Nayak MK (2012) Managing resistance to phosphine in storage pests: Challenges and opportunities. In: Navarro S, Banks HJ, Jayas DS, Bell CH, Noyes RT, Ferizli AG, Emekci M, Isikber AA, Alagusundaram K, [Eds.] Proc 9th. Int. Conf. on Controlled Atmosphere and Fumigation in Stored Products, Antalya, Turkey. 15 – 19 October 2012, ARBER Professional Congress Services, Turkey pp: 609-619

MANAGING RESISTANCE TO PHOSPHINE IN STORAGE PESTS: CHALLENGES AND OPPORTUNITIES.

Manoj K Nayak*

[1]Department of Agriculture, Fisheries and Forestry, EcoSciences Precinct, GPO Box 267, Brisbane, QLD 4001, Australia

[2]Cooperative Research Centre for National Plant Biosecurity, LPO Box 5012, Bruce, ACT 2617, Australia

*Corresponding author's e-mail:*manoj.nayak@daff.qld.gov.au*

ABSTRACT

Over several decades, two fumigants (methyl bromide and phosphine) have been used extensively to disinfest stored commodities across the world. In 1992, the landmark decision of the Montreal Agreement to phase out the fumigant methyl bromide by 2015 triggered research into development of alternative fumigants. During this period, industries have become highly dependent on phosphine, a unique fumigant with several positive attributes including its cheap price, versatility and ease in application and most importantly, its broad acceptance as a residue-free treatment. Over reliance on phosphine, however, has resulted in development of resistance in a range of stored product pest species. Several alternatives that have been developed so far (e.g. ethyl formate, ethanedinitrile, carbonyl sulphide and sulfuryl fluoride) cannot match the benefits offered by phosphine.

For these reasons, the focus has shifted to managing resistance to phosphine to ensure its future sustainability. This paper will present a critical appraisal of the challenges associated with resistance to phosphine and recent research advances in its management. An insight into the opportunities offered by the alternative fumigants and other pest management tactics towards alleviating phosphine resistance will also be discussed. The key components of a national phosphine resistance management program that has been adopted in Australia to combat resistance problems will be presented as a case study. This will include research highlights from the characterisation of strong-level of phosphine resistance in key pest species and rapid diagnosis for their detection; development of new phosphine fumigation protocols and the potential for using alternative fumigants such as sulfuryl fluoride as a 'phosphine resistance breaker'.

Key words: Fumigants, phosphine, stored product pests, resistance management, sulfuryl fluoride, eradication strategies.

1. INTRODUCTION

Disinfesting stored commodities including grain, dried fruits, nuts, cocoa, coffee, processed food and food products using fumigants is a practice spanning over a 100 years. According to Bond (1984) fumigants are chemicals which, at a required temperature and pressure, can exist in the gaseous state in sufficient concentration to be lethal to a given pest organism. Moreover, for its broad acceptance, apart from being economically affordable, an ideal fumigant should also have minimal effect on environmental aspects, health of human beings, non-target organisms and structures. These particular properties required of fumigants have meant that among several chemicals developed over the years for fumigation purposes, relatively few chemicals have ever been considered suitable. Among them, methyl bromide and phosphine are the ones with the longest history of use with a high degree of effectiveness against a broad range of pests. Due to the steady increase in global concern about the effects of fumigants and chemicals on the environment, human and animal health, scrutiny of their use is becoming stricter. Unfortunately, industry is on the verge of losing methyl bromide due to its ozone-depleting nature and phosphine is threatened by the development of strong levels of resistance in key pest species. This paper will present a critical appraisal of the challenges and opportunities that have arisen from the major issue of resistance to phosphine in key pest species in view of the complete phase-out of methyl bromide in 2015. While examples will be provided from various parts of the world, the core examples on resistance management will be from Australia.

2. CHALLENGES

2.1. PHASE-OUT OF METHYL BROMIDE

Methyl bromide has been used as a fumigant for the 80 years since its insecticidal properties were first reported by Le Goupil (1932). The uniqueness of this fumigant has been its effective use against a broad range of pests across many fields of application including soil, durable and perishable commodities and structures (mills and buildings). Compared to other fumigants, methyl bromide delivers disinfestation quite rapidly, which enabled the industry to use it effectively in short-term fumigations, where a successful disinfestation is achieved within 24 h including the clearance of the gas. This aspect of methyl bromide has made it very attractive for industry, particularly for use in large storages in export terminals and shipping containers; where long-term fumigations are considered very expensive due to high demurrage costs involved in delaying of shipping. Successful control of stored product pests by methyl bromide is well documented (Estes, 1965; Howe and Hole, 1966; Bell and Glanville, 1973).

In the 1990s, several reports (SORG, 1990; WMO, 1990, 1992) have drawn attention to the ozone depleting properties of methyl bromide, and environmental organizations around the world, particularly in the U.S.A., have called for a rapid phasing out of the fumigant (Friends of the Earth, 1992). This movement culminated in the landmark decision under the Montreal Protocol on Substances that Deplete the Ozone Layer, which led to the gradual phase-out of methyl bromide from 1995 with exemptions for use for quarantine and pre-shipment purposes and for developing countries. While the developed nations have completely banned this fumigant in 2005 (with the exception of quarantine use), the worldwide phase-out of methyl bromide is scheduled to be completed in 2015 (Taylor, 1994); which will leave a major hole in the fumigant armoury. Moreover, it will put extra pressure on phosphine, which will be left as the only major fumigant for use by industry in disinfesting stored products across the globe.

2.2. INCREASE IN STRENGTH OF RESISTANCE TO PHOSPHINE

Like methyl bromide, phosphine as a fumigant has a history of nearly 80 years and its use across the globe in disinfestation of durable commodities has been well established (Taylor, 1989; Rajendran and Narasimhan, 1994; Zettler et al., 1989; Collins et al., 2005; Lorini et al., 2007). As expected with the use of any chemical treatment, development of resistance to phosphine in several stored product pests has occurred over time and was first highlighted during the global survey for susceptibility to pesticides undertaken by the Food and Agriculture Organisation of the United Nations (Champ and Dyte, 1976). This survey detected resistance in stored product pests in 33 out of 82 countries and after this first survey; Chaudhry (2000) listed roughly eleven species of major stored product pests that had developed resistance to phosphine by 2000. In the last decade, however, new pests such as psocids have also been reported to have developed a high level of resistance to this fumigant (Nayak et al., 2003b). Overall, the situation has worsened during the last two decades in terms of both frequency and strength of resistance (Srivastava, 1980; Mills, 1983; Taylor, 1989; Zettler, 1997; Nayak et al., 2003a, 2003b; Collins et al., 2005; Lorini et al., 2007). In most of the cases, the development of resistance is presumed to be related to inadequate fumigation practices involving poorly sealed structures and repeated fumigations.

Recent studies in Australia and Brazil have reported the strongest level of resistance detected so far in any stored product pests for this fumigant in these countries (Collins et al., 2005; Nayak et al., 2003b; Lorini et al., 2007; Nayak et al., in press). As registered rates of phosphine have failed to control these resistant pests, new fumigation protocols are being developed to manage them. The increase in strength in resistance means that the traditional FAO method (FAO, 1975) to detect resistance is inadequate for diagnosis of such strongly resistant insects and new methods need to be developed (see section 2.1).

2.3. RESISTANCE TRENDS NOT KNOWN IN MOST COUNTRIES

A key to the successful management of resistance to phosphine is its early detection and proper characterisation. Australia is probably unique in that it has had a national resistance monitoring program that has been operating successfully since the 1980s. Apart from providing early warning to the industry on new resistance developments, this monitoring program also provides the annual trend in resistance frequency across Australia (Emery et al., 2011). The program runs concurrently in three laboratories across the country representing three grain growing regions (northern, southern and western), where thousands of insect samples representing a range of stored grain pests are tested for resistance to phosphine using a nationally agreed statistically robust monitoring protocol. Throughout the year trained staff collect samples from farms and central storages through random and targeted sampling apart from samples being sent directly to the laboratories from storage operators for resistance testing. All resistance data are being stored in an integrated database (Australian Grain Insect Resistance Database) for future reference on trends and frequencies of resistance. The program has been accredited for providing early warning of strong resistances in the lesser grain borer *Rhyzopertha dominica* (F.) (Collins et al., 2005), the psocid *Liposcelis bostrychophila* Badonnel (Nayak et al., 2003b) and most recently the flat grain beetle *Cryptolestes ferrugineus* (Stephens) (Nayak et al., in press). Moreover, research has established two levels of resistance to phosphine ('weak' and 'strong') and it has been suggested that once the frequency of 'weak' resistance reaches 80% in populations of a particular pest species across a particular region, there is a strong possibility of development of strong resistance in that species (Collins and Emery, 2002). This was proven to be the case in the development of strong resistance in *Tribolium castaneum* (Herbst) in Western Australia in 2010 (Emery et al., 2011).

The only other comprehensive survey of resistance published since the FAO survey (Champ and Dyte, 1976) was undertaken by Benhilima et al. (2002) across several grain storages in Morocco and reported very high frequency of phosphine resistance in *T. castaneum*, *R. dominica* and *Sitophilus oryzae* (L.). In another small-scale survey, the frequency of strong resistance was found to be alarming in central grain storages in Brazil, where 14 of the 19 populations of *R. dominica* collected were detected with strong resistance (Lorini et al., 2007). Due to the lack of a country-wide resistance survey, however, the exact frequency of this resistance in Brazil over time and space is not known.

It is important that countries using phosphine to disinfest stored commodities need to monitor for resistance in key pest species. Late detection of 'strong' resistance doesn't help the case for sustainability of phosphine as early detection would help in eradication of the resistant populations as well as allow time to develop new fumigation protocols to manage them (see section 2.1.2).

2.4. LIMITATIONS OF ALTERNATIVE FUMIGANTS

In view of the development of resistance to phosphine in key pest species and imminent phase out of methyl bromide, there has been significant research undertaken in recent years to explore the potential of other fumigants as alternatives. Unfortunately, almost all of them fail to match the combined advantages that have been offered by phosphine. The limitations of some of the alternatives are discussed here briefly and detailed information on their effectiveness against stored product pests is deliberately omitted.

Among several alternatives, sulfuryl fluoride (SF), a broad spectrum fumigant commercialised by DowAgroSciences seems to be the most promising. It is currently registered in Australia to fumigate flour mills, food factories, dried fruits and stored grain. With the limited published data available, this fumigant has been shown to have poor efficacy against the egg stage of storage pests (Drinkall et al., 1996; Bell, 2000). Moreover, the potential of SF as a greenhouse gas and its fluoride residue on treated food materials are becoming major concerns, which may jeopardise its future use. Another fumigant, carbon dioxide (CO_2), has been shown to have excellent potential for rapid disinfestation only at high pressure and has limitations due to logistical constraints such as high construction and operating costs of pressure chambers (Prozell et al., 1997).

Carbonyl sulphide (COS), although found to be highly effective against a range of stored product pests (Desmarchelier, 1994) fails to control the rice weevil *S. oryzae* (Rajendran, 2001). In addition, fumigation with COS can affect germination of a range of cereals, can leave off odours in walnuts and milled rice and can cause discolouration of soybeans (Navarro, 2006). Ethyl formate (EF) can be effective against a range of pests but only when combined with CO_2 (Haritos et al., 2006). When used independently, it failed against several of these pests. The other major drawback with this fumigant is that it is highly volatile and flammable at normal ambient temperature, which raises serious safety concerns.

Hydrogen cyanide (HCN), a very old and highly toxic fumigant, has limited use on grain due to its high sorptive nature (Navarro, 2006). Ethyl dinitrile (EDN) (also called as cyanogen), a broad spectrum fumigant, has the major drawback of being phytotoxic, which affects seed germination ((Ducom, 2006; Navarro, 2006).

Although not considered as a fumigant, modified atmospheres (MA) involving elevated CO_2, lowered oxygen or an atmosphere with nitrogen generated from cylinders or by separation of air on site has shown excellent effects against range of stored product pests (Adler et al., 2000). However, these methods are not cost effective in large scale operations and compared to fumigants, MA has the serious limitation of needing a very long time to achieve required levels of disinfestation (Donahaye et al., 1994).

3. OPPORTUNITIES

3.1. PHOSPHINE REMAINS VIABLE IF RESISTANCE IS MANAGED

As discussed earlier, the development of high levels of resistance to phosphine has been documented in several major insect pests of stored products. With the imminent complete phase out of methyl bromide by 2015, and market reluctance to accept chemical residues; it is inevitable that phosphine will continue to play an important role in stored product disinfestation for the foreseeable future. The following are ways that will help in maintaining the viability of this unique fumigant in stored product protection.

3.1.1. CHARACTERISATION OF STRONG RESISTANCE AND DEVELOPMENT OF NEW FUMIGATION PROTOCOLS

One key feature of phosphine is that concentration and exposure period can both be altered to maximise its efficacy (Daglish et al., 2002). Research has also clearly demonstrated the influence of temperature on phosphine efficacy (Bell, 1992; Nayak and Collins, 2008). These aspects have direct implications for management of strong level of resistance to phosphine in key pest species. For example, with the detection of strong resistance to phosphine in *R. dominica* and *L. bostrychophila* in the 1990s in Australia, extensive research was undertaken (Collins et al., 2005; Nayak et al., 2003b) to characterise these resistances and development of new fumigation protocols to manage them. After their field validation, these protocols are being incorporated into the phosphine label in Australia (APVMA, 2012) and are used by bulk storage operators in successful control of strongly resistant pest populations. However, compared to these resistances, a much stronger resistance was detected in *C. ferrugineus* in 2006, which was not been controlled by the current registered rates of phosphine (Nayak et al., 2010; Nayak et al., in press). Like previous resistances, this new resistance is now being characterised and new fumigation protocols are being developed to manage it (Nayak et al., 2010). This means that the phosphine label in Australia will need to be modified again to incorporate these new protocols. In a similar study, Lorini et al. (2007) characterised the strong resistance in *R. dominica* from Brazil and determined effective rates of phosphine for its control.

On another front, a population of *S. oryzae* from China was imported under quarantine permits to Australia for resistance research that was characterised to be almost 10% more resistant to phosphine than the local resistant population of this pest (Daglish et al., 2002). Several fumigation protocols have been developed against this resistant strain in an attempt to prepare the Australian industry for combating this resistance in the future if it develops locally (Nayak et al., 2003a).

3.1.2. RAPID DIAGNOSIS FOR STRONG RESISTANCE

The FAO method (FAO, 1975) is a standard procedure that is followed by researchers around the world to detect resistance to phosphine in a particular pest species by exposing a field population to a fixed concentration of phosphine over a 20-h period and assessing mortality after another 14 days. In addition, some researchers have shown that a longer exposure period (e.g. 48 h) is needed for some types of resistance (Collins et al., 2002; Daglish et al., 2002). Recent research by Nayak et al. (in press), however, characterised the strong resistance in *C. ferrugineus* and established a rapid method that can discriminate between susceptible, weak and strongly resistant individuals in a population. This method provides industry with advice on resistance status of samples of *C. ferrugineus* within 7 h of its receival in the laboratory, including the preparation and processing of insects. The same day advice on strong resistant populations enables the storage operators to make quick decisions on implementing an eradication plan.

There is enormous opportunity to develop rapid tests to diagnose strong resistance to phosphine in other key pest species including *R. dominica* and *T. castaneum* for establishing a more robust early warning system for their detection and timely management.

3.1.3. IMPROVED UNDERSTANDING OF MOLECULAR GENETICS AND ECOLOGY OF RESISTANCE

Understanding of the genetic basis of development of resistance provides valuable information towards development of any pest management strategy. Our understanding of the molecular aspects of phosphine resistance has grown significantly in recent years. It has been established now that at least two major genes control high level phosphine resistance in *R. dominica* (Collins et al., 2002) and *T. castaneum* (Jagadeesan et al., 2012). More comprehensive genetic and molecular analysis of *R. dominica* (Schlipalius et al., 2008) revealed the presence of two loci, *rph1* and *rph2*, responsible for phosphine resistance in this insect. *Rph1* controls the "weak" resistance phenotype providing moderate resistance to phosphine, whereas *rph2* conferring only very low level of resistance by itself. *Rph2* was not discovered in the field until *rph1* had become common and when both are combined in the same individual, they synergise to produce a much higher level of resistance known as the 'strong' resistance phenotype. These findings have led to a series of genetic complementation experiments to establish whether populations of *R. dominica* across widely separated geographic locations in Australia share the same genes for development of strong resistance (Mau et al., 2012a, b). The findings from this research have concluded that resistance in each of the three populations under investigation was derived independently from others despite genetic analysis being consistent with two major genes being responsible for resistance in each case. Research in this area is in progress involving pest populations from Australia and India to confirm whether resistance mechanisms in *R. dominica* and *T castaneum* across continents are the same. The outcomes of this research will be significant in that resistance management strategies developed in one country will be relevant to the other country.

Research is also in progress in Australia on stored grain pest ecology for better understanding of movement of resistance populations and their implications on the grain biosecurity. This research is combining trapping with population genetics analysis to investigate insect dispersal and gene flow (Ridley et al., 2011). Basic information on these aspects would contribute to more effective grain hygiene practices leading to lower infestation threat and reduced need for chemical treatment. Data on how ecological processes contribute to the development and spread of phosphine resistance will be incorporated into a strategy for reversion to susceptibility under reduced selection pressure.

3.2. DEVELOPMENT OF ERADICATION STRATEGIES

In 2006, when the strong level of phosphine resistance was detected in several populations of *C. ferrugineus* in bulk storages in Australia, it was established in laboratory research that registered rates of phosphine failed to control *C. ferrugineus* with resistance of this type. To maintain its 'nil tolerance' principle for live insects in export grain, the industry urgently developed an action plan to combat this resistance problem. This plan was developed collaboratively by the bulk handling companies and researchers aimed at eradicating infestations of phosphine resistant *C. ferrugineus* populations from bulk storages and preventing their spread (Nayak et al., 2010, Nayak et al., in press). The key components of this plan include use of an alternative fumigant such as SF (see section 2.3), strategic application of grain protectants (chlorpyrifos-methyl, fenitrothion) and adoption of an intensive hygiene program, monitoring of insect populations and resistance testing.

This strategy has been instrumental in the eradication of strongly resistant *C. ferrugineus* populations in at least 60 bulk storages since the implementation of the plan in 2009 (MK Nayak, unpubl. data).

3.3. STRATEGIC USE OF ALTERNATIVE FUMIGANTS

Industry around the world should take advantage of the available alternative fumigants and use them strategically to overcome the phosphine resistance and associated problems. Reliance on a single treatment should be avoided and the strength of each alternative fumigant should be manipulated to fit it to an integrated pest management program. A recent example of successful implementation of this approach has been the use of SF as an alternative to phosphine for managing strongly phosphine resistant *C. ferrugineus* populations in bulk storages in Australia, after this pest's emergence as a major problem in the bulk storage system in recent years. The number of incidences of strongly phosphine resistant populations in these storages has been halved within a year after exclusive use of SF in 2010. The current registered rate of a ct-product (concentration x exposure period) of 1500 $mg.h.L^{-1}$ of SF was found to be quite effective in controlling strongly phosphine resistant flat grain beetle populations for a minimum of 3 months in large-scale bunker (pad) storages (MK Nayak unpubl. data).

After the initial success of SF, research and consultations are in progress in Australia to maximise the potential of this fumigant as an alternative to relieve the ever growing pressure on phosphine. It is suggested that this fumigant should only be used as a 'phosphine resistant breaker' and be used exclusively where phosphine fails to control infestations and that the number of fumigations should be limited in a calendar year to delay the development of resistance. Moreover, with the increasing use of SF by the industry, it is important that a resistance monitoring protocol be established now to prepare industry for detection of resistance as and when they emerge in the future.

4. CONCLUSION

To protect phosphine and extend its usefulness into the future, a strategy including several approaches as described above needs to be in place. This has been well demonstrated by the Australian grain industry. A National Phosphine Resistance Management Strategy was developed over several years of consultation between researchers, key bulk grain storage operators, farmers, extension specialists and other end-users and policy makers of key state and federal government agencies that are relevant to the registration and use of phosphine in Australia (Collins, 2009). Under the umbrella of the National Working Party on Grain Protection, these stakeholders unanimously agreed to adopt and implement this strategy that provides a foundation to achieve the goal of ensuring the long-term sustainability of phosphine. The key components of this strategy are: a national resistance monitoring program, identifying the factors responsible for development of resistance, reducing selection (eg. limit number of fumigations), destroying resistant populations through use of alternative methods and implementation of eradication plans and adherence to recommended use of phosphine by all industry users. This holistic approach to manage phosphine resistance has already shown encouraging results as reflected in the effective management of strongly resistant *C. ferrugineus* populations in bulk storages through use of SF as an alternative fumigant and implementation of an eradication plan.

To conclude, industry around the world should look at the case of Australia and develop similar programs to help protect phosphine and maintain its viability in the future.

ACKNOWLEDGEMENTS

The author acknowledges Greg Daglish for his valuable comments on the manuscript and the support from the Australian Government Cooperative Research Centres Programs.

REFERENCES

Adler C, Corinth HG, Reichmuth C (2000) Modified atmosphere. In: Subramanyam Bh, Hagstrum D (eds) Alternatives to pesticides in stored product IPM. Kluwer Academic New York, USA, pp 105-146.

APVMA (2012), Australian Pesticide and Veterinary Medicine Authority, Product No 50177, $ECO_2FUME^{®}$ Phosphine Fumigant. http://services.apvma.gov.au. Accessed 15 June 2012.

Bell CH (1992) Time, concentration and temperature relationship for phosphine activity in tests on diapausing larvae of *Ephestia elutella* (Hubner) (Lepidoptera: Pyralidae). Pestic Sci 35: 252-264.

Bell CH (2000) Fumigation in the 21st century. Crop Prot 19: 563-569.

Bell CH, Glanville V (1973) The effect of concentration and exposure in tests with methyl bromide and with phosphine on diapausing larvae of *Ephestia elutella* (Hubner) (Lepidoptera, Pyralidae). J Stored Prod Res 9: 165-170.

Benhalima H, Chaudhry MQ, Mills KA, Price NR (2002) Phosphine resistance in stored-product insects collected from various grain storage facilities in Morocco. J Stored Prod Res 40: 241-249.

Bond EJ (1984) Manual of Fumigation for Insect Control. FAO Plant Production and Protection Paper 54, FAO, Rome.

Champ BR, Dyte CE (1976) Report on the FAO global survey of pesticide susceptibility of stored grain pests. FAO Plant Protection and Production Services No. 5, FAO Rome.

Chaudhry MQ (2000). Phosphine resistance: a growing threat to an ideal fumigant. Pesticide Outlook, June 2000: 88–91.

Collins PJ (2009) Strategy to manage resistance to phosphine in the Australian grain industry. An initiative of the National Working Party on Grain Protection. Cooperative Research Centre for National Plant Biosecurity Project CRC70096, http://www.crcplantbiosecurity.com.au/publications/npb1437, accessed June 20, 2012.

Collins PJ, Emery RN (2002) Two decades of monitoring and managing phosphine resistance in Australia. Credland PF, Armitage DM, Bell CH, Cogan PM, Highley E (eds) Proc 8th Int Wkg Conf Stored Prod Protection, CAB International, Wallingford, UK pp 570–575.

Collins PJ, Daglish GJ, Pavic H, Kopittke RA (2005) Response of mixed-age cultures of phosphine-resistant and susceptible strains of lesser grain borer, *Rhyzopertha dominica* to phosphine at a range of concentration and exposure periods. J Stored Prod Res 41: 373-385.

Collins PJ, Daglish GJ, Bengston M, Lambkin TM, Pavic H (2002) Genetics of resistance to phosphine in *Rhyzopertha dominica* (Coleoptera: Bostrichidae). J Econ Ent 95: 862-869.

Daglish GJ, Collins PJ, Pavic H, Kopittke RA (2002) Effects of time and concentration on mortality of phosphine-resistant *Sitophilus oryzae* (L) fumigated with phosphine. Pest Manag Sci 58: 1015-1021.

Desmarchelier JM (1994) Carbonyl sulphide as a fumigant for control of insects and mites. In: Highley E, Wright EJ, Banks HJ, Champ BR (eds) Proc 6th Int Wkg Conf Stored Prod Prot, CAB International, pp 78-82.

Drinkall MJ, Dugast JF, Reichmuth C, Scholler M (1996) The activity of the fumigant sulfuryl fuoride on stored product insect pests. In: Wildey KB (ed) Proc 2nd Int Conf Insect Pests in the Urban Environment, Cambridge, UK, BPC Wheatons Ltd, Exeter, UK, pp 525-528.

Donahaye E, Navarro S, Rinder M (1994) The influence of temperature on the sensitivity of two nitidulid beetles to low oxygen concentrations. In: Highley E, Wright EJ, Banks HJ, Champ BR (eds) Stored Products Protection. Proc 6th Int Wkg Conf Stored Prod Prot, CAB International, Wallingford, UK, pp 88-90.

Ducom PJF (2006) The return of the fumigants. In: Lorini I, Bacaltchuk B, Beckel H, Deckers D, Sundfeld E, Santos JP, Biagi JD, Celaro JC, Faroni LR, Bortolini LOF, Sartori MR, Elias MC, Guedes RNC, Fonseca RG, Scussel VM (eds) Proc 9th Int Wkg Conf Stored Prod Prot, Brazilian Postharvest Association, Campinas, Brazil, pp 510-516.

Emery RN, Nayak MK, Holloway JC (2011) Lessons learned from phosphine resistance monitoring in Australia. Stewart Postharvest Review, 3:6: 1-8. doi: 10.2212/spr.2011.3.6.

Estes PM (1965) The effects of time and temperature on methyl bromide fumigations of adults of *Sitophilus granarius* and *Tribolium confusum*. J Econ Ent 58: 611-614.

FAO (1975) Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Tentative method for adults of some major pest species of stored cereals with methyl bromide and phosphine – FAO method no. 16. FAO Plant Prot Bull 23: 12-25.

Friends of the Earth (1992) In: Straub D (ed) Into the Sunlight, Exposing Methyl Bromide’s Threat to the Ozone Layer, Friends of the Earth, Washington, DC.

Haritos V, Damcevski K Dojchinov G (2006) Improved efficacy of ethyl formate against stored grain insects by combination with carbon dioxide in a dynamic application. Pest Manag Sci 62: 325-333.

Howe RW, Hole BD (1966) The susceptibility of the developmental stages of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) to methyl bromide. J Stored Prod Res 2: 13-26.

Jagadeesan R, Collins PJ, Daglish GJ, Ebert PR, Schlipalius DI (2012) Phosphine resistance in the rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae): Inheritance, gene interactions and fitness costs. PLoS ONE 7 (2): e31582. doi: 10.1371/journal.pone.0031582.

Le Goupil P (1932) Les proprietes insecticides du bromure de methyl. Rev Path Veg Agric France 19: 169-172.

Lorini I, Collins PJ, Daglish GJ, Nayak MK, Pavic H (2007) Detection and characterisation of strong resistance to phosphine in Brazilian *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae). Pest Manag Sci 63: 358-364.

Mau YS, Collins PJ, Daglish GJ, Nayak MK, Pavic H, Ebert PR (2012a) The rph1 gene is a common contributor to the evolution of phosphine resistance in independent field isolates of *Rhyzopertha dominica*. PLoS ONE 7(2): e31541. doi:10.1371/journal.pone.0031541.

Mau YS, Collins PJ, Daglish GJ, Nayak MK, Ebert PR (2012b) The rph2 gene is responsible for high level resistance to phosphine in independent field strains of *Rhyzopertha dominica*. PLoS ONE 7(3): e34027. doi:10.1371/journal.pone.0034027.

Mills KA (1983) Resistance to the fumigant hydrogen phosphide in some stored-product species associated with repeated inadequate treatments. Mitt dtsch ges allg angew Ent 4: 98-101.

Navarro S (2006) New global challenges to the use of gaseous treatments in stored products. In: Lorini I, Bacaltchuk B, Beckel H, Deckers D, Sundfeld E, Santos JP, Biagi JD, Celaro JC, Faroni LR, Bortolini LOF, Sartori MR, Elias MC, Guedes RNC, Fonseca RG, Scussel VM (eds) Proc 9th Int Wkg Conf Stored Prod Prot, Brazilian Postharvest Association, Campinas, Brazil, pp 495-509.

Nayak MK, Collins PJ (2008) Influence of concentration, temperature and humidity on toxicity of phosphine against strongly phosphine-resistant psocid *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae). Pest Manag Sci 64: 971-976.

Nayak MK, Collins PJ, Pavic H (2003a) Development in phosphine resistance in China and possible implications for Australia. In: Wright EJ, Web MC, Highley E (eds) Stored Grain in Australia 2003, Proc Australian Postharvest Tech Conf, CSIRO Entomology, Canberra, Australia, pp. 156-159.

Nayak MK, Collins PJ, Pavic H, Kopittke RA (2003b) Inhibition of egg development by phosphine in the cosmopolitan pest of stored products *Liposcelis bostrychophila* (Psocoptera: Liposcelididae). Pest Manag Sci 59: 1191-1196.

Nayak MK, Holloway JC; Emery RN, Pavic H, Bartlet J, Collins PJ (in press) Strong resistance to phosphine in the rusty grin beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae): its characterisation, a rapid assay for diagnosis and distribution in Australia. Pest Manag Sci**:** DOI: 10.1002/ps.3360.

Nayak MK, Holloway J, Pavic H, Head M, Reid R, Collins PJ (2010) Developing strategies to manage highly phosphine resistant populations of rusty grain beetles in large bulk storages in Australia. In: Cavalho MO, Fields PG, Adler CS, Arthur FH, Athanassiou CG, Campbell JF, Fleurat-Lessard F, Flinn PW, Hodges RJ, Isikber AA, Navarro S, Noyes RT, Riudavets J, Sinha KK, Thorpe GR, Timlik BH, Trematerra P, White NDG (eds) Proc 10th Int Wkg Conf Stored-prod Prot, Julius-Kuhn-Archiv 425, pp 396-401. DOI: 10.5073/jka.2010.425.052.

Prozell S, Reichmuth C, Ziegleder G, Schartmann B, Matissek R, Kraus J, Gerard D, Rogg S (1997). Control of pests and quality aspects in cocoa beans and hazel nuts and dilution experiments in compressed tobacco with carbon dioxide under high pressure. In: Donahaye EJ, Navarro S, Varnava A (eds) Proc Int Conf Controlled Atmosphere and Fumigation in Stored Prod, Printco. Ltd, Nicosia, Cyprus, pp 325-333.

Rajendran S (2001) Alternatives to methyl bromide as fumigant for stored food commodities. Pesticide Outlook 12: 249-253.

Rajendran S, Narasimhan KS (1994) Phosphine resistance in the cigarette beetle *Lasioderma serricorne* (Coleoptera: Anobiidae) and overcoming control failures during fumigation of stored tobacco. Int J Pest Manag 40: 207-210.

Ridley AW, Hereward JP, Daglish GJ, Collins PJ, Raghu S and Walter GH (2011) The spatiotemporal dynamics of *Tribolium castaneum* (Herbst): adult flight and gene flow. Molecular Ecology 20, 1635-1646.

Schlipalius DI, Chen W, Collins PJ, Nguyen T, Reilly P, Ebert PR (2008). Gene interactions constrain the course of evolution of phosphine resistance in the lesser grain borer, *Rhyzopertha dominica*. Heredity 100: 506-516.

SORG (U.K. Stratospheric Ozone Review Group) (1990) Stratospheric Ozone 1990. U.K. Department of Environment and Meteorological Office.

Srivastava JL (1980) Pesticide residues in food grains and pest resistance to pesticides. Bull Grain Technol 18: 65-76.

Taylor RWD (1989) Phosphine - a major fumigant at risk. Int Pest Cont, 31: 10-14.

Taylor RWD (1994) Methyl bromide – Is there any future for this noteworthy fumigant? J Stored Prod Res 30: 253-260.

WMO (1990) Scientific Assessment of Ozone Depletion: 1989. World Meteorological Organization Report No. 20, WMO, Geneva.

WMO (1992) Scientific Assessment of Ozone Depletion: 1991. World Meteorological Organization Report No. 25, WMO, Geneva.

Zettler JL (1997) Influence of resistance of future fumigation technology. In: Donahaye EJ, Navarro S, Varnava A (eds) Proc Int Conf on Controlled Atmosphere and Fumigation in Stored Prod, Printco Ltd. Nicosia, Cyprus, pp 445–454.

Zettler JL, Halliday WR, Arthur FH (1989) Phosphine resistance in insects infesting stored peanuts in the south eastern United States. J Econ Ent 82: 1508-1511.