

A novel approach to limit the development of phosphine resistance in Western Australia

Newman, C.R.*#

Department of Agriculture and Food Western Australia, Bougainvillea Avenue, Forrestfield, Western Australia 6058. Email: chris.newman@agric.wa.gov.au

* Corresponding author

Presenting author

DOI: 10.5073/jka.2010.425.072

Abstract

Escalating development of resistance to phosphine is of concern to grain storage operators world wide. In Western Australia 85% of grain produced is exported with a guarantee through legislation that it is free of all grain insects. Phosphine plays a vital part in shore-based fumigations to achieve this insect-free status but it is also available for unrestricted use by growers for grain stored on farms. For more than 20 years a campaign has been in place to encourage better use of phosphine. Central to the program is the preservation of phosphine for long-term use by exporters and growers. Over this period weak resistance frequency has increased to the current rate of about 48% averaged across all species. Strong resistance has been confirmed in two strains of *Tribolium castaneum*. The three key components of the strategy are:

1. Inspection of central and farm storages for grain insects, and testing insects to discover phosphine resistance.
2. Education of grain-storage managers on farms and commercial premises on effective management of grain stocks and correct use of phosphine.
3. Eradication of highly resistant insect colonies found on farms and commercial premises as well as management of strains with elevated levels of tolerance to phosphine.

Keywords: Farm silo, Fumigation, Phosphine resistance, Extension, Western Australia.

1. Introduction

Australia is one of the few countries in the world where phosphine is available to farmers for the protection of grain in their own storage. The continued effectiveness of phosphine at the farm level in Australia, however, will depend on the ability to slow or arrest the development of resistance to this fumigant in all the major grain-storage insect pests present in the country.

Phosphine has been available to farmers in Australia since the 1950's when the label recommendations included the use of the product in unsealed storages and admixture to a grain stream. In 2008, the label was changed and the two practices removed from the recommended-use table. It is suggested the continued use of phosphine in this manner for many decades in Australia has led to an escalating resistance in stored-grain insects.

In the 1980's, the Western Australian central grain-storage operator, Cooperative Bulk Handling (CBH) abandoned the use of contact insecticides and created sealed storages in which to use phosphine exclusively for protection of export grain.

However, it was of concern that evidence of resistance to phosphine was being found. Champ and Dyte (1976) reported in an FAO survey that 10% of the insect strains tested had an increased tolerance to phosphine. Following this investigation, other reports were presented of phosphine resistance in laboratory strains. The first report of control failure was provided by Tyler et al. (1983) where survival of a number of stored-product insect pest species was discovered in food warehouses in Bangladesh.

This potential for fumigation failure required a sampling regime be put in place in Western Australia to find out if phosphine resistance was present. Grain insects were taken by sampling and screening grain from farm and central storages since 1985 and submitted for bioassay to determine tolerance to phosphine and this provides information on the status of phosphine resistance. The objectives of this paper are to demonstrate how an inspection, education and eradication program assist in mitigating resistance and extending the effectiveness of phosphine as a fumigant in Western Australia.

2. Materials and methods

2.1. Inspection and testing

The inspection and testing program was facilitated by an existing network of District Officers attached to the Agriculture Protection Board were already visiting farms to advise on and write programs for control of pest plants and animals that were 'Declared' under an Act of Parliament. All major stored-grain insect species were added to the 'Declared' list and all Officers were required to inspect farm-grain storages, sample grain, collect grain insects and offer advice or write programs for the reduction of grain pests. The number of individual strains submitted for phosphine resistance varied annually from 750 to 2500.

District Officers select farms at random and extract approximately 0.5 kg of grain from the base or bagging chute. In some cases, a grain-sampling probe was used to obtain samples from the surface of the grain. The grain is shaken over a 0.9-mm screen and the insects that fall through are collected from a tray beneath. The insects are placed in plastic vials with some of the grain and sent to the stored-grain resistance testing laboratory at the Department of Agriculture and Food Western Australia (DAFWA) for testing. There the insects are separated by species from the grain and approximately 100 of each species are put into a desiccator which is injected with phosphine at 1 mg/L for 30 min. If any insects survive this test it indicates a resistance to phosphine, but not whether the resistance is 'weak' or 'strong'. Another sample of the insects captured are put into culture on a medium relevant to their feeding habits and placed in a controlled temperature room at 28°C and 60% r.h. The progeny produced are subjected to the FAO 'discriminating-dose test' where the insects are exposed to a concentration of the fumigant under controlled conditions that approximates the amount needed to kill 99.9% of the adult insects of a fully susceptible strain (Taylor, 1986). Survivors of this test are classified as having 'strong' resistance.

The classification of 'weak' or 'strong' resistance was developed by Ebert et al. (2003) who demonstrated that the tolerance to phosphine in *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) is controlled by two major genes and the insects can carry either of these genes. One gene is responsible for 'weak' resistance and the other 'weak' resistance gene on its own has little effect, but in combination enhances the effect of the other gene. It is assumed that over a series of fumigations in poorly sealed silos, insects carrying the 'weak' resistance gene survive and as they increase in numbers there is greater chance they will mate with the carriers of the other gene resulting in the progeny expressing the 'strong' resistance characteristics. For the purposes of this work, it is assumed that all stored-product insect pest populations will behave in a similar fashion.

2.2. Eradication of strong resistant strains and education

Identification of a strong resistant strain initiated a process to eradicate the insects at the source. This action by the DAFWA was to demonstrate the process needed to achieve eradication of the strongly resistant insects to the grain store manager to keep the strain in check and to create procedures for future control work.

The procedure is as follows:

- Re-sample insects from the property where strong resistance has been determined and re-test in the laboratory for resistance factor;
- Send sample of insects to a collaborative testing laboratory of the Cooperative Research Centre for National Plant Biosecurity in Brisbane, Queensland for confirmation testing;
- Confirmation of strong resistance initiates a farm visit to assess the scale of the problem and plan a clean up and fumigation with the owner;
- DAFWA personnel visit the farm to commence a hygiene program. This involves sealing silos where possible, fumigating any grain in situ and cleaning around the silo complex to remove food and harbourage for stored grain insects outside the silos;
- A further visit to the farm prior to harvest is required to ensure hygiene procedures have been completed and the silos sprayed internally with a contact insecticide. In addition, contact insecticide is applied underneath and around the silos and in any former derelict grain storage areas that might provide refuge for grain insects. Grain-handling equipment, including harvesters are also treated with contact insecticide. Silos are checked for gas tightness by replacing rubber seals as needed, checking the oil in the pressure relief valve and conducting a pressure test on the silo which should be able to hold an introduced pressure of 250 Pascals decaying to 125 Pascals for five min or longer;

- After harvest and shortly after the farmer has finished loading grain into the silos, DAFWA personnel revisit the property to fumigate the grain. This involves a further pressure test of the silo and loading phosphine, aluminium phosphide (AIP) at the label rate of 1.5 g/m³ into the headspace onto a wide tray that allows the AIP tablets to lay one deep and facilitate release of the phosphine. Grain is usually hot and dry (>25°C, <12% m.c.) and fumigation protocol is 7 d at concentrations greater than 100 ppm when temperature is greater than 25°C and 10 d when temperature is less than 25°C, assuming the silo is appropriately sealed;
- Over the course of the fumigation, monitoring is conducted using a CanaryTM brand ‘Silo Chek’ phosphine monitor. On well-sealed silos, only one point is monitored at the base just above the lower seal plate, which is the most difficult point for gas to penetrate and the point at which the fumigation is most likely to fail. (Newman et al., 2004) On the less well sealed silos, a headspace reading is also taken. The aim is to achieve >200 ppm for 9 d at all monitored points. In less-well-sealed silos this usually requires additional AIP to be applied to achieve protocol;
- To validate the treatments, traps are baited around the silos with whole and crushed grains and grain samples removed from the silos and sieved.

3. Results

Since commencement of the testing program, results indicate there has been a steady escalation in grain insects showing a resistance to phosphine from approximately 10% to over 40% in the 20 years that the program has been in effect (Fig. 1). In Western Australia, the average across all species shows that up to 48% of insects tested have a ‘weak’ resistance to phosphine with a range of 15–70% between species. The selection of grain farms for sampling is completely random, and the variability each year in the numbers found to have weak resistance is most likely the result of sampling intensity (Emery, personal communication, 2010). In the eastern states of Australia 70 to 100% of insects in the Northern GRDC region (North New South Wales (NSW) and Queensland) and 53–83% in the southern GRDC region (NSW, Victoria and South Australia) exhibit a weak resistance (Collins, personal communication, 2006). In 2007, strong resistance to phosphine had been detected in the Northern region but remained below 10% of the 253 insect samples analysed. (Collins, personal communication, 2007).

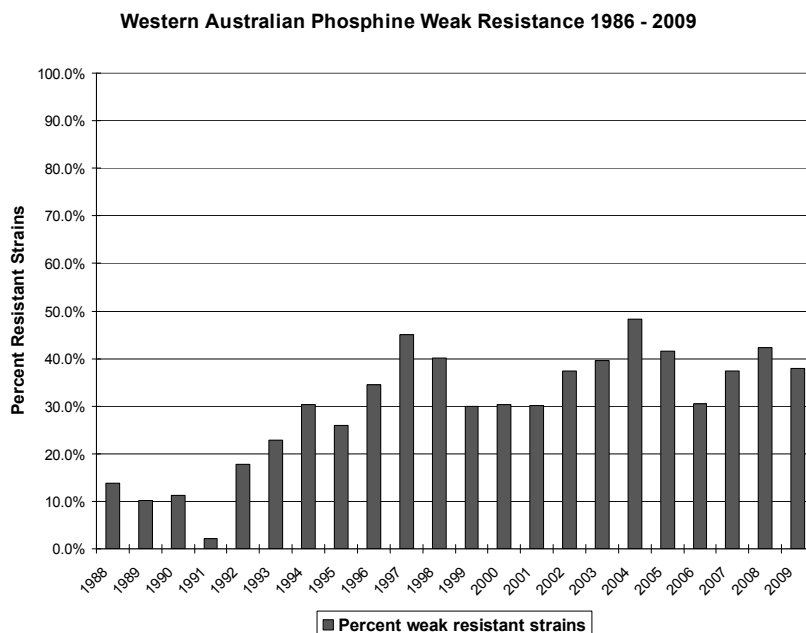


Figure 1 The occurrence of strains with weak resistance in Western Australia, Australia, from 1988 to 2009.

When test results of individual insect species are tabled for Western Australia (Fig. 2), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) showed the highest weak resistance and two cases up to 2009 have been found to have 'strong' resistance (Emery and Chami, personal communication). Few samples of *Cryptolestes* species (Coleoptera: Laemophloeidae) collected prior to 1999 demonstrated resistance and as the population becomes more numerous they appear to be exhibiting trends similar to *R. dominica* and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) (Fig. 2).

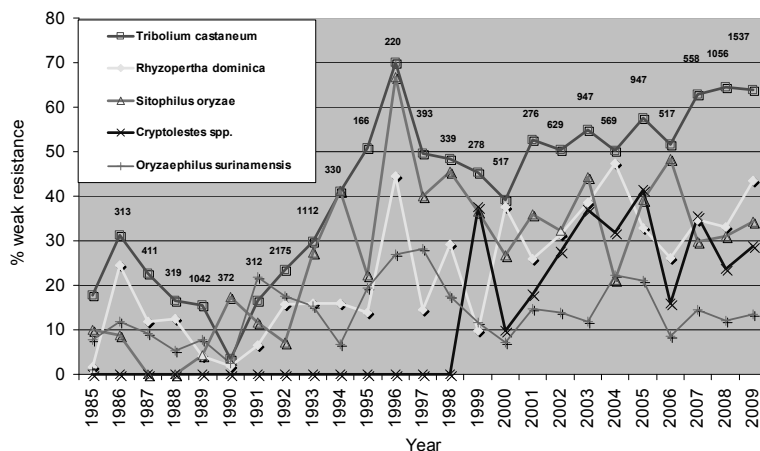


Figure 2 Occurrence of weak resistance to phosphine of stored product insects found in Western Australia from 1985 to 2009.

4. Discussion

Stored-product insect pest resistance to phosphine is important in Western Australia and in order for phosphine to remain an effective pest-control product in the future, action must be continued to slow the development of weak resistance. The emergence of strong resistance is an indication of continued selection of the weak resistant genes in farm silos. In both cases, *T. castaneum* is the dominant species making it more likely that a high proportion of the insect population contains the 'weak' resistance gene and that there will be a cross fertilisation of the two major genes. A failure to effectively eliminate an identified strong-resistant strain during subsequent fumigations will further select the most resistant individuals in the population.

The Western Australia program has been successful in avoiding the levels of resistance observed in eastern Australia and the program will likely need to remain in effect for as long as phosphine or any other single pest-control product is put into use. Slowing the development of phosphine resistance is the key to extending the economic life of the fumigant. In Western Australia, extension of information on improved grain storage and fumigation was put in place soon after stored-grain insects were 'Declared'. Written information was published and distributed to the farmer as insect samples were being collected.

The change from contact insecticide application to aluminium phosphide fumigation by CBH in the early 1980's initiated a new phase in the prevention of phosphine resistance. As more grain is being initially stored on the farm, quality fumigation is essential to managing grain quality and maintaining the effectiveness of phosphine. To elevate the standard of grain storage across the state, manufacturers of farm silos were approached to alter silo designs to enable them to be sealed easily and more effectively and, therefore, retain gas long enough to eliminate all life stages of grain insects in the silo. Silo manufacturers modified their production processes to enable silos to be pressure tested to a standard that would hold phosphine long enough to eliminate all species of insects. (Newman, 1996) However, this change has been somewhat reduced in its effectiveness by poor maintenance of the silos on farm, effectively preventing a higher level of control of the resident insect population than has been observed. (Newman, 1989). The extension message moved to focus on repair and maintenance of farm silos and correct fumigation. Phosphine monitors are uncommon on Western Australia farms so the aim has been to promote 'set and forget' fumigation. From earlier work by Newman et al. (2004) it was effective if

the silo can pass the standard pressure test and the correct dose is applied. The standard pressure test requires that the silo hold an introduced pressure of 250 down to 125 Pascals for five min. Failing this test means it is unlikely phosphine gas will be retained at the required concentration x time factor to eliminate all life stages of all insects in the grain.

The silos are produced from the factory with robust, long-lasting sealing between the wall sheets and all major joints. The vulnerable points are the rubber seals in the inlet and outlet ports and the oil in the pressure relief valve which needs to be kept at a level that allows air within the silo to expand and contract, thus preventing vacuum pressures on the roof that could be potentially damaging. Replacement of damaged seals and checking oil levels is a relatively simple and inexpensive task but it is this lack of maintenance that is the most significant factor behind the development of resistance in grain insects.

The author conjectures that the instructions on the first available containers of aluminium phosphide in the 1950's are an important contributor to the continued use of phosphine in unsealed and poorly maintained, sealable silos. The instruction to use in unsealed storages and admixture with a grain stream has fallen into 'folklore' and passed on through generations. In addition, the purchaser of the silos expected that the manufacturer would provide very long lasting seals and that the silo should remain sealed for life.

The extension campaign continues to encourage time be allocated to inspection, repair and maintain the hygiene of grain storages though written material, press articles, radio interviews and most importantly, through on-farm workshops where essential maintenance techniques are demonstrated (Figures 3 and 4).

The funding for extension came from the state government in the early years but since 2000 has been partly funded by the GRDC though several defined programs. These focused on better use of phosphine, aeration for quality control and reduction of grain-insect populations and early harvesting and drying of grain. The current extension program is funded until 2012, providing 75% full time equivalent to promote quality grain-storage principles. Campaigns of this nature collectively raise the awareness of the importance and principles of grain storage as part of the farm business and are now invaluable since Australian growers have the opportunity to store and market their own grain directly off farm.

From 2008, the abolition of the centralised marketing system in Australia has allowed growers opportunity to market their own grain. However, as the length of time that grain is stored on farm increases, there is a need to ensure the product meets buyer specifications. In the case of export from Australia, this specification is that it is free of stored-grain insects. Prior to 2008, the central grain-handling and warehousing system in each state had exclusively received the grain, protected quality and marketed the product domestically and overseas.



Figure 3 Replacing old worn seals of grain silo hatch to improve fumigation efficacy.



Figure 4 Demonstration of airtight silos.

This new aspect to the farm-grain business will focus the importance of high-quality grain storage for farmers who adopt a self-marketing policy. There remains the need to provide information to growers who continue to deliver to the central system but also use phosphine for protecting their own seed and feed in farm storage.

5. Conclusions

Through the efforts of inspection, program development and implementation of eradication programs, fumigation with phosphine is maintaining effectiveness in Western Australia. However, the results of this work would suggest that an insect-management model as explained here, needs to continue if strong resistance is to be limited. The unique approach to the control of stored-grain insects taken by DAFWA, the sealing of the central storage system by CBH, the response by silo manufacturers to seal transportable silos and the ongoing extension campaign have all contributed to the lower level of phosphine resistance encountered in Western Australia. In addition, other mitigating factors may also have played a part in slowing the development of phosphine resistance. For example, the smaller amount of grain held on farms for domestic trading compared to eastern Australia and the majority of grain grown in Western Australia is delivered direct from field to the central system. Of this 85% of grain held under high quality, central storage conditions is exported, allowing better monitoring and control implementation from professionally trained staff.

With the dismantling of the centralised marketing system, it is anticipated there will be more storage installed on farms, larger amounts of grain traded in small parcels and greater use of phosphine. To ensure the grain meets customers specifications, there will need to be more professionalism applied to farm-grain management than in previous years. This includes more effective fumigation measures to avoid resistance selection. Continued extension of quality grain storage principles is a primary method in creating change along with sampling and testing for resistance of stored-grain insects. Both will play a major role enabling the grain-storage manager to deal with escalating phosphine resistance.

The novel component of this strategy is that a large proportion of the funds to conduct this work are derived from grain growers through a levy on delivered grain. The Grains Research and Development Corporation (GRDC) receive this levy and invest the funds in defined research and extension projects maximising the return for the grain growers who contributed the levy. Eradication of strong resistance outbreaks at source cannot continue to be funded by government and industry in the long-term and will most likely revert to advice provided on site by qualified personnel to enable individual growers to manage the problem.

Into the future there will be a need for independent advisers to provide this service and it is unlikely that this will be funded from government sources as the activity is considered a private good. Funding from industry should be sought for this work beyond 2012.

Acknowledgments

Thanks to Dr Yonglin Ren for reviewing this paper and to Rob Emery and Michelle Chami for providing the data for Figure 2.

References

- Champ, B.R., Dyte, C.E., 1976. Report of the FAO global survey of pesticide susceptibility of stored grain pests. Food and Agriculture Organization Plant Protection Service. Series No. 5.
- Ebert, P.R., Schlipalius, D.I., Collins, P.J. 2003. The molecular genetics of phosphine resistance in the lesser grain borer and implications for management. In: Wright, E.J., Webb, M.C., Highley, E. (Eds), *Stored Grain in Australia: Proceedings of the Postharvest Technical Conference*, 25–27 June 2003, Canberra, Australia. Stored Grain Research Laboratory, CSIRO Entomology, Canberra, Australia, pp. 152-155.
- Newman, C.R., 1989. On-farm sealed storage in Western Australia: a progress report. In: Champ, B.R., Highley, E., Banks, H.J., (Eds), *Fumigation and controlled atmosphere Storage of Grain*. Proceedings of the International Conference, Singapore, 14-18th February 1989. ACIAR Proceedings No 25. p. 272.
- Newman, C.R., 1996. The response of the silo manufacturing industry in Australia to the sealing of transportable grain silos. In: Donahaye, E.J., Navarro, S., Varnava, A. (Eds), *Proceedings of the International Conference on Controlled Atmosphere and Fumigation in Stored Products*, Nicosia, Cyprus, 21-26 April 1996, Printco Ltd., Nicosia, Cyprus, pp. 393-406.
- Newman, C.R., Daglish, G.J., Wallbank, B.E., Burrill, P.R., 2004. Investigation into control of phosphine-resistant *Rhyzopertha dominica* in sealed farm silos fumigated with phosphine. In: *Proceedings of an international conference on Controlled Atmosphere and Fumigation in Stored Products*, Gold Coast, Queensland, August 2004.
- DAFWA report to National Working Party on Stored Product Protection, Canberra August 12 – 13, 2009.
- Taylor, R.W.D., 1986. Response to phosphine of field strains of some insect pests of stored products. In: *Proceedings of the GASGA Seminar on Fumigation Technology in Developing Countries*, 18-21 March 1986, TDRI Slough, UK, pp. 132-140. Tyler, P.S., Taylor, R.W.D., Rees, D.P., 1983. Insect resistance to phosphine fumigation in food warehouses in Bangladesh. *International Pest Control* 25, 10-21.