_3 in the environment than might be expected from allowing the formulation to decompose in air.

The economic feasibility of PH_3 recirculation using "closed-loop fumigation" systems in sealed bins was presented by Ron Noyes. The improvements obtained using this method of fumigation were reported to have provided savings of approximately US$0.50 per t for a single fumigation.

The potential for using CO_2 under high pressure to control pests in cocoa beans and hazelnuts was outlined by Sabine Prozell. It was suggested that this technique could provide an alternative non-residual disinfestation process for these and similar commodities.

Christoph Reichmuth, using the same technique, reported on control of the tobacco beetle (*Lasioderma serricorne*). He indicated that the process tended to be temperature dependent, and further trials were required to refine the technique in this application.

The final paper in this session, also presented by Christoph Reichmuth, described the rate at which O_2 is replaced in bales of compressed tobacco disinfested with N_2 or CO_2. In the examples described, 6 h were required to obtain the target distribution.

SESSION 7: INTEGRATED COMMODITY MANAGEMENT METHODS WITH CA AND/OR FUMIGATION

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Rapporteur's Report

The international conference on controlled atmosphere and fumigation in stored products is a great event for every specialist in this field. Each of us comes here with his own ideas and opinions and the results of his own investigations. Each of us leaves the conference enriched by dozens of ideas and opinions, as well as the results of work undertaken by the best brains of many countries. Here we have a chance to meet old friends and be introduced to many new ones.

Although only four papers were presented, in my opinion session 7 was one of the key sessions of the conference because in it an approach towards the rationalization of the insect control system was pursued.

R.J. Hodges, representing the research team of the NRI from the UK, in cooperation with H. Haled of Indonesia, managed, by developing a fumigation decision support system, to solve the difficult problem of when to fumigate. This system consists of an insect monitoring technique, an insect growth model and a pragmatic pest control threshold. Using these three elements it is possible to predict when future fumigations will be required and determine whether previous fumigations were successful. The present results are limited, being applicable only to *Tribolium castaneum* in the humid tropics, but the approach seems to be very important.

The next report was presented by Paul Flinn, who worked with David Hagstrum in the USA, and was entitled "Simulation model of low O_2 atmospheres on insect population dynamics in stored grain." Its approach is close to that in the previous paper. It has become possible, on the basis of their experimental results, to predict the effects of low O_2 atmospheres, grain temperature and insect stage on insect mortality, to predict the duration of fumigation required to produce a given mortality using low O_2 levels and to predict insect density in grain 1 to 2 months after fumigation.

Two other reports, presented by Australian participants, detailed research that was no less pragmatic. Barry Bridgman's investigations showed that the CO_2 fumigation of organic grain, followed by refrigerated aeration, enabled insect-free grain to be outloaded over a storage period of 24 months.

Sarah Hilton and J. Banks carried out investigations on the sorption rate of ethyl formate on sultanas and raisins as well as studying the effectiveness of this fumigant against six pests. They concluded that ethyl formate, where not limited by sorption behaviour, has excellent potential as a replacement for MB in the treatment of durable commodities.

SESSION 8: POTENTIAL THREATS TO CONVENTIONAL CA AND/OR FUMIGATION (REGULATORY AND INSECT RESISTANCE)

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Rapporteur's Report

Nine papers were presented during this session. The first paper, presented by L. Zettler of the USA, provided an overview of the influence of resistance on future fumigation technology. In his presentation, L. Zettler mentioned the problems arising from PH_3 resistance and the incidence of PH_3-resistant insects in the world. He stressed PH_3-control failures and recommended doses for controlling resistant strains. Measures to combat insect resistance, through efficient control, include increasing exposure periods, using proper fumigation techniques, using hermetically sealed storage structures, employing recirculation technology, using combination treatments, developing alternative fumigants, using biotechnology and using post-harvest techniques. He came to the conclusion that insect resistance to fumigants will continue to develop but can be managed with existing alternative methods.

The second paper, presented by C. Adler of Germany, was entitled "Resistance — a threat to the use of controlled atmospheres for stored-product protection?" C. Adler mentioned that there are no reports of field resistance to CA treatments. Comparison of eight laboratory and two field strains of *S. granarius* from six countries showed no significant variation in their susceptibility to controlled atmospheres of N_2 and CO_2. Tolerance in the insect populations may be stable for a long period. Indications were that the weight of both adults and pupae of laboratory-selected strains resistant to CA is higher than that of susceptible strains. He concluded that there is no proof that resistance is threatening this technology but suggested strategies to minimize the risk that this might happen in the future.

The third paper, presented by F.A. El-Lakwah of Egypt, dealt with the selection of *T. castaneum* for resistance to a combination of PH_3 + CO_2 and with biological observations on the resistant strain. The results of his work indicate that the lethal time values achieved to obtain a certain mortality were significantly longer for the 16th generation, selected for resistance to a mixture of 40 ppm PH_3 + 46% CO_2, than for the parent stock. At the 16th generation, the selected strain showed 19.4-fold resistance at 26°C, and 18.5-fold resistance at 6°C, at the LT_{50} level. In number of eggs, larval mortality and developmental period, the resistant strain also revealed significant differences from the parent strain.

In a presentation on PH_3 measurement at environmental levels, S. Pratt of Australia reviewed the present situation regarding environmental levels of PH_3 and the limits for its detection. He then reported on sensors and amplification techniques, concluding that by

using several techniques and novel sensors, laboratory detection of PH_3 in ppb is feasible, but this must be done in the field since detection must be made as soon as possible after sampling.

A paper entitled "Does underdosing select for resistance to PH_3?" was presented by P. Collins of Australia. He reported on farmers' use of tablets for fumigation and mentioned that fumigation by farmers is generally poor because most of their silos are not well-sealed. Several farm fumigations were monitored and assays were carried out in the laboratory on resistant, heterozygous and susceptible strains of *S. oryzae* and *T. castaneum*. Analysis of the response of these insects to PH_3 leads to the conclusion that any underdosing, producing incomplete kill, will select for resistance. However, poor fumigations will kill only the homozygous susceptibles, leaving the heterozygous resistants to maintain the susceptible gene in the population. Furthermore, good fumigations would use doses high enough to also kill the heterozygous resistant insects, thus eliminating the susceptible genes. Therefore, unless the dose is sufficiently high to kill all resistant stages (heterozygous and homozygous), selection pressure will be greater for the good fumigation than for the poor one.

Chris Bell of the UK presented a paper on the limitations on infestation control in cooled bulk grain and on a strategy to overcome inherent sealing and gas distribution problems by using PH_3. He mentioned that a sensor-controlled, automated dosing system (originally developed for MB mill fumigations) has been modified for use in the PH_3 fumigation of bulk grain. This system potentially maintains adequate PH_3 gas concentrations throughout the long exposure times required for PH_3 treatments at low temperatures.

Perlina Sayaboc of the Philippines presented a paper on the status in her country of resistance of *R. dominica* to PH_3. She reported on tests of field strains (collected from various areas) that showed high levels of resistance to PH_3, and she attributed this phenomenon to poor fumigation practices.

The next paper, presented by C. Reichmuth of Germany, was entitled "There is no resistance of stored-product moths against treatment with CO_2 under high pressure." He reported on experiments conducted to examine whether, during 12 generations of selection, eggs of *Plodia interpunctella* develop resistance to CO_2 under pressure. His findings showed that no significant change in sensitivity occurred over this period.

Lastly, S. Ignatowicz of Poland presented a review of the current status of MB and PH_3 fumigation in his country. He provided a detailed account of the commodity quantities treated since 1990, the registration of fumigants and the quantities employed. He concluded by remarking that, since PH_3 will continue to play a role in the fumigation of agricultural products in Poland and there are indications of development of insect resistance, a comprehensive research program has been planned to monitor resistance to this gas.

SESSION 10: SAMPLING AND TRAPPING TO MONITOR INSECT POPULATIONS IN RELATION TO CA AND/OR FUMIGATION

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Rapporteur's Report

Insect detection and the estimation of population size are of fundamental importance when deciding if a control measure has been effective or if treatment is needed. Sampling and trapping are the tools most often used to provide this information on stored-product pests (both in commodities and in stores). Poor detection methods must, at the very least, lead to an overestimate of the efficacy of treatments. There are also implications for the early detection of resistance and the survival of low numbers of resistant insects. Therefore, it is very appropriate that a Sampling and Trapping Session was included in this conference. It is important that researchers working on control methods remain aware of the best options for detection as well as the risks associated with less efficient options. Trappers and samplers, particularly at a practical level, need to be aware of the problems they are expected to solve. This conference provides an ideal forum for such an interchange.

The point was made during questions that good detection methods are not always popular. Well, science must rule, and trappers and samplers must boldly go (splitting infinitives on the way) — but watch out for the men in suits!

The papers in this session covered pest detection and population estimation in grain, under laboratory and practical conditions, and the detection of pests in freight containers filled with cocoa. Several different approaches were covered, and different methods and equipment were compared.

Three of the papers presented (and one relevant poster) compared different traps or equipment, giving valuable information directly applicable to practical studies. Trematerra and Mancini showed that there are significant differences, both in terms of numbers caught and the response of different species, between trap types. However, the work also showed a high level of variability in trap-catch results, even under controlled laboratory conditions.

The theoretical view of sampling, presented by Bhadriraju Subramanyam, Hagstrum, Meagher Burkness, Hutchison and Naranjo, was supported by some practical results. Wilkin and Van Natto gave a practical account of the comparison of two methods and, at the same time, showed the disadvantage of using small samples in estimating insect populations or detecting insects at low densities.

Trematerra, Fontana and Mancini showed that natural insect pheromones, as well as the presence of dead insects in a trap, can affect trap catch. In the final paper, Cogan discussed the use of traps in freight containers, specifically to assess the efficacy of earlier fumigations. He also compared three different types of trap in his presentation.

The conclusions seem to be that traps must be calibrated and compared and that we also need to set objectives for detection regimes and to be cautious of spot data.

LATE-COMER

Donahaye, E.J., Navarro, S. and Varnava, A. [Eds.] (1997) Proc. Int. Conf. Controlled Atmosphere and Fumigation in Stored Products, 21–26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 691–697

CARBON DIOXIDE FUMIGATION OF ORGANIC GRAIN FOLLOWED BY 'REFRIG-AERATION'

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ABSTRACT

Organic grain was fumigated on arrival in storage with carbon dioxide (CO_2). Concentrations were held above 30% for 15 d by continuously topping up with additional CO_2 through a recirculation system. Subsequent aeration with refrigerated air enabled long-term storage without the need for re-fumigation. This integrated commodity management strategy provided the means to outload 60-t batches of insect-free grain over a total storage period of 24 months. The results of insect trapping in the storage area are discussed, and the equipment and operational costs of implementing this strategy are described.

INTRODUCTION

Grainco (Queensland, Australia) specialises in storing grains in a sub-tropical to tropical environment. Clients include the Australian Wheat Board, flour mills, feed mills, brewers and growers. The greatest challenge in accomplishing this task has been to prevent damage caused by stored-grain insects. In the 1970's, grain was protected in Queensland by admixture of residual grain protectant insecticides and by aeration. These strategies are no longer employed due to the development of insect resistance to protectants and, more particularly, to consumer demand for low (or zero) chemical residue food. They were replaced during the 1990's by sealed storage and the use of fumigants.

The increased demand over the past decade for clean, "green" food has provided the challenge to develop commercially viable strategies for storage of large bulks of grain in hot humid environments without reliance on chemicals (fumigants or residual pesticides). The technology of grain disinfestation with controlled atmospheres (CA) using nitrogen

(N_2) or carbon dioxide (CO_2) has been well-researched (Boland, 1985; Hoey, 1981), and suitable procedures are readily available (Bond, 1989; Annis and van S. Graver, 1989; Banks and Annis, 1977).

Grainco (like its predecessors, the State Wheat Board and Bulk Grains Queensland) has been actively involved in developing this research into viable commercial grain protection strategies. Trials carried out in the past (Boland, 1985; Hoey, 1981) centred on large bulks in gastight steel or concrete bins. The large tonnages involved provided some economy of scale to spread costs. However, whereas in an export situation larger storage structures are preferred and the entire contents are out-turned for delivery once disinfested, for the domestic grain market the situation is more demanding. Mills require reception of grain a truckload at a time, so the grain must be drawn from the same bulk over a period of 18 months or more. This requirement, in itself, adds a significant new dimension to the challenge of providing insect-free storage in a chemical-free environment.

To achieve this level of control, grain needs to be disinfested as soon as possible after intake. In the case reported here rejection of infested deliveries was not an option. Once disinfested, the challenge to keep the grain insect-free could theoretically be met by aeration (Bridgeman and Collins, 1994; Navarro and Calderon, 1980; Hunter, 1981). Unfortunately, in Queensland, the period when grain is delivered into storage coincides with hot, humid climatic conditions. These are the optimal conditions for insect population development, and there is only a very short period during the day when conditions are suitable for aeration with ambient air. For this reason an air conditioning system ("refrig-aeration") was used to aerate the grain.

The concept of cooling grain with refrigerated air had been applied previously by Grainco (Taylor and Elder, 1981) but was not extended due to the energy costs of the operation. In earlier successful trials, the process resulted in uninfested grain. In the case reported here, it was considered that the extra cost involved in pulling the grain temperature down to below 9°C in order to disinfest it would be unnecessary if the aeration process was used in an integrated plan with another process, namely a CA treatment.

The client was 'Daydawn', a manufacturer of organic breakfast cereal. Its requirement for an uninterrupted periodic out-turn of insect-free, organic grain in small tonnages was achievable in theory. The bins would be protected from reinfestation by climatic manipulation. There would be no need to re-gas bins when only partly full. Most importantly, none of the planned strategies would compromise the organic nature of the product.

METHODS AND MATERIALS

The client

The client requested Grainco to store its supply of organic wheat for 18 months or longer so that this new line of business could be established. 'Daydawn' wished to draw

on the stocks on an "as required" basis, stipulating that they be insect-free and that the organic status of the grain not be compromised. The organic nature of the product was expected to command a premium in the market.

The grain

The grain (1,000 t of premium quality wheat) was delivered from on-farm storage. It was graded and checked for organic authenticity prior to intake. Only grain grown by properties certified by the organic authorities and produced by sustainable farming practices without assistance from artificial chemicals was acceptable. Growers were paid a premium for the grain. However, on reception most of the deliveries (70%) were found to be infested.

The plan

In theory, the plan was to disinfest the grain completely by CA treatment with CO_2 within 2 weeks of delivery. After a 15-d exposure period, aeration with refrigerated air ("refrig-aeration") would be used to reduce the temperature of the grain to below 20°C (ideally 15°C). This would effectively prevent insects from recolonising the grain and prevent subsequent population growth. Periodic inspection of the grain would verify the success of the procedure.

The storage

The Haunstraup multi-bin complex at Grainco's Malu depot near Toowoomba, Queensland, was selected for the trials. This complex consists of a single 1,600-t capacity cell which is surrounded by eight 500-t bins. The complex was selected because of its central location and the multiple segregations that it offered. The bins used were 600-m^3 concrete structures with conical bases, each with a storage capacity of 510 t premium grade wheat. The bins were constructed in the 1950's, and none of them conformed to standard pressure tests (Banks and Ripp, 1983). The two bins to be used (bin Hm205 and Hm206) were sealed on the cone and lower walls and at the roof to wall junction. Three-metre lengths of 35-cm half-round perforated ducting were installed down the cone to facilitate the CA fumigation and aeration. The bins were pressure tested for pressure decay from 100 to 50 mm of water gauge at 3 and 2 min, respectively.

The major leakage was from longitudinal cracks in the concrete walls. This level of airtightness would disqualify the bins from the traditional static type fumigation.

The CA fumigation system

"BOC Gases" supplied CO_2 as a liquid in pallet tanks. The CO_2 was delivered via a vaporiser to the bin recirculation system. A "3b Dawn" fan powered the recirculation through the ducting described above. Gas sampling lines were located in the base of the bin, at the grain surface and in the ducting. Because of the leaky nature of the bins, a

continuous-addition system was set up to maintain the concentration above the target concentration of 30% for 15 d.

The refrig-aeration system

The refrig-aerator was set up to cool both bins simultaneously. The power usage of the unit was approximately 31 kw/h. The cost of power was AU$ 0.115 per kw/h. During the initial cooling phase, the unit operated for 20 h per day. Once the cool front had moved through the grain and stabilised (5 d), the aeration time was reduced to a maintenance phase of 2 h per day during summer and 1 h per day during winter. The target was to achieve a grain temperature of about 15°C.

Population monitoring

After the CO_2 fumigation had been completed, a composite grain sample of 3 kg was taken from the bulk of each bin and monitored monthly to detect any insect development. These samples were stored at ambient conditions for 6 months. The grain was also inspected monthly when it was turned in the bin, and average grain temperatures were measured at the same time. The grain was finally inspected for the presence of insects on out-turn before delivery.

RESULTS

General

The planned process was successful. No insects were detected in either of the two test bins during the 24-month storage period although large populations of insects were detected in silos adjacent to the test bins and in facilities close by.

Controlled atmosphere treatment

The CO_2 concentrations recorded during the CA treatment were plotted on a daily basis (Fig. 1). It can be seen from the figure that the target dose could only be maintained by continuous topping up. This procedure enabled the treatment to be carried out successfully. Had the bins been properly sealed, however, only about half the gas would have been required. The approximate cost of CO_2 treatment, including labour and gas, was AU$ 2/t of grain.

"Refrig-aeration"

The grain temperature was monitored at the silo base once a week and also once a month during bin-turning activities. The readings were tabulated against weekly minimum and maximum ambient temperatures. The number of fan hours per week was also recorded (Fig. 2). The refrig-aeration unit operated on a timer. This made costing the process very straightforward. The cost of cooling was approximately AU$ 1/t/month over the 24-month storage period.

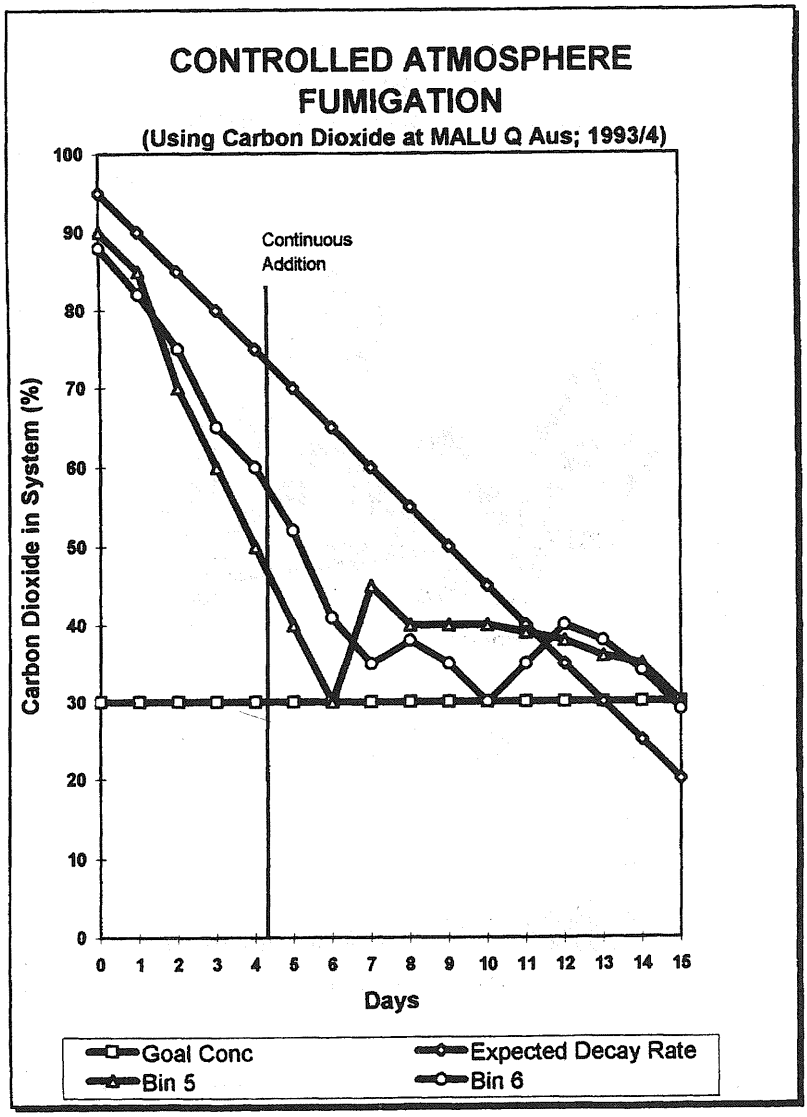


Fig. 1. CO_2 concentrations during the initial CA treatment of organic wheat.

Insect population monitoring

No insects were detected in the bin samples taken at post-harvest reception into storage (and disposed of after 6 months of checking), nor were insects detected during monthly bin-turning activities of the test bins though insects were detected in other bins within the complex, on the grain path outside the bins and in the surrounding areas. No insects were detected in loads of grain on out-turn for delivery.

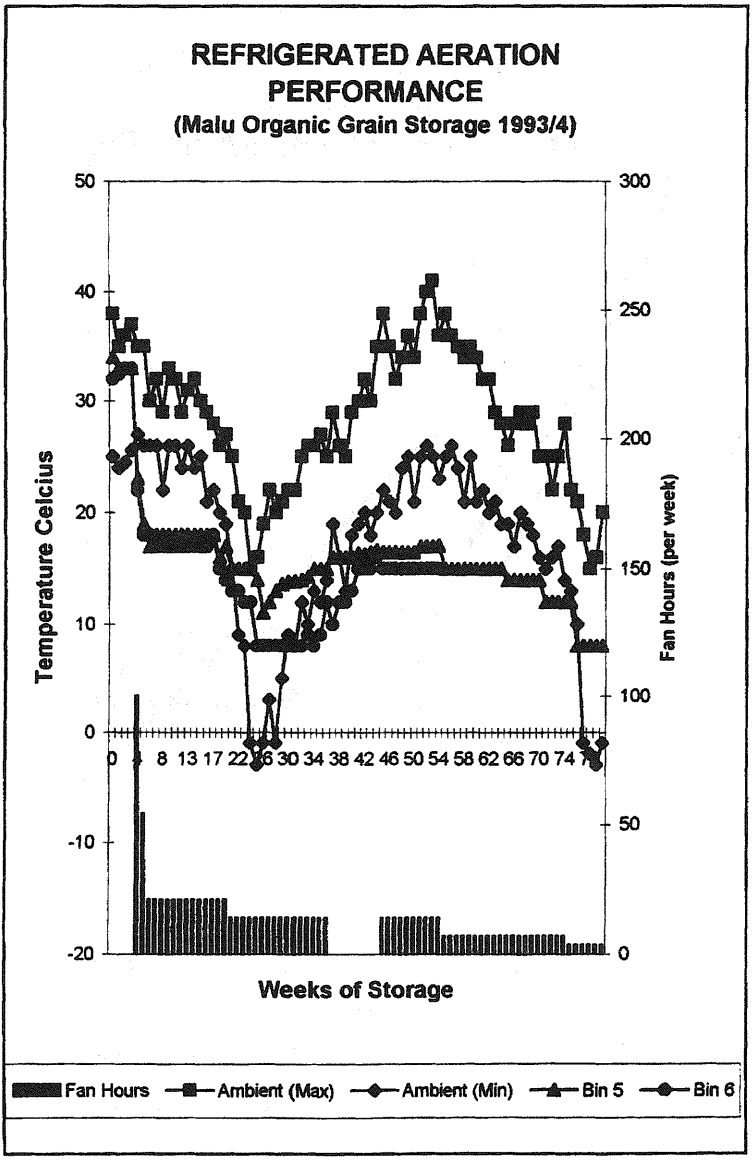


Fig. 2. The refrig-aeration performance as indicted by weekly measurements of grain temperature and weekly readings of fan hours.

Costs

The actual costs of the full grain protection process in this project are deemed commercially sensitive and have therefore been omitted from this paper. The approximate costs were about four times those of periodic tri-monthly fumigation with phosphine (PH_3).

DISCUSSION

The success of this process has led to additional clients who are looking for organic grain storage. There is, however, scope for significant improvement. The cost of the operation, although significantly higher than that of PH_3 fumigation, still appeals to clients seeking a chemical-free storage environment. The ability to maintain continuous, periodic out-turn is also a significant service. Although the costs are apparently acceptable to the clients who want to store a special commodity, they can be substantially reduced. Both the CA and the refrig-aeration process can be improved on. Improvements in the bin sealing, for example, would halve the amount of CO_2 used.

ACKNOWLEDGEMENTS

I wish to thank both my colleagues in the Grainco Fumigation team and the Malu silo operators, Wayne Spinks and Lance Weiss, for their patient assistance during this work. I am also grateful to BOC Gases for their cooperation in this project.

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