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Monitoring and managing phosphine resistance in Australia

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Abstract. The Australian grain industry relies heavily on phosphine to meet domestic and international market demand for high-quality grain, free of insects. Phosphine usage has increased markedly over the last 10 years because of market reluctance to accept protectant chemical residues, as well as resistance in target pests to grain protectants. The threat that insects may also develop resistance to phosphine led to resistance-monitoring projects being initiated across all cereal-growing regions of Australia as a pro-active response to develop strategies to combat resistance as it evolved.

With industry support, these projects have now amalgamated to form a national phosphine resistance monitoring and management program underpinned by a database of over 35,000 assays.

Over the past two decades, the incidence of weak resistance has increased from 5% to 34% of strains tested along with the emergence of strong resistance in 1997. This latter resistance occurs in only 1.7% of strains from eastern states tested over the past two years, but poses the greatest threat to the industry.

The Australian approach is unique in that it has drawn together primary producers, bulk handlers, chemical companies, industry-funded organisations and government research institutions from across the country to combat the national threat of phosphine resistance.

Introduction

The Australian grain industry continues to rely heavily on the use of phosphine to disinfest stored grain. Phosphine is currently used to disinfest about 80% of Australian grain whereas grain protectants (contact insecticides) are now used on less than 20%. Western Australia (WA) is particularly reliant on phosphine because contact insecticides have not been used on any export grain since 1990 and over 60% central storage capacity is sealed. Sealed storages are used on about 60% of farms in WA, although the status of their sealing is a point of conjecture.

Before the 1980s, application of grain protectants was the dominant chemical strategy used in Australia. However, the importance of phosphine increased as grain protectants became vulnerable due to the occurrence of resistance strong enough to cause control failures in many species.

This resulted in two different approaches across Australia to combat the protectant resistance problem. The eastern states embarked on a rigorous program of evaluating and delivering new protectants and protectant combinations under the auspices of the Australian Wheat Board's Working Party on Grain Protectants. The Western Australians, on the other hand, developed a regulatory system that minimised the chemicals available for farmers, thereby holding some in reserve for exclusive use by the central handling system and eradication of outbreaks of resistance.

All states recognised that the farm was often the source of resistance and noted the importance of detecting resistance before control failures occur. Accordingly, very successful protectant resistance monitoring and management programs were developed in all states.

This gradual but steady development of protectant resistance and the global move away from contact insecticides due to concern about residues meant that interest in phosphine was growing. The stage was set for phosphine following the international agreement to phase out methyl bromide.

Phosphine has several other advantages that have made it attractive for use in the Australian grain industry. It is a relatively easy to apply (compared with other fumigants), versatile and inexpensive, with international acceptance as a near residue-free treatment. Although phosphine resistance was detected in the 1970s, it was believed that this

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resistance could be controlled with a properly applied fumigation, preferably in a sealed storage. Of course, monitoring to determine the extent of resistance was an essential component of any management strategy.

As phosphine became more important to the grain industry, attention focused on the future of this material. The industry realised that loss of phosphine because of resistance would be a major disaster as there was no viable replacement. Resistance to phosphine had already been detected in some locations in Australia (Champ and Dyte 1976; Attia and Greening 1981; Herron 1990; White and Lambkin 1990; Emery 1994), but the incidence was low and the resistance level regarded as 'weak' and thought to be controllable if phosphine was correctly applied. More significant were reports by reputable scientists of the occurrence of much stronger resistances overseas (Mills 1983; Taylor and Halliday 1986).

The value of resistance monitoring to the grain industry had already been demonstrated during the late 1970s when the Australian grain industry was reliant on protectants to maintain its nil-tolerance for insects in export grain. The industry began to introduce new grain protectants to replace malathion (Bengston et al. 1983), the use of which had been jeopardised by widespread resistance. To ensure early warning of any incipient resistance problems with these newer materials, the grain industry requested that state government departments of agriculture provide a resistance-monitoring service. The grain protectant resistance-monitoring program was highly successful in that it not only provided early warning of emerging resistance problems (Collins 1994; Dean 1994; Wallbank et al. 2002), it also generated data on which to base resistance management strategies.

Clearly, the only means to learn what is happening in the field with respect to phosphine resistance is to monitor farms, merchants and central storages so that resistant strains can be eradicated *before* control failures occur. International resistance researchers have recommended that this type of resistance monitoring be conducted on a national basis for many years (Subramanyam 1996) and this was reinforced in 2002 at the International Working Conference on Stored Product Protection where a resistance monitoring and management workshop was convened (Collins et al. 2003).

Although continuous resistance monitoring has been conducted since 1984, the national dependence of all sectors of the grain industry on phosphine, along with the threat of stronger resistances occurring, prompted the industry through the Grains Research and Development Corporation to make funds available from 1996 to integrate state-based programs to facilitate continued and extensive monitoring for phosphine resistance.

This industry support is unique in the world in that it has brought together three state government organisations across the nation. The Western Australian Department of Agriculture collects and tests samples from the western region, New South Wales Agriculture the south and south-east, and the Queensland Department of Primary Industries looks after samples from the north of the grain belt.

These three laboratories are now closely integrated so that information can be readily shared and methods standardised. These procedures are published annually and benchmarked at resistance-monitoring workshops. Transparent and standardised test data are stored on an interactive, web-enabled database that is regularly updated by each group and can be easily accessed by each of the contributing laboratories. Resistance data are also continuously available to the six major bulk handling companies to support their operations.

Leaders of the three research groups present their findings to industry annually at meetings of the National Working Party on Grain Protection.

Methods

Sampling

Project staff collect samples from farms and other sectors of the industry, including grain merchants and flourmills, on a random basis—although if resistance is detected or storage practices suspect, these locations will be targeted in future surveys. Samples are also collected by staff of bulk handling companies when insects are detected in bulks. Other sources include quarantine and household samples.

Testing methods

In most instances, resistance is tested using the standard Food and Agriculture Organization of the United Nations (FAO) technique of injecting phosphine into gastight desiccators (FAO 1975). 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 (Daglish and Collins 1999). We have adapted the discriminating doses (DDs) listed in the original method based on responses of our own laboratory reference strains. Insects believed to be homozygous for phosphine susceptibility are used to determine the lower DD, while strains homozygous for weak resistance are used to determine the upper DD.

Two other assay methods are also used. The 'rapid test', originally developed by Reichmuth (1991) and Bell et al. (1994), is used to give a quick yes–no answer with field-collected insects—i.e. resistant or not—allowing immediate action (control, eradication, quarantine) to be taken where appropriate. The drawback with this type of method is that it is difficult to determine the strength of resistance in some species (Daglish and Collins 1999). 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 (Winks and Hyne 1997; Daglish et al. 2002). This method is very laborious and lengthy but it gives an accurate prediction of the time required for complete extinction of an insect population at

a nominated phosphine concentration (Daglish and Collins 1999). It is used to characterise the resistance and predict concentrations and exposure periods needed to control insects in the field.

Data management

In addition to results of assays, other data including location (latitude and longitude), grain type, storage type, application method, previous treatments, and other parameters are collected. This information, together with bioassay results, is stored on an Internet-based database called Australian Grain Insect Resistance Database (AGIRD) (Emery and Tassone 1998). A demonstration version of AGIRD can be viewed at <http://www.agric.wa.gov.au/ento/agird1.htm>. This database currently holds the results of 35,149 bioassays on 17,800 grain insect strains from 6362 locations. The number of weak and strong resistance tests conducted each year since 1982 are shown in Figure 1.

The advantage of an Internet-based system is that information is immediately available and is stored in a form that can be readily manipulated and analysed for reporting purposes. Each of the three laboratories regularly updates a replicate database in Brisbane, Wagga Wagga or Perth, and later synchronises with the master database maintained at the WA Department of Agriculture. This avoids delays with real-time transmission of data for individual strains, but allows access by registered clients to data on a secure link.

Project integrity

The three laboratories share a common laboratory manual and regularly meet to standardise testing and other methodologies. In addition, yearly blind exchanges and testing of reference strains is undertaken to ensure compatibility of results. When new or significant resistances are found, the suspect strain is sent to at least one sister laboratory for confirmation of the diagnosis.

Results

Results of monitoring are reported at industry meetings on an annual basis and can be downloaded from AGIRD at any time by project participants. Bulk handling companies have password-protected Internet access to filtered results of resistance tests of insects that they have supplied. Farmers and others who permit sampling on their properties are informed (in writing) of the results of testing, along with extension material on grain management practices.

The large number of assays on strains collected from such diverse and dispersed locations means that many analyses of resistance development can be made. Figure 2 shows the steady increase in the frequency of weak phosphine resistance since 1982 and a similar build-up in strong resistance since 1997. These data are taken from 22,099 resistance assays. This extensive dataset allows for more complex analyses and presentation—for example, by geographical distribution, by industry segment, or by application method. Geographical distribution of sampling sites and locations where strong resistance has been detected—all species since 1997, when strong resistance was first detected—are illustrated in Figure 3. It is important to have consistent criteria for determining resistance and in this study we have assessed greater than 5% survival in the weak discriminating dose test as weak resistance, and more than one survivor in the strong resistance discriminating dose test as strong resistance.

Strongly resistant insects have also been intercepted by Australian quarantine inspectors (indicated by the point over Perth, WA in Figure 3), highlighting the need for bioassays to be used to test not only local cosmopolitan

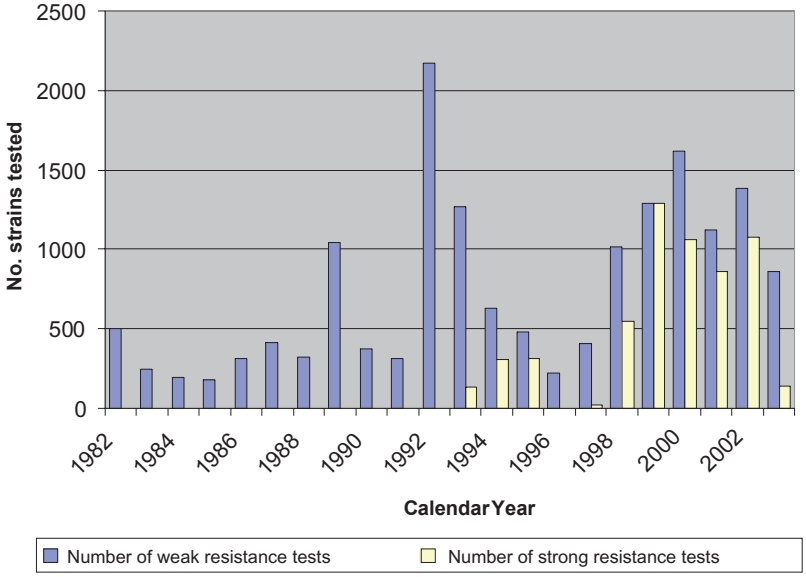

Figure 1. Number of grain insect strains tested for resistance to phosphine 1982–2003 (to June 2003).

species, but exotic, potentially resistant strains of grain insects as well.

Targeted regional maps can also be produced showing areas of high and low risk (e.g. Figure 4). This information is used by bulk handling companies and state agriculture departments for planning future pest and resistance management strategies in particular regions, although care is taken not to discriminate against individual growers.

Figures 5 and 6 show trends in resistance frequency from 18,534 weak resistance assays. Such illustrations allow comparisons can be made between states. The percentages tend to move up and down over time depending on how random the field collections are, but few would argue that there is an increasing trend for weak resistance—from around 5% of samples tested nationally to over 40% (Figure 5). The percentages over the last few years are not as indicative because the focus and testing in the eastern states is now on strong resistance. The data from 6039 strong resistance assays (Figure 6) are variable. Strong resistance is yet to be confirmed in WA and none has been detected in the eastern states during the first half of 2003, attesting to the value of monitoring and early intervention. The very high result for South Australia in 1999 (Figure 6) was because only four samples were collected in that year.

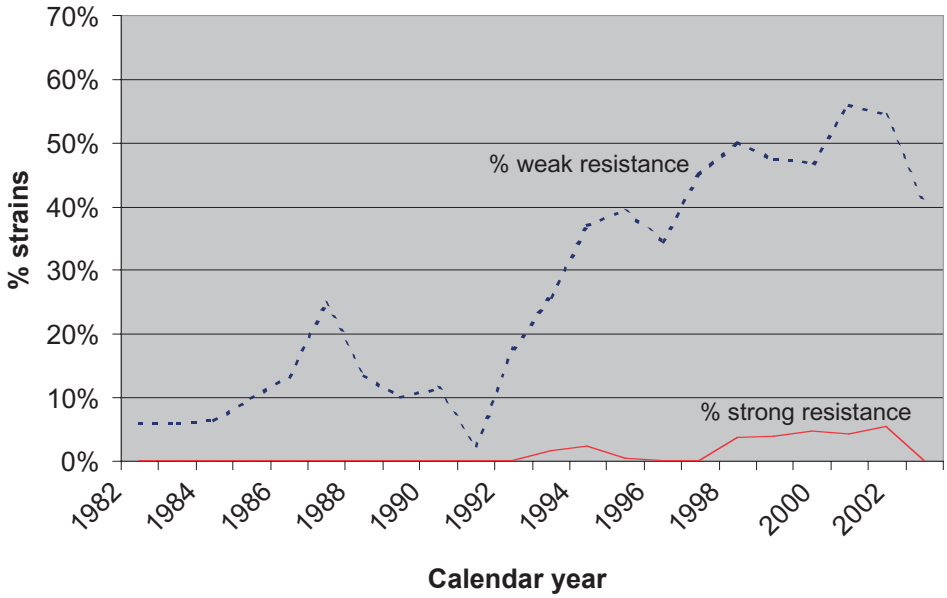

Figure 2. Percentage of grain insect strains with strong and weak phosphine resistance in Australia 1982–2003 (to June 2003).

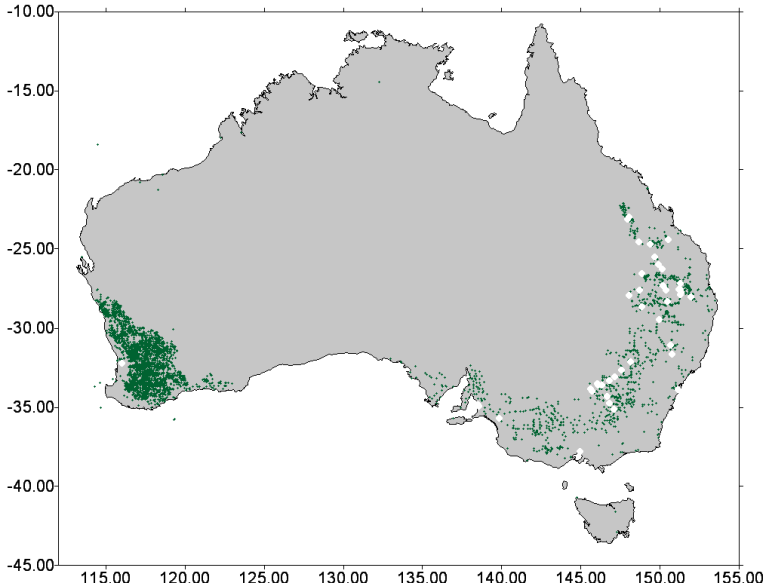

Figure 3. Grain insect sample sites and detection of strong resistance (white dots) to phosphine in Australia.

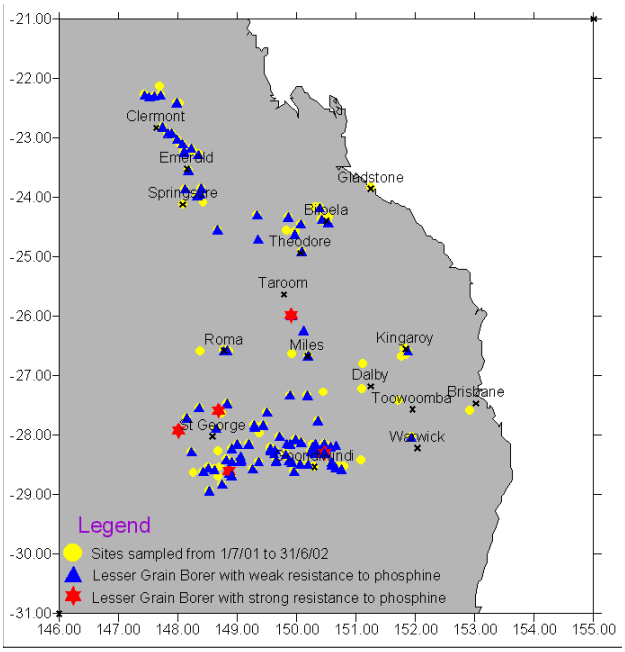

Figure 4. Detection of strong resistance to phosphine in the lesser grain borer, *Rhyzopertha dominica*, in Queensland, Australia, 2001–2002.

Figure 7 shows the frequency of weak resistance across industry sectors taken from 2540 central storage strains, 10,154 farm strains, 253 flour millers and 530 merchants. There are indications that a problem may be developing post-farm gate, with only 23% of farm strains having resistance, compared with 47% for flour millers and 35% for central storage. The data for strong resistance by industry (Figure 8) show that the incidence of strong resistance is marginally higher in central storages than farms or flour millers and continued monitoring of central storages for strong resistance should continue to be a priority.

Figures 9 and 10 provide an insight into which grain insect species are the ones likely to threaten the industry. The lesser grain borer (*Rhyzopertha dominica* (F.)) enhances its pest status by not only being the most damaging species in Australia, but one of the most likely to develop phosphine resistance as well.

This extensive survey also provides the opportunity to study the frequency of resistance in strains collected from different storage types. Figure 11 compares weak phosphine resistance in 214 strains collected from sealed farm storages and 2487 strains collected from unsealed farm storages. Unsealed storages appear to be selecting for weak resistance. Unfortunately, the small number of strongly resistant strains collected from sealed storages did not allow comparison across storage types for this more serious resistance.

Discussion

Laboratory confirmation and measurement of resistance

Once a new or high-level resistance is detected in an insect population sample, a subsample is sent to at least one sister laboratory for confirmation. If resistance is confirmed, the next step is to determine its likely impact on the grain industry, particularly whether it can be controlled. The resistant insects are selected in the laboratory with phosphine to produce a strain homozygous for the resistance genes involved. This allows the full potential of the resistance to be measured. Resistance is best characterised by exposing mixed-age populations living in grain to concentration and exposure periods that mimic real-world fumigations. To do this, we use a flow-through technique modified from one developed by Winks and

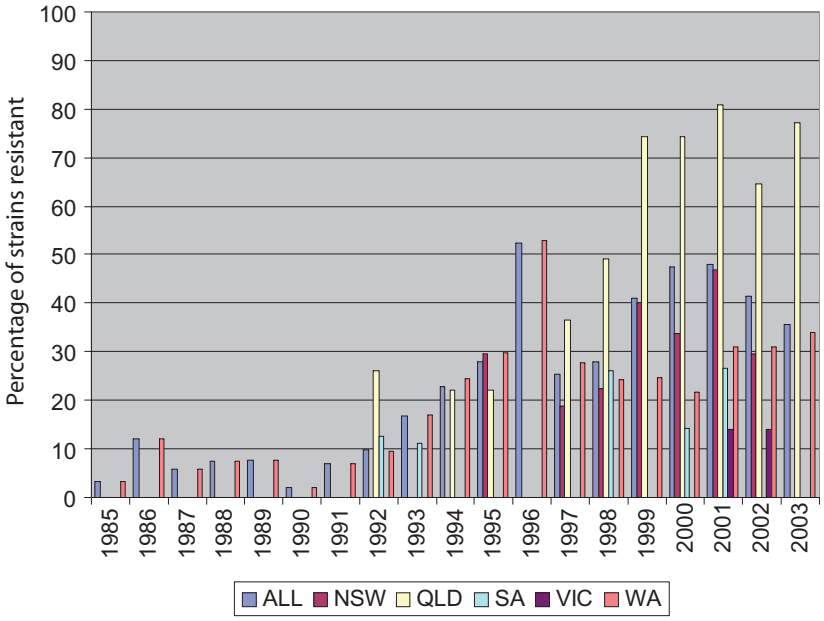

Figure 5. Percentage of strains containing insects with weak resistance to phosphine by state, 1985–2003 (to June 2003).

Hyne (1997). This method applies a constant concentration of phosphine in air to the test population. Results are expressed as time to population extinction at a particular concentration. We have used this method to devise fumigation protocols necessary to control strongly resistant *Rhyzopertha dominica* (Collins et al. 2002). Unlike many other insecticides, resistance to phosphine can usually be overcome with either longer exposure periods or higher concentrations or a combination of both. Proposed concentrations and exposure periods are then tested in field trials under commercial fumigation conditions (Bridgeman et al. 2002a).

Field action

In addition to laboratory work, other initiatives have been undertaken to combat resistance. In WA, weak resistance is managed by reporting results directly to the collecting field officer who then provides extension information and advice if the strain is considered to be a threat.

Strong resistance is still relatively localised, so that eradication can usually be implemented urgently before the infested bulk is moved to further sites or placed into the market. If the sample comes from a bulk handling company, then the company will generally take immediate action. This includes turning the grain into another silo

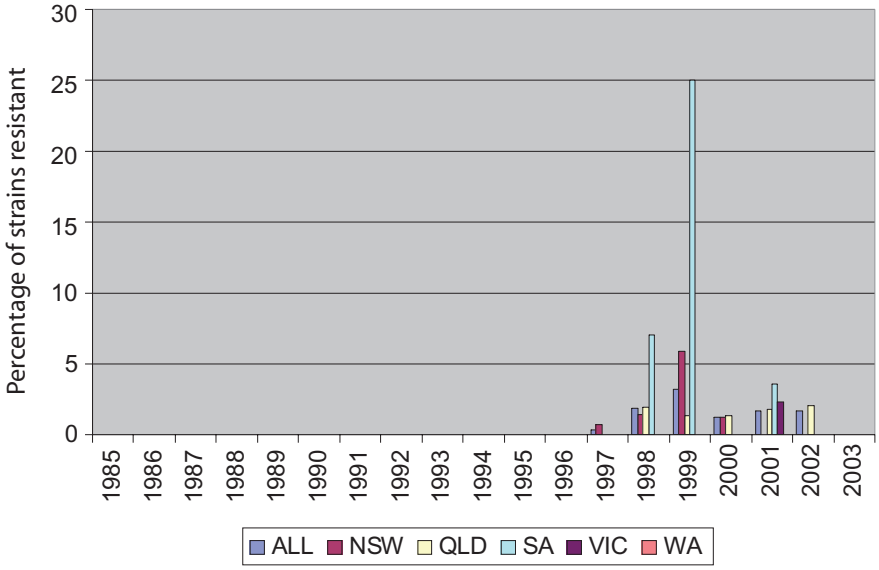

Figure 6. Percentage of strains containing insects with strong resistance to phosphine by state, 1985–2003. (Notes: 1999 figure for South Australia was for four samples only; 2003 data to June only.)

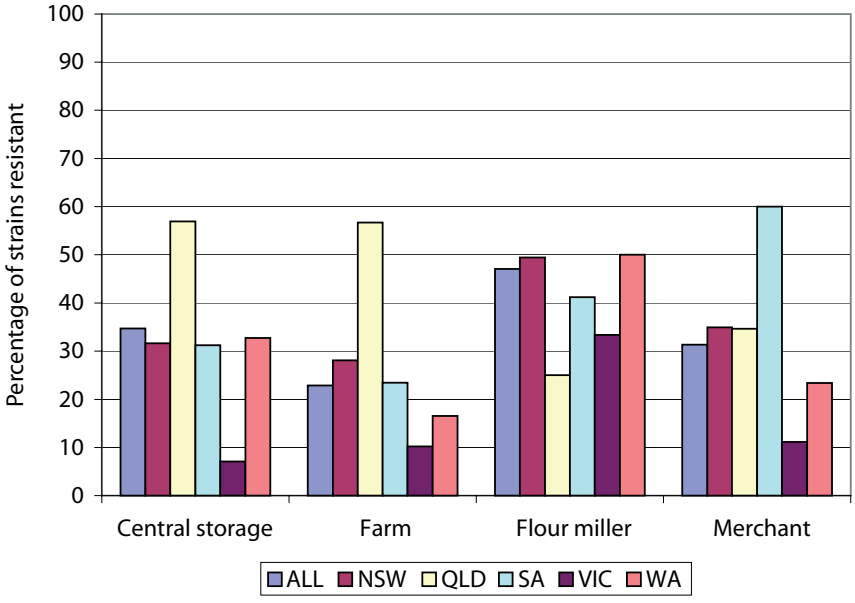

Figure 7. Percentage of strains containing insects with weak resistance to phosphine by industry sector and state, 1985–2003.

and treating it with an effective grain protectant. The empty storage bins are then subjected to detailed cleaning protocols and treated with a residual chemical such as azamethifos (Bridgeman et al. 2002b; Wallbank and Farrell 2002). In some cases, phosphine application equipment that fails to deliver appropriate doses is replaced with better equipment (Bridgeman et al. 2002b). Eradicating resistant populations from farm silos or grain merchant premises is more difficult. In the past, all buildings may have been fumigated with methyl bromide on a farm where resistance was detected (Dean 1994). More recently, where strong resistance is discovered, farmers are advised personally by entomologists of the severity of the situation and offered assistance with treatment of storages and equipment. Most farmers will then willingly cooperate in eliminating the infestation—usually by treating the grain with dichlorvos and grain protectants. Eradication appears to be successful in almost all cases, since we have been unable to detect resistant insects in follow-up sampling of storages.

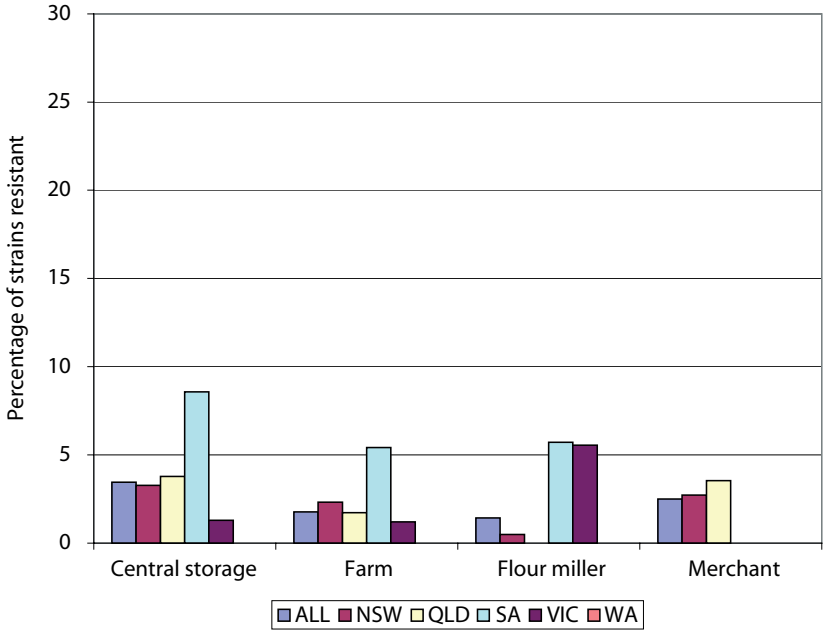

Figure 8. Percentage of strains containing insects with strong resistance to phosphine by industry sector and state, 1985–2003.

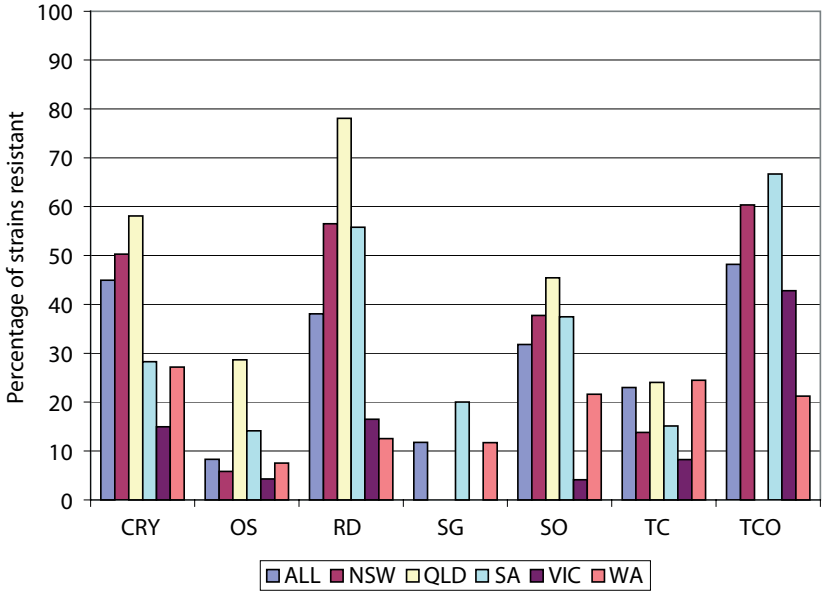

Figure 9. Percentage of strains containing insects with weak resistance to phosphine by species and state, 1985–2003 (CRY = *Cryptolestes* sp., OS = *Oryzaephilus surinamensis* (L.), RD = *Rhyzopertha dominica* (F.), SG = *Sitophilus granarius* (L.), SO = *S. oryzae* (L.), TC = *Tribolium castaneum* (Herbst), TCO = *T. confusum* J. du Val).

The future of phosphine resistance monitoring is at a turning point. Current bioassay methods used to monitor resistance to phosphine have several disadvantages. The standard FAO method (FAO 1975) requires more than two weeks before an answer is available and action can be taken. This does not include time to culture the insects before testing. Moreover, if a significant resistance is detected, then confirmation tests are required. These are usually undertaken on subsequent generations, resulting in delays of a further two months at least. Although resistance types can be separated using discriminating doses, it is not possible to distinguish intermediate genotypes (Collins et al. 2002).

The 'rapid assay' method attempts to reduce the time constraint. This method is extensively used in WA because this state is in a position to take immediate action, given the network of nearly 100 field protection/extension officers, and to delay result reporting would be a waste of this significant resource. There are, however, problems with the method and we have found that it is difficult to use with some species and, although resistance and susceptibility can be clearly separated, it is not possible to distinguish resistance levels or types (Daglish and Collins 1999).

Recent advances in deoxyribonucleic acid (DNA) technology have provided the tools to develop new

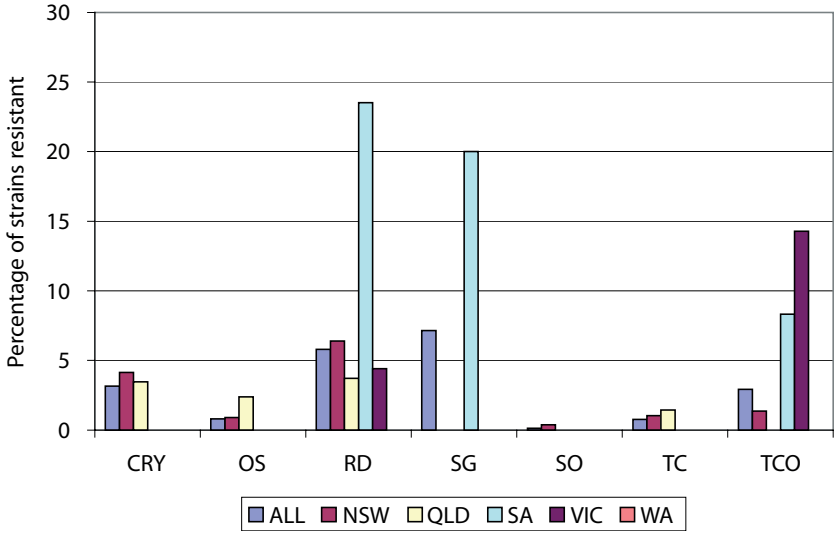

Figure 10. Percentage of strains containing insects with strong resistance to phosphine by species and state, 1985–2003 (CRY = *Cryptolestes* sp., OS = *Oryzaephilus surinamensis*, RD = *Rhyzopertha dominica*, SG = *Sitophilus granarius*, SO = *S. oryzae*, TC = *Tribolium castaneum*, TCO = *T. confusum*).

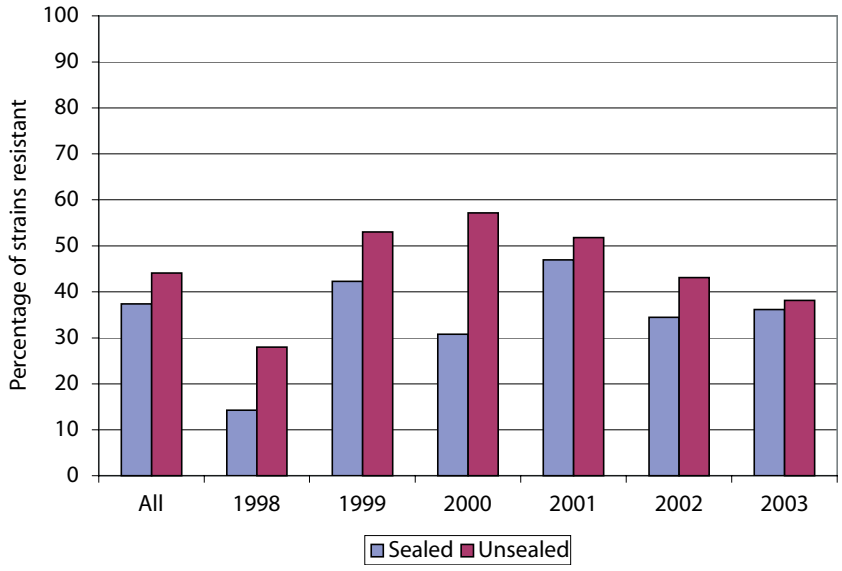

Figure 11. Percentage of strains containing insects with weak resistance to phosphine collected from sealed and unsealed storages (sealed storages are defined as those that were purchased as sealed).

approaches to resistance monitoring. Resistance genes can be mapped using DNA markers. In this way, strong resistance to phosphine in *Rhyzopertha dominica* has been determined to be controlled by two genes which arose independently (Schlipalius et al. 2002). Once a marker can be identified that is always linked to the primary resistance gene, then resistance can be very rapidly and precisely diagnosed using the polymerase chain reaction (PCR) technique. In addition, once the gene is identified, a biochemical test can be developed specifically for that resistance. This may lead to the development of an 'on-site' test. The potential is that decisions could be made about fumigation treatment regimes on the spot where this is not possible currently.

Conclusions

With support from the Australian grain industry, the current insect resistance-monitoring program has successfully drawn together the resources of three laboratories to ensure an effective system for combating resistance. The integrity of the program is underpinned by common assay methods, inter-lab confirmation of results, good communication, and sharing of information through the Australian Grain Insect Resistance Database.

The ultimate aim of this program is to extend the useful life of phosphine. The best way to do this is to have effective early warning systems in place to enable a strong response when commercially damaging resistances appear. The program has made possible a 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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