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Lessons learned from phosphine resistance monitoring in Australia

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Abstract

Purpose of review: Heavy reliance on phosphine to disinfest stored grain over the last few decades has led to the development of resistance in key stored grain insect pests around the globe jeopardising the long-term sustainability of this key fumigant. Australia is the only country in the world with a national resistance monitoring program which has, for over two decades, diagnosed, recorded and managed resistance to phosphine. The purpose of this review is to highlight the lessons learned from the Australian experience.

Recent findings: Recent findings from the national resistance monitoring in Australia include: (1) strong resistance to phosphine in the lesser grain borer, *Rhyzopertha dominica* (F.); the psocid, *Liposcelis bostrychophila* Badonnel; and the flat grain beetle, *Cryptolestes ferrugineus* (Stephens); (2) strong resistances in *R. dominica* and *L. bostrychophila* have now been successfully managed through development of new fumigation protocols and changes to phosphine label; (3) identified resistance in *C. ferrugineus*, that is the highest level recorded so far for any stored grain pest and cannot be controlled with the current recommended dosages; (4) a nationally coordinated collaborative program balancing applied and basic research along with development and extension is the key to successful management of resistance to phosphine.

Directions for future research: Research towards developing fumigation strategies to manage the strong level of resistance in *C. ferrugineus* at a range of temperatures is a priority as is development of an appropriate molecular phosphine resistance test.

Keywords: phosphine; grain insect; resistance; monitoring; management; database

Abbreviations

ACT	Australian Capital Territory
AGIRD	Australian Grain Insect Resistance Database
CBH	Cooperative Bulk Handling (CBH Group)
CRCNPB	Cooperative Research Centre for National Plant Biosecurity
FAO	Food and Agriculture Organization
GRDC	Grains Research and Development Corporation
NSW	New South Wales
NWPGP	National Working Party on Grain Protection
PDA	Personal Digital Assistant
Qld	Queensland
WA	Western Australia

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Introduction

Australia is the seventh largest wheat producer in the world with an average annual production of 22 million tonnes valued at US\$4 billion [1]; a remarkable achievement given that much of the 7,686,850 square kilometre continent is desert with no agricultural production. Australia is the oldest continent on Earth; erosion has heavily weathered Australia's surface leaving poor soils over much of the country. Australian farmers, despite these difficulties, have shown extraordinary resilience, perseverance and inventiveness over the last 200 years.

During the First World War Australia was forced to stockpile tens of millions of grain bags, and Winterbottom eloquently describes "when walking through the lanes between the infested stacks, there was a regular hissing noise to be heard caused by the movement of the weevil in the bags" [32]. The enormous impact grain insects had on this inadequately stored grain drove government and farmers to look for better storage methods. As a result all Australian grain-producing states formed farmer cooperatives with the single-minded aim of developing world-class grain bulk storage and handling facilities. A fine example is the Cooperative Bulk Handling (CBH) Group in Western Australia. During the Great Depression of the 1930s it was clear that farmers' costs could be reduced if the use of bags was minimised. In 1933 the cooperative was registered with an authorised capital of US\$160,000. In 1932-33, CBH received just 42,565 tonnes of wheat; however, by the 2003-04 seasons this had risen to 14,695,392 tonnes stored by a company with net assets of \$1 billion.

Much of the Australian grain crop is harvested during summer when temperatures are often well over 30°C and the mild climate maintains temperatures in grain bulks around 28°C, optimal for grain insect development and reproduction. Grain storage managers have had to continually deal with infestations and it was not until the 1960s when malathion became available that grain could be stored and delivered relatively free of insects. To further develop Australia's reputation as an exporter of premium grain, the Australian government mandated a 'nil-tolerance' for live grain insects at export.

Collins describes how, soon after the introduction of this mandate, resistance was found in the two major pests the red flour beetle, *Tribolium castaneum* (Herbst), and the lesser grain borer, *Rhyzopertha dominica*, (F.) and control began to collapse [10]. In response, two differing approaches were used in Australia [10]. The eastern states pursued the quick development and registration of alternative protectants for all the grain industry, while Western Australia pursued a regulatory path and restricted protectant use for on-farm stored grain to delay the development of new cases of resistance. The intent was to give CBH a transition time to seal its bulk storages and commit to phosphine fumigation [15]. By 1980 CBH had embarked on a silo sealing program of several hundred million dollars. Sealed storages were also beginning to find their way onto Western Australian farms, both as new units and retro-sealed existing storages. There was concern

that the widespread use of a single fumigant both on-farm and in the central grain-handling system, could result in rapid increase of phosphine resistance through the value chain and threaten the industry. To combat this the Western Australian Silo Manufacturers Association agreed that they would only produce sealed storages so that no company would be at a competitive disadvantage.

A critical prerequisite to resistance management is anticipation of resistance before control measures actually fail [4], and with this in mind the Western Australian Department of Agriculture began monitoring both farm and central storages for phosphine resistance in 1984 [16]. This work involved a network of about 200 field staff across the grain-producing areas of the state and a central laboratory located in South Perth. Initially the work was funded by the state government until 1996 when the industry-funded Grains Research and Development Corporation (GRDC) supported an Australia-wide phosphine resistance monitoring program, with the Western Australian, New South Wales and Queensland state governments responsible for different grain growing regions of Australia. In 2007, GRDC along with the major bulk handling companies and state government departments came together to join the Cooperative Research Centre for National Plant Biosecurity (CRCNPB) to obtain additional support from the federal government for research. Since then the resistance monitoring project is being run as a national project. This National Grain Insect Resistance Monitoring project has undoubtedly been the largest of its kind in the world and demanded cooperation, collaboration and coordination across both the country and the grains industry. Any potential patriotism or competition between states and companies was ignored in the interest of national plant biosecurity and maintaining Australia's enviable reputation as an exporter of insect- and residue-free premium quality grain [18].

The most significant achievements of this project, in terms of industry benefits, have been the early detections of strong resistance to phosphine in *R. dominica* [9], the psocid *Liposcelis bostrychophila* Badonnel [23] and most recently in flat grain beetle, *Cryptolestes ferrugineus* (Stephens) [24]. This has provided opportunities for researchers to assess the potential impact of these resistances on the grain industry through resistance characterisation in the laboratory. Moreover, the early warning of new resistances has enabled industry to develop appropriate remedial strategies, such as those recently developed to tackle the strong resistance in *C. ferrugineus* [25, unpublished]. Research parallel to the resistance monitoring program also resulted in development of new fumigation protocols to control strongly resistant pest populations that required changes to the phosphine label [8, 22].

Lessons learned

Strategy

Before embarking on a phosphine resistance monitoring project it is essential to have a resistance management strategy in

place. Collins in his definitive “Strategy to manage resistance to phosphine in the Australian grain industry” provided the goal of ensuring the long-term sustainability of phosphine through strategic adoption and implementation of commercially viable, practical and scientifically-based management strategies [11]. This strategy document was developed in consultation with researchers and the grain industry through the Australian National Working Party on Grain Protection (NWPGP) to ensure that practical and commercial constraints inherent to this industry were accommodated without loss of the resistance management aim. The NWPGP is an Australian grains industry body formed by the Australian Wheat Board in 1973 to provide industry leadership in the areas of postharvest storage, chemical use, market requirements, infestation and resistance levels, as well as chemical regulation. The phosphine resistance management strategy applies to all sectors and is consistent with current best practices in integrated pest management. However, a drawback is that participation in the strategy is voluntary, and its success depends on industry commitment and widespread compliance. So there is a concern that some parts of the grain industry may not see the document as ‘prescriptive’. One of the main problems facing the Australian export grain industry is that bulk handling companies are expected to have adequate stocks of ‘nil-tolerance’ grain ready for export at short notice. This ‘just-in-time’ approach can result in some grain bulks being fumigated multiple times while waiting for a ship to arrive. These fumigations are additional to whatever has already been conducted on-farm and through the value chain.

Collaboration

Prior to the GRDC national program, the bulk handling companies were in competition with each other and wary about sharing information lest they find themselves at a competitive disadvantage. The national resistance monitoring program tied the industry funding to collaboration and transparent exchange of infestation and resistance levels information. Obviously grain types, quantities and locations remain confidential.

Collaborating government laboratories across Australia now share a common laboratory manual and technical staff meet regularly to ensure standardised testing procedures, other methodologies and to update their knowledge of overall resistance trends both at regional and national levels. Blind exchange of reference strains is undertaken to ensure compatibility of results and when new or significant resistances are found, the suspect strain is sent to at least one sister laboratory for confirmation of the diagnosis [7]. Consideration is given to the danger of sending resistant strains to laboratories in locations where the resistance may not exist. Where there is no alternative ‘biosecure’ packaging is used [20].

Sampling

Resistance monitoring project staff visit and collect samples from farms, central storages and other sectors of the industry including grain merchants and flour mills. In Western Aus-

tralia a network of about 50 Department of Agriculture and Food field staff are used with an allocation of six days each. The eastern states have a smaller number of dedicated staff from Department of Employment, Economic Development and Innovation, in Queensland; and the New South Wales Department of Primary Industries who service the northern and southern regions of Australia, respectively. These differences reflect, in part, the differing historical approaches to managing pesticide resistance within each jurisdiction.

The Western Australian approach permits more samples to be taken by local staff who are familiar with local growers, but staff may be hundreds of kilometres from their counterparts and only meet once a year to compare sampling procedures. The advantage of the dedicated staff approach is that more consistent sampling methods are employed, because staff work closely together. Field staff should have phytosanitary inspector status to allow them to enter farms, because some growers may refuse entry especially if they are aware that they have poor hygiene and storage conditions and may incur some penalty, quarantine or requirement to perform storage maintenance at their own expense. While this is not the case in the eastern states, most companies and growers are happy to allow grain samples to be taken, and it allows the inspector a chance to educate the staff on procedures that may improve their storages and insect control.

Staff training manuals and online as well as face-to-face courses are essential to maintain interest and ensure that consistent methods are used that provide the best chances of finding grain insects. It is important to ensure that all staff submit insect population samples even if only one or two insects are found. Samples should be submitted to the laboratory in a timely fashion so that the insects arrive in good condition ready for bioassay or culturing. Insects are usually collected by sieving grain and care should be taken to ensure that concentrated dusts and residues are not concentrated in the sample vial. Where possible, biosecure packaging should be used to minimise the chances of the vial breaking en-route and spreading what could be resistant insects.

Strains collected from different storages should not be bulked otherwise there is no chance of determining from which storage a resistant sample was collected. Samples collected from adjoining storages should be recorded separately, because the storage conditions and commodities can be quite different. Similarly insect populations collected from the same storage but 12 months later should be classified as a new population. GPS coordinates must be recorded for both sites and individual storages to facilitate trace-back and mapping the spread of resistance.

Farm samples should be defined and labelled as ‘targeted’ or ‘random’. Summary results of random surveys can be used to compare resistance frequencies between storage methods and states. Targeted samples are taken from sites with a history of poor storage practice or resistance, or confirmatory samples

collected. These data give the best chance of providing early warning of resistance.

Samples from bulk handling companies are collected by company staff during hygiene inspections and on every occasion that insects are detected in storage facilities, grain bulks or shipments. As with farm samples, it is important that bulk handler samples be sent off for resistance testing even if only one or two insects are found in a population. These individuals could be a result of control failure due to resistance and early detection underpins any resistance monitoring and management project. It is important that storage managers are not made to feel that the presence of insects indicates a failure on their part otherwise they may not report an infestation. Other sources of insects for testing include household samples and quarantine interceptions. There have been a number of occasions where grain insects detected in imported goods were subsequently found to be highly resistant to phosphine, with some categorised as high-risk to the local industry. It can be very costly to isolate and eradicate these pests and there is little point in putting the grain industry through the expense of sealing storages, monitoring and managing resistance if pre-selected resistant strains are allowed to enter the country through ports.

Culturing

Resistance monitoring programs should give consideration to project objectives. Is the work being conducted to determine trends or compare the impact of storage and fumigation regimes on resistance? Or is it being conducted to detect and respond to early resistance development? These considerations will influence how best to deal with small numbers of insects.

Biometricians will always demand a larger sample size but this is not always possible when infestation levels are low or where there may be large numbers of one species and only a trace of others. How best to deal with these strains? Culturing sufficient numbers to test will provide a larger sample size and more significant results but, at best, will delay the diagnosis of resistance until after grain has moved or insects spread. At worst, the culture may die out and no data at all is obtained. Either way, the extent of culturing (F_1 , F_2 , etc) must be recorded to allow appropriate interpretation of results as the resistance frequency in the F_1 or F_2 generation can reflect that of the original small sample. However, this may not always be the case, because the proportion of females in the original sample, whether or not they had mated with males outside the sample, and differential survival and reproduction between females in a small sample may also have significant effects.

Testing

The established 'industry standard' test method is the Food and Agriculture Organization (FAO) technique of injecting phosphine into gas-tight desiccators [2]. However there are now a suite of tests available and resistance monitoring project leaders should consider how quickly a result is required.

If some action is to be taken when resistance is detected a quick test should be used to obtain a same-day result; if data are being collected to compare locations or plan strategies, the 15-day FAO procedure is more suitable; if research data collection is for product registration or to determine end-point mortality, the flow through technique against mixed age cultures is best.

With the FAO technique two discriminating doses are used. A lower one discriminates between susceptible and resistant insects and a higher one is designed to detect resistances higher than the common 'weak' resistance [12]. This work details adaptation of discriminating doses from the original method based on responses of laboratory reference strains. Insects believed to be homozygous for phosphine susceptibility were used to determine the lower discriminating dose while strains homozygous for weak resistance were used to determine the upper dose.

Reeves [unpublished, 27] notes that with small numbers of test insects, FAO assays that fail to cause mortality reveal the presence of resistance, and provide a conclusive result—in this case. Unfortunately, if all insects submitted have been used in a test for weak resistance, there will be no opportunity to check for strong resistance and strong resistance must be assumed whether it is present or not. However, if a small number of insects are tested for resistance, and all die, then the test is inconclusive, as the proportion of insects which are resistant may be low enough that there are no resistant specimens in the sample at all. In this scenario also, strong resistance should be assumed. Therefore there is little point testing very small numbers of insects with FAO bioassays, as whatever the outcome, one cannot rule out strong resistance. Another way to look at this situation, as elaborated by Reeves, is to determine a confidence interval for the proportion of resistant individuals given the test results. Standard confidence intervals are unreliable for data where no successes are observed and a Bayesian probability interval is recommended based on an uninformative Jeffreys' prior, as outlined in Brown *et al.* [6]. On this basis, Reeves gives 95% confidence intervals for the proportion of resistant individuals and finds that a 100% mortality rate in a test of only five insects is consistent with an underlying proportion of resistant individuals in the population of up to 23%. On the other hand a 100% mortality rate for 100 insects, is consistent with an underlying proportion of resistant individuals of up to 1.3%. For the purpose of definitively diagnosing strong resistance in Australia with the FAO method, at least 150 insects are tested with three replicates.

A second method, known as the 'rapid test', was originally developed by Reichmuth [28] has been widely used in Western Australia so that results can be quickly returned to field staff who, as required, will revisit infested properties and direct hygiene, control or eradication depending on the severity of the resistance. This test was further refined by Bell and others [3] and is used to give a quick yes or no answer with

field-collected insects, ie, resistance at an undefined level or no resistance. This approach allows immediate action (control, eradication, quarantine) to be taken where appropriate. The drawback with the rapid method is that it is difficult to determine the strength of resistance in some species [12]; however, as the rapid test is a knock-down test, it is possible to culture the survivors and retest later using other methods.

The recent discovery in eastern Australia of *C. ferrugineus* with the strongest phosphine resistance ever reported for any stored product pests required development of a new ‘rapid test’ to diagnose this very strong resistance [25, unpublished]. A third method is the flow-through technique that exposes mixed age cultures of insects to a continuous flow of phosphine at a constant concentration [13, 31]. This method is very laborious and lengthy but it gives an accurate prediction of the time required for complete control of an insect population at a nominated phosphine concentration [12]. It is used to characterise the resistance and predict concentrations and exposure periods needed to control insects in the field.

Exciting work has been conducted at the University of Queensland mapping resistance with DNA markers. Strong resistance to phosphine was first determined to be controlled by two genes which arose independently [29]. Researchers hope that a marker can be identified that is always linked to the primary resistance gene and that resistance can be rapidly and precisely diagnosed using PCR. In addition, once the gene is identified, it may be possible to develop a biochemical test for that resistance leading to an ‘on-site’ test whereby decisions can be made about fumigation treatment regimes. A molecular test would mean that dead insects and even insect fragments with intact DNA could be tested for resistance. This DNA work has discovered much about the toxicity of phosphine resistance and the mechanisms involved [21].

Database

An important early step to engage all collaborators in national phosphine resistance monitoring programs is the development of a database with data on sites visited, strains collected and assays conducted. This permits standard data recording methods and analysis as well as transparent exchange of results between collaborating laboratories. In 1996, the Australian Grain Insect Resistance Database (AGIRD) was developed by the Department of Agriculture and Food to include all data from disparate resistance databases and spreadsheets [17]. As of 2011, the AGIRD database held the results of over 60,000 assays on 30,000 samples from more than 9,000 sites across Australia. Sophisticated hardware, software and expertise were not required for development and management of AGIRD. The AGIRD database has three main tables for sites, samples and assays along with fifteen other lookup tables to normalise the data. Referential integrity is enforced by following two simple rules: (1) assays cannot be assigned to a sample that does not exist or a sample assigned to a non-existent site; and (2) records cannot be deleted from a primary table if matching records exist in a related table. For exam-

ple, deleting a site record will delete all related samples and assay details. The AGIRD database fields, structure and relationships are given in Emery and Tassone [17]. The AGIRD database has not been upgraded since 1997, and will be modified soon to incorporate two new tables into the database structure. We now regularly revisit many sites and storages and the addition of storage and inspection tables will allow recording of multiple inspections of individual storages. The new structure will be Site > Storage > Inspection > Sample > Assays with determination of resistance category recorded in the Sample table, rather than in the Assays table.

A standard database used by all participants ensured that consistent diagnoses were made. For example, some laboratories follow the FAO method closely and only classify a strain as resistant if more than one insect survives out of 80 tested; other laboratories will diagnose resistance if there are any survivors out of any number tested. Some laboratories summarise resistance frequency by counting sites with resistance while others record resistant strains or assays. Other laboratories may only classify a strain as resistant only when a second confirmatory sample has been collected and gives a positive result. These different interpretations impact significantly on data reporting of resistance frequencies and can make some locations appear far worse than others. Standard database queries, cross-tabulations and reports shared by laboratories will ensure consistent reporting. Resistance data are securely shared fully between collaborating government laboratories. However, the results of resistance assays for bulk-handling companies are delivered over password-protected web pages filtered by the company for privacy and security. One limitation of cooperative or grower-owned bulk handling companies is that shareholder privacy must be maintained, and companies will not release grower details even when damaging infestations are found. If strong resistance is found, however, the company will perform trace-back and follow-up action.

Handheld computers, PDAs, smartphones and the like are changing how field surveillance data are collected and should be integrated into any resistance monitoring program. In the past nearly all field-collected information was recorded manually on paper reducing the rate of capture, integrity, conformity as well as security of the data. Emery [19] outlined the development of stored grain pest surveillance data collection software and hardware using smartphones to provide auditing, validation, chain of evidence and increase the volume of data collected as well as its integrity through relational databases and seamless data transfer to central databases. Data collection is supported by digital voice navigation itineraries, GPS-located traps, digital time and date stamps as well as field printed barcode labels and site imagery – all in a single hand-held unit.

Action

Resistance monitoring should serve a purpose, be it a contribution to the resistance management strategy or to support eradication/containment action. Monitoring facilitates early

detection that can give information about causal influences and future action that may need to be taken. Where initial resistance is localised, early warning allows eradication to be implemented before the infested bulk is moved or placed into the market.

It is important to look at resistance frequency within a species. Currently in Western Australia weak resistance is around 45% across all species, however considering *T. castaneum* alone, it is over 70%. Collins and Emery [7] noted that once weak resistance frequency approaches 80% of samples, strong resistance will soon ensue. This was the case in Queensland in 1993 when strong resistance was first detected in Australia and in 2010 with the first three samples in Western Australia.

If the resistant sample comes from a bulk-handling company storage facility, the company will generally take immediate action. Unlike many other insecticides, resistance to phosphine can usually be overcome with either longer exposure periods or higher concentrations or a combination of both. This attribute of phosphine led to label rate changes in recent years to ensure control of insect species with strong phosphine resistance. The current exception is strong resistance in *C. ferrugineus*. However, protocols have now been developed and further changes to the phosphine label rates will occur [24]. In some cases storage sealing, re-sealing or phosphine application equipment replacement and re-treatment is sufficient to eradicate these resistant insects. Other methods may include turning (moving) the grain from one silo into another silo and treating it with an effective grain protectant. The empty silos are then subjected to detailed cleaning protocols and treated with a residual chemical.

Eradication of resistant populations from farm silos or grain merchant premises is more difficult. In the past, structures may have been fumigated with methyl bromide on farms where resistance was detected [15]. More recently, where serious resistance is discovered, farmers are advised by entomologists of the severity of the situation and offered assistance with treatment of storages and equipment. Most farmers willingly cooperate in eliminating the infestation – usually by re-sealing storages, moving grain to a sealed storage and treating the surrounding area and equipment with grain protectants. Sinclair and Alder [30] explored various management practices on farms which confirmed the importance of physical farm clean up, especially of the header, in reducing pest numbers in grain.

Intensified follow-up sampling of storages for two years following eradications is recommended to confirm success. In Western Australia during 2010 weak resistance frequency was over 40% of the samples tested. Strong resistance has been found and eradication efforts were undertaken at three sites. Eventually, as the frequency of weak resistance approaches 80% and detections of strong resistance increase, a containment strategy will become the best option.

Resistance monitoring programs need to consider the source of resistance. Bulk-handling companies usually feel that farms are the source of all resistance and that the problem becomes ‘concentrated’ at their facility. It is possible that the bulk handlers are doing their own selections through calendar-based fumigations. Some researchers have suggested that poorly maintained sealed storages will select for higher resistance than fumigations in totally inadequate storages; so far this seems not to be the case. Generally speaking, farm storages in the eastern states are very poorly sealed and appear to have less incidence of strong resistance. However, this may be due to the fact that growers tend not to re-fumigate their grain as often as bulk handlers.

In the eastern states of Australia during the early 1990s almost all strong resistances were initially found in unsealed central storages where a low concentration flow-through phosphine application (Siroflo®) system was used. Bridgeman outlined field trials in bulk storages that provide conclusive evidence that low concentration fumigations with phosphine are not effective in controlling target pests and that relevant data about known resistant samples should have been incorporated in the Siroflo® label directions [5]. Similarly, Nayak and colleagues describe how the national resistance monitoring program showed that, although weak resistance is widespread in Australia, very strong resistance in *C. ferrugineus* occurred almost exclusively (97%) in central storages in eastern Australia [25, unpublished]. In Western Australia where sealed storage is widely used both on-farm and in the central handling system, strong resistance is yet to be found in bulk storages. However, the first three incidences of strong resistance, discovered during 2007-10, were on farms within 100 kilometres of each other and in marginal country.

The ecology of grain insects may be able to answer important questions about the development and spread of resistance and contribute to resistance monitoring programs. For example, is resistance evolving separately in widely dispersed locations or spreading independently? Do resistant samples spread through the value chain with transport or do they fly there by their own means? Daghlish and colleagues investigated flight activity and gene flow in *T. castaneum* in a grain growing district in Australia [14]. They showed that dispersal was important on a scale of at least tens of kilometres and that this must be contributing to gene flow. This type of work will contribute to future resistance monitoring projects by highlighting situations where limited resources should be focused.

Extension

Resistance monitoring programs can provide useful information for grain storage extension effort by highlighting locations and practices that may be selecting for resistance. For the past decade the Australian GRDC has funded a national extension campaign aimed at raising grower awareness of the safe and effective use of phosphine. The extension strategy

included media releases, brochures, bumper stickers, training courses, field days and negotiation with the Australian Pesticides and Veterinary Medicines Authority to improve the phosphine label. This project enables extension officers specialised in stored-grain protection to disseminate important information and key messages on safe handling of phosphine and accurate fumigation techniques to growers through extension workshops across Australia. The GRDC-supported 'Growers Update' is another excellent platform for researchers and extension specialists to educate growers on these issues. Newman outlines the relationship between resistance monitoring program and extension projects and proposes a novel approach where grower fees collected for the resistance monitoring service can be reinvested in defined research and extension projects maximising the return for the grain growers [26].

Lessons learned

1. A nationally coordinated program balancing applied and basic research along with development and extension is the key to the successful management of resistance to phosphine.
2. 'Duty of Care'. It is the responsibility of all players in the grain value chain (growers, feedlots, food processors, small retailers and bulk handlers) to protect this unique fumigant and prolong its useful life by adopting the recommendations of the established Resistance Management Strategy [11]. Any lapse along the chain will trigger a collapse of the strategy.

Conclusions

Collaboration between the Australian government and Australian grain industry through the National Working Party on Grain Protection, and with support of both the GRDC and CRCNPB, the Australian phosphine resistance monitoring program has successfully drawn together the resources of three laboratories across the country incorporating over a quarter of a century of resistance data to ensure an effective system for combating resistance.

The integrity of the program is underpinned by common assay methods, inter-laboratory confirmation of results, open communication and sharing of information through the Australian Grain Insect Resistance Database. Collins and Emery note that the ultimate aim of the program is to extend the useful life of phosphine through an early warning system to enable an uncompromising response to insects with incipient resistance [7]. The program has made possible a truly national strategic approach to managing resistance to phosphine. Benefits to industry include not only early warning of emerging resistance problems, but also dynamic research into strategies to manage or combat resistance and targeted extension campaigns.

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References

1. Agricomber. World top 10 wheat producers 2010, accessed October 20, 2011. <<http://www.agricomber.com/world-top-ten-wheat-producers-2010/>> 2010.
2. Anonymous. Recommended methods for the detection and measurement of resistance of agricultural pests to pesticides. Method number 16. Tentative method for adults of some major species of stored product insects with methyl bromide and phosphine. FAO Plant Protection Bulletin 1975: 23:12–25.
3. Bell CH, Savvidou N, Mills KA, Bradberry S and Barlow ML. A same-day test for detecting resistance to phosphine. Proceeding of the 6th International Working Conference on Stored-product Protection, (Edited by Highley E., Wright EJ, Banks HJ and Champ, BR), Canberra, Australia, CAB International, Wallingford, U.K. 1994: 41–44.
4. Brattsten LB, Holyoke CW, Leeper JR and Raffa, KF. Insecticide resistance: a challenge to pest management and basic research. Science 1986; 231: 1255–1260.
5. Bridgeman B and Collins PJ. Monitoring fumigation effectiveness using bioassays of mixed aged cultures of target pests. Proceedings of the International Conference on Controlled Atmospheres and Fumigation in Stored Products, Fresno, California, USA 2000: 679–684.
6. Brown LD, Cai T and DasGupta A. Interval Estimation for a Binomial Proportion. Statistical Science 2001; 16(2): 101–133.
7. Collins PJ and Emery RN. Two decades of monitoring and managing phosphine resistance in Australia. Proceedings International Working Conference on Stored Product Protection, York, UK 2002: 570–575.
8. Collins PJ, Daglish GJ, Pavic H and Kopitke RA. 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. Journal of Stored Product Research 2005; 41:373–385.
9. Collins PJ, Daglish GJ, Bengston M, Lambkin TM and Pavic H. Genetics of Resistance to Phosphine in *Rhyzopertha dominica* (Coleoptera: Bostrichidae) Journal of Economic Entomology 2002; 95(4):862–869.
10. Collins PJ. Resistance to chemical treatments in insect pests of stored grain and its management. Proceedings of the 9th International Working Conference on Stored Product Protection 2006: 277–282.
11. Collins PJ. 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, accessed October 20, 2011. <<http://www.crcplantbiosecurity.com.au/publications/npb1437>> 2009.
12. Daglish GJ and Collins PJ. Improving the relevance of assays for phosphine resistance. Proceedings of the Seventh International Working Conference on Stored-Product Protection (Edited by Jin, Z, Liang Q, Liang, Y, Tan, X and Guan, L), Beijing, PR China 1999: 584–593.
13. Daglish GJ, Collins PJ, Pavic H and Kopitke RA. Effects of time and concentration on mortality of phosphine-resistant *Sitophilus oryzae* (L) fumigated with phosphine. Pest Management Science 2002: 58

- (10):1015–1021.
- 14 Ridley AW, Hereward JP, Daglish GJ, Collins PJ, Raghu S and Walter GH. The spatiotemporal dynamics of *Tribolium castaneum* (Herbst): adult flight and gene flow. *Molecular Ecology* 2011; 20: 1635-1646.
 - 15 Dean KR. An Integrated approach to stored-grain protection in Western Australia. *Proceedings of the 6th International. Working Conference on Stored Product Protection* (Edited by Highley, E, Wright, EJ, Banks, HJ and Champ, BR), Canberra, Australia.1994: 1179–1181.
 - 16 Emery RN. A Western Australian farm survey for phosphine-resistant grain beetles. *Proceedings of the 6th International. Working Conference on Stored Product Protection* (Edited by Highley, E, Wright, EJ, Banks, HJ and Champ, BR), Canberra, Australia 1994: 98–103.
 - 17 Emery RN and Tassone RA. The Australian grain insect resistance Database - a national approach to resistance data management. *Proceedings of the Australian Postharvest Technical Conference*, (Edited by Banks, HJ, Wright, E, and Damcevski, KA), Canberra, Australia 1998: 312–317.
 - 18 Emery RN, Collins PJ and Wallbank BE. Monitoring and managing phosphine resistance in Australia, *Stored Grain in Australia 2003*. *Proceedings of the Australian Postharvest Technical Conference*. (Edited by Wright EJ, Webb MC and Highley E), Canberra, Australia 2003: 142–151.
 - 19 Emery RN, Chami M, Garel N, Kostas E and Hardie DC. The use of hand-held computers (PDAs) to audit and validate eradication of a post-border detection of khapra beetle in Western Australia. *10th International Working Conference on Stored Product Protection*, Estoril, Portugal. 2010: 1031–1037.
 - 20 Glocke P and Hall B. Biosecure packaging for the Transport of Emergency Plant Pest samples - Final report. Cooperative Research Centre for National Plant Biosecurity. Project CRC40121, accessed October 20, 2011 <<http://www.crcplantbiosecurity.com.au/publications/npb1601>> 2010.
 - 21 Nath NS, Bhattacharya I, Tuck AG, Schlipalius DI and Ebert PR. Mechanisms of Phosphine Toxicity. *Journal of Toxicology*. Volume 2011, Article ID 494168 2011.
 - 22 Nayak MK and Collins PJ. Influence of concentration, temperature and humidity on toxicity of phosphine against strongly phosphine-resistant psocid *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae). *Pest Management Science* 2008: 64:971–976.
 - 23 Nayak MK, Collins PJ and Pavic H. Resistance to phosphine in psocids: Challenges ahead! In Wright EJ, Banks HJ and Highley E [Eds], *Proceedings of the 2nd Australian Post-harvest Technical Conference*, Adelaide, Australia 2002: 113–118.
 - 24 Nayak MK, Holloway JC, Pavic H, Head M, Reid R and Collins PJ. Developing strategies to manage highly phosphine resistant populations of flat grain beetles in large bulk storages in Australia, in *Proc 10th Int Working Conference on Stored-product Prot.*, (Edited by 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 and White NDG), *Julius-Kuhn-Archiv*, 425, 2010: 396–401.
 - 25 Nayak MK, Collins PJ, Holloway JC, Emery RN, Pavic H and Bartlet J. Strong resistance to phosphine in flat grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae): its characterisation and a 'rapid test' for diagnosis. *Pest Management Science* 2011 (submitted).
 - 26 Newman CR. A novel approach to limit the development of phosphine resistance in Western Australia. *Proceedings of the 10th International Working Conference on Stored Product Protection*, Estoril, Portugal 2010: 1038–1044.
 - 27 Nayak MK, Holloway JC, Emery RN, Head, M, Reid R, Reeves, R, Collins, PJ. Resistance monitoring and protocol development: key components in ensuring the biosecurity of post-harvest. Cooperative Research Centre for National Plant Biosecurity, Science Exchange 2009.
 - 28 Reichmuth CA. Quick test to determine phosphine resistance in stored product pests. *GASGA Newsletter* 15, 1992: 14–15.
 - 29 Schlipalius DI, Qiang C, Reilly PEB, Collins PJ and Ebert PR. Genetic linkage analysis of the lesser grain borer *Rhyzopertha dominica* identifies two loci that confer high-level resistance to the fumigant phosphine. *Genetics*, 2002: 161:773–782.
 - 30 Sinclair ER, Alder J. Development of a computer simulation model of stored product insect populations on grain farms. *Agricultural Systems* 1985: 18(2):95-113.
 - 31 Winks RG and Hyne EA. The use of mixed-age cultures in the measurement of response to phosphine. *International Conference on Controlled Atmospheres and Fumigation in Stored Products*, (Edited by Donahaye E, Navarro S and Varnava, A) Nicosia, Cyprus 1997: 3–16.
 - 32 Winterbottom DC. *Weevil in Wheat and Storage of Grain in Bags: a record of Australian experience during the war period (1915-1919)* Publisher Fast Books, 1994 ISBN 0642193762, 780642193766, 1922: 122 pages.