Resistance considerations for choosing protectants

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Abstract
Resistance has been a major constraint on the continued success of many grain protectants. Only a restricted number of these chemicals are available commercially and none will control all resistant strains of all pest species. As a consequence, potential resistance development should be considered when choosing protectants for new applications or when replacing obsolete materials. Key steps in this process include the accurate identification of the pest species complex, and an understanding of known resistance and cross-resistance patterns and the factors that may influence the development of resistance. In addition, the effective exploitation of grain protectants requires an efficient resistance monitoring program, the early introduction of practical strategies to delay the development of resistance and the formulation of procedures to respond to resistance if it occurs.

A number of resistances have occurred in major pests in Australia and elsewhere. Resistance to malathion is widespread and has been detected in many species. Resistance to other organophosphates strong enough to cause control failures occurs in Rhizopertha dominica and Oryzaephilus surinamensis. Significant pyrethroid resistance has evolved in R. dominica, Tribolium castaneum, Sitophilus oryzae and S. zeamais. Other important resistances include carbaryl resistance in R. dominica, general lindane resistance, and resistance to Bacillus thuringiensis formulations in Lepidoptera. Routine monitoring is essential to determine if resistance is implicated in control failures. Early detection allows time to respond appropriately to incipient resistance problems.

How rapidly resistance develops depends on a number of factors. Most important of these are the insect species involved, and the characteristics of the chemical and its use pattern.

Although various chemical solutions have been suggested, such as alternating or rotating protectants, it is more likely that resistance can be delayed using methods aimed at reducing selection. These methods include use of alternatives to protectants (such as amorphous silica), assiduous hygiene and cooling protectant-treated grain.

Once resistance has been detected the next step is to determine its significance. This is most effectively done by exposing homozygous resistant insects to protectant-treated grain. These assays can also be used to evaluate potential alternative chemicals. Finally, it is important that a range of alternatives, acceptable to registration authorities, be available.

Patterns of Resistance
In Australia the intensive use of protectants for many years to control a range of cosmopolitan pests, coupled with a warm climate, has resulted in numerous resistance problems, some of which have not occurred elsewhere. Much of the following outline of resistance is based on this experience.

Organophosphorous chemicals
Chemicals belonging to the organophosphorous (OP) group have been the most successful and versatile grain protectants. Control failures due to resistance, however, have occurred with most OPs currently used as protectants. Malathion was intensively used until high level resistance was detected in many species (Champ and Dyte 1976). Despite widespread resistance, however, this chemical is still used in many areas. High level resistance to malathion is limited to compounds containing carboxylester groups so that other OP's are still effective against 'malathion-specific' resistant insects. A second resistance to malathion, 'malathion non-specific' resistance or 'multi-resistance' also occurs in many stored products pest species. In most species, this resistance is weak compared with malathion-specific resistance and, as the name implies, resistance extends to a range of OPs, carbamates, DDT (Champ and Campbell-Brown 1970) and juvenile hormone analogues (Dyte 1972). Multi-resistance will not cause control failures with organophosphorous grain protectants such as fenithion, pirimiphos-methyl or chlorpyrifos-methyl. It may be important, in some species, however, where underdosing occurs, for example with Tribolium castaneum (White 1988). An exception to this general pattern is Rhizopertha dominica. Multi-resistant strains of this species are not effectively controlled by OPs so that alternative chemicals such as a pyrethroid must be used.

Resistance to OPs other than malathion-specific and multi-resistance include resistance in Sitophilus oryzae and Oryzaephilus surinamensis. Laboratory assays indicate that OP-resistant S. oryzae should not be controlled by protectants such as fenithion or chlorpyrifos-methyl (Collins et al. 1993) but experience in Australia where this insect is a major pest on farms shows that it only rarely causes control failures in OP-protected bulk grain. These are always associated with patches of underdosed grain. A very high resistance discovered in Western Australia and maintained in the laboratory as strain CS0231 has not been detected in the field since.

Species Identification
Accurate identification is a fundamental requirement in the choice of protectants and in the effective management of resistance. Species vary in their natural tolerances and in their response to selection. Special care must be taken when identifying closely related species such as the Cryptolestes, Tribolium, Sitophilus groups and others.

In addition, to the untrained observer, grain infesting insects all look much alike. Non-specialists need training and clear taxonomic aids such as illustrated keys and computer-aided learning facilities to make accurate identifications. Decisions about appropriate protectants to use where resistance is suspected require accurate identification of infesting insects.

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Strong resistance to fenitrothion, chlorpyrifos-methyl (Collins et al. 1993), pirimiphos-methyl (Collins, unpublished data; Wallbank, unpublished data), and resistance to etrimphos and methacrifos (Muggleton et al. 1991) have been recorded in *O. surinamensis*. There is some evidence that at least two resistance mechanisms are involved (Wallbank and Rose 1987; Collins et al. 1993). There appears to be a link between chlorpyrifos-methyl and etrimfos resistance while resistance to malathion seemed to be linked to pirimiphos-methyl, etrimphos and fenithrothion resistance and resistance to methacrifos seemed to be separate (Muggleton et al. 1991). Herron et al (1994), however, found no link between pirimiphos-methyl and fenithrothion resistance in their survey.

Because of its high vapour pressure dichlorvos is an important disinfestant. Resistance to this material is fairly widespread in *R. dominica* in parts of Australia. Resistance is strong enough to make control incomplete with this chemical. Dichlorvos resistance may be a result of the malathion non-specific or ‘multi-resistance’.

**Pyrethrins and pyrethroid insecticides**

Resistance to pyrethrins has been detected in *Sitophilus oryzae*, *S. granarius* (Champ and Dyte 1976), and *Ephesia cautella* (Zettler et al. 1973) and we recently detected resistance to this material in *R. dominica* in Australia (Collins and Mackay, unpublished data). Pyrethroid resistance was detected after about 12 years use of biorethemethrin/piperonyl butoxide mixture specifically to control multi-resistance *R. dominica* (Collins et al. 1993). Resistance to pyrethrins appears to be independent of the resistance to biorethemethrin in *R. dominica*. Biorethemethrin resistance extends to other pyrethrins such as phenothrin, fenvalerate and deltamethrin but not to other chemical groups (Collins and Lambkin, unpublished data). Pyrethroid resistance also occurred in *Tribolium castaneum* (Collins 1990). This resistance includes other pyrethrins particularly the α-cyano containing materials deltamethrin, cyfluthrin and cypermethrin. Once again resistance did not extend to the OPs. Weak resistance to pyrethroids has been detected in several species (Champ 1986; Lloyd and Ruczkowski 1980) and may be a result of the multi-resistance mechanism.

Heather (1986) reported high resistance to pyrethroids in a laboratory strain of *S. oryzae*. This was probably a result of previous selection with DDT. It demonstrates, however, that this species has the potential to evolve resistance to these materials. Significant resistance to deltamethrin was also detected in *S. zeamais* from northern Australia (P. Samson, unpublished data).

**Other protectant groups**

Metolphene has been in use in Australia for 2–3 years and there has been no resistance detected. It is effective against pyrethroid and OP-resistant *R. dominica*. OP and pyrethroid-resistant *T. castaneum* and OP-resistant *O. surinamensis*.

Carbaryl has been used for many years to control *R. dominica* on feed grain. Resistance to this material in *R. dominica* results in a reduced protection period.

Lindane still has some use as a seed protectant. Resistance was recorded in most of the world’s important pest species by the time of the FAO world survey of resistance (FAO 1974) and in many minor species since (Champ 1986).

Resistance to the synergist piperonyl butoxide has occurred in *T. castaneum* (Rowlands and Dyte 1979) and in *R. dominica* (Collins and Lambkin, unpublished data).

Resistance to the biological insecticide *Bacillus thuringiensis* is unexpectedly frequent in the pyralid *Plodia interpunctella* (McGaughhey 1985) and can occur in other moth pests (McGaughhey and Beeman 1988).

**Resistance Detection and Monitoring**

Usually, the first sign of resistance to a grain protectant is a reduction in the period of protection previously enjoyed. Resistance, however, is only one of the possible causes of control failure with these materials. More often than not failures are caused by other reasons, such as mechanical failure of application equipment, incorrect dilution, inadequate mixing, degradation of chemicals during storage or after mixing, or temperature effects on pesticide decay. A control failure may be a result of more than one factor, one of which may be resistance. Therefore, routine testing of insects associated with control failures is essential to determine if resistance is implicated. Moreover, early detection gives the industry time to respond appropriately to an incipient resistance problem with minimal economic loss.

The standard insecticide susceptibility test method for stored products Coleoptera involves exposure of insects to filter-papers impregnated with technical grade insecticide in oil. This method, published by FAO (FAO 1974), is used extensively with a wide number of insecticides (Champ 1986; Taylor 1991) and gives relatively rapid and reliable indications of changes in response to the test chemical. Standard tests for resistance in larval and adult moths have also been published (Busvine 1980). Both these standard tests recommend use of a discriminating dose based on the 99.9% response level of insecticide-susceptible laboratory strains. The use of the discriminating dose technique is the most efficient way to process samples when monitoring for resistance (Roush and Miller 1986), but it is a mistake to use published discriminating concentrations without verification in local conditions. Experience in Australia with several laboratories testing for resistance is that, although superficially objective, these tests are in reality subject to human interpretation of response criteria. Therefore, each laboratory undertaking resistance monitoring should develop its own discriminating doses. These should be developed, initially, from known susceptible strains. If necessary these can be imported. However, tolerance to insecticides can vary significantly from sample to sample and through generations in normal, susceptible populations. Some reports of low level resistance are, on further investigation, due to variations in tolerance of normal, susceptible populations. Thus, it is important to also test local strains that have recently been collected from the field over several generations.

Many field populations will have had contact with insecticides and some resistant insects will be present. These insects may have some weak cross-resistance to new compounds as a result of previous selection. For example *T. castaneum* in Australia shows some resistance to fenithrothion probably as a result of previous selection by malathion of the multi-resistance mechanism. At concentrations used in the field fenithrothion, overcomes this resistance mechanism. However, discriminating doses of fenithrothion developed from reference susceptible strains will score these insects as resistant to fenithrothion even though this result is meaningless in the field. Thus, when setting up a monitoring program, current levels of resistance occurring in field populations should be investigated and discriminating doses based on these results should be established. The purpose of monitoring, in this case, is to look for new resistance. This cannot occur unless old resistances are adequately characterised and taken into account.

The major advantage of establishing discriminating doses is that a large number of individual insects can be tested quickly and economically. For an accurate diagnosis of resistance,
insect population samples that fail a discriminating dose test should be submitted to a full bioassay for confirmation (FAO 1974). Despite its advantages the standard impregnated-paper assay (IPA) has a major drawback — it bears no direct relation to the use of protectants in the field. Thus it cannot indicate the importance of a resistance for control of insects. To assess the practical importance of a resistance insects must be exposed to grain treated with commercially formulated insecticides. Correlating the response of insects in impregnated-paper assays against their response in protectant treated grain assays can also give insights into the practical significance of results from IPAs. Using this method we have provisionally developed IPA discriminating doses for O. surinamensis that will predict the response of various resistant strains to chlorpyrifos-methyl admixed with grain (Fig. 1).

Finally, the results of discriminating dose assays should always be regarded as provisional. The more resistance testing that is undertaken the more variation in response in insect samples will be detected so that in some cases discriminating doses have to be revised upwards to minimise the problem of ‘false positives’.

![Graph showing correlation between LD99.9 values and impregnated-paper assay percentages.](image)

**Fig. 1.** Correlation between LD99.9 values obtained from impregnated-paper and grain assays of various resistant population samples of *Oryzaephilus surinamensis* against chlorpyrifos-methyl. Each pair of assays was performed on the same generation of insects. Correlation coefficient $r = 0.83$. Equation of line of best fit: $\log(y) = 2.00928 \times \log(x) - 0.15185$.

**Factors Effecting the Development of Resistance**

The very property of grain protectants that makes them so effective also contributes significantly to the rapid development of resistance in grain insect pests. Grain protectants are admixed with grain as it is augured into storage. Chemical is admixed initially at a high concentration so that the grain is protected from insect infestation over an extended period. From the beginning of the storage period to its conclusion protectant residues are declining. Furthermore, application is uneven and there are often pockets of underdosed grain. Thus, not only are insects exposed to the insecticide over long period of time they are also exposed to a range of concentrations. In many areas chemicals are also used to treat storages after the grain has been removed. Consequently, insects are continuously exposed to various levels of chemical concentrations over a period of many generations. These conditions are ideal for selecting resistance. This problem is compounded when insect populations are treated several times with protectants. For example, extensive research in Western Australia (Moulden 1987) showed that malathion resistance was selected in several species of grain pests on farms before it was detected in central storages. Similarly, chlorpyrifos-methyl resistance was present in half the *O. surinamensis* population samples collected from central storages in Queensland although this material had rarely been used in these silos (Collins et al. 1993). In both cases the extensive use of a protectant on farms jeopardised the efficacy of this material in central storage.

In addition to the operational use of protectants, other factors including agronomic practices, climate and genetics may contribute significantly to selection of resistance. The importance of these factors varies from species to species.

Agronomic practices are considered to be important in the development of resistance to OPs in *O. surinamensis*. This insect occurs on farms throughout the grain-growing regions of Australia. Populations of *O. surinamensis* have developed resistance to OP insecticides in most areas, but it is particularly high and more frequent in southern New South Wales and north-western Victoria (Herron 1990; Wallbank, unpublished data; Collins and Mackay, unpublished data) than in other regions. Farmers in these districts store grain, notably oats for stock feed, on their properties for relatively long periods. This grain is usually treated with a protectant, and surveys have shown that *O. surinamensis* is particularly common in these farm silos (Herron 1990; Price et al. 1988). This insect is much less common in regions where less grain is stored on-farm and oats are only a minor crop (Herron 1990; Sinclair 1982; Collins 1985). Figures 2 and 3 show the distribution of resistance to fenitrothion and chlorpyrifos-methyl resistance, respectively, in *O. surinamensis* in southeastern Australia.

Climate can also be a significant factor. A recent illustration of the importance of climate is the occurrence of resistance to biocresmethrin in *R. dominica* in 1990 in Australia (Collins et al. 1993). Despite the extensive use of this material in grain storages in much of eastern Australia to control OP-resistant *R. dominica*, resistance to biocresmethrin has been detected only in Queensland (Fig. 4) (Collins et al. 1993; Collins and Mackay, unpublished data; Wallbank, unpublished data). The major reason for the geographical distribution of resistance appears to be the climate. Central Queensland, where resistance was first detected, is the hottest section of the eastern grain growing belt. Grain is routinely stored at $\geq 30^\circ C$ and it remains at this temperature for several months longer than in southern Queensland. In contrast, grain temperatures of $25^\circ C$ are more typical in the southerly part of the grain belt. The higher temperatures in the north favour rapid development and increased reproductive rate in *R. dominica* (Birch 1953) and stimulate migration and flight of adults (Sinclair and Hadddrell 1985).

Genetic factors are also significant in determining the rate of development of resistance. For example, the relatively high initial frequency of pyrethroid resistance gene in *T. castaneum* (Collins, unpublished data) meant that resistance to cyfluthrin arose rapidly and precluded the use of pyrethroids against this species in Australia (Collins 1990). On the other hand, the major fitness disadvantage associated with this resistance (Collins, unpublished data) opens up the possible use of these protectants either in mixtures with, for example, OPs or in predetermined rotations.
Is it Possible to Manage Resistance to Protectants?

The concept of long-term chemical protection and the requirement for nil insects in the grain limit the practical implementation of many potential resistance management options (Collins 1991).

Despite this situation, some positive steps can be taken in an attempt to delay resistance development. The most important of these are reducing insecticide use and minimising the numbers of insects exposed to the insecticide. Both these measures are aimed at reducing selection. Finally, alternative approaches to the use of protectants that might extend their effective life should be considered. Are these chemicals, for example, best used in sequence, as mixtures or rotated?

Reducing selection

The more often a protectant is used the more insects are exposed to selection and, consequently, the more likely that resistance will be selected. A break in the year round exposure of insects to insecticides has been achieved in the central grain handling system in Queensland by replacing residual spraying of storage structures with amorphous silica treatment (Bridge-man and Collins, these proceedings).

The numbers of insects exposed to selection will also influence the rate at which resistance can develop. Therefore, assiduous hygiene should be a cornerstone of resistance management strategy. Grain storage hygiene refers to the removal of all grain residues and grain particles from inside and around silos. This minimises the likelihood that insects will survive between storage seasons thus preventing the continuous selection of the same insect population resident in a silo. Recently, Herron et al. (1994) found that highest levels of resistance in *O. surinamensis* occurred on farms with poorest hygiene.

A third method of reducing selection is the use of protectants on grain cooled by aeration (Longstaff and Desmarchelie 1983). The value of cooling protectant-treated grain is that the number of generations an insect population may pass through in the grain is minimised. This greatly reduces the resistance gene frequency at the end of the storage period (Longstaff 1988) thus avoiding control failures.
Alternative chemical approaches

In general, unless other factors intervene, grain protectants are used year after year until resistance necessitates a change. For example, the development of resistance to malathion in *T. castaneum* and *R. dominica* prompted an intensive search for possible alternatives to this chemical. In Australia most bulk-handling organisations replaced malathion with fenitrothion plus synergised bioresmethrin. Subsequently, chlorpyrifos-methyl was used in storages with a history of fenitrothion-resistant *O. surinamensis*.

This sequential use of protectants raises the question, however, as to whether this is the most appropriate way to use these materials. There has been wide discussion in the literature on the merits of using two or more chemicals in a predetermined pattern, such as in a mixture, a rotation in time or in a mosaic (Comins 1986, Leeper et al. 1986; Roush 1989; Tabashnik 1989). Computer simulation models show that for these alternative approaches to be successful two preconditions must be met. First, the mechanisms of resistance that develop to each of the components of a chemical strategy should be different and independent (i.e. no cross-resistance). Second, the frequency of resistance genes in target populations must be low and they should not occur together in the same individual. Each tactic relies, furthermore, on its own set of assumptions. For example, rotation strategies require that individuals resistant to a particular insecticide have fitness substantially lower than susceptibles, so that the frequency of resistance declines between applications of that chemical. To be successful rotation strategies also should be co-ordinated over a large area so that insects functionally belonging to the same gene pool are not simultaneously selected for resistance to the different pesticides used (Roush 1989). To many people, rotating insecticides seems intuitively better than using chemicals in a sequence. Computer simulation studies, however, do not support this. Curtis (1987), Curtis et al. (1993) and Roush (1989) concluded that, unless there were large fitness differences associated with resistance, then rotations were no better at delaying resistance than using the same chemicals in sequence. Because they are no worse than sequences, however, rotations, may have some practical advantages. For example, it is much easier to get chemical company representatives to agree to their product being used in a rotation than being held back to be used only when another chemical fails.

Mixture strategies also have special requirements. To be successful each component of a mixture must have the same decay rate and be equally effective (Roush 1989). In addition, mixtures have the greatest chance of delaying resistance if both resistance genes are effectively recessive, i.e. that only resistant homozygotes survive an insecticide treatment (Tabashnik 1989). Simulation models show that violation of any of these assumptions and the general preconditions mentioned earlier will result in the development of resistance.

Mosaics involve the separation of chemicals in space. The aim is to avoid selection for the same resistance mechanism by treating adjacent regions simultaneously with different insecticides (Georgiou 1983; Tabashnik 1990). There is little experimental support for this tactic (Tabashnik 1989) and it is not well supported by simulation modelling (Curtis 1985).

Space precludes an in-depth analysis of each tactic as it relates to particular storage pest complexes but it is obvious that it is very difficult to meet the conditions required for the success of the various tactics. Nevertheless, some rotations or mixtures may be successful in particular circumstances for
individual pests. In general, however, there is no evidence that tactics such as rotations and mixtures are superior to a rational use of protectants in sequence. Significant progress in delaying the development of resistance is therefore more likely to arise from the implementation of non-chemical pest management techniques discussed in the previous section.

**Responding to Resistance**

Once resistance has been detected the next step is to determine its significance. This is most effectively done by exposing test insects, preferably homozygous for the resistance in question, to protectant-treated grain in the laboratory. Homozygosity of resistance allows the full potential of a resistance to be measured. Treated grain assays simulate application of protectants in the field as closely as is feasible in the laboratory. These assays allow estimation of adult mortality and suppression of progeny development and give a reasonable indication of field doses involved. A new resistance may or may not jeopardise field control. If it has been established that a new resistance is strong enough to be of concern then an alternative protectant will need to be found. Treated grain assays can be used to assess the efficacy of potential replacement compounds against the resistant strain, and a recommendation made. The implementation in the field of recommendations from the laboratory requires that suitable protectants, acceptable to registration authorities, be available. This presupposes the existence of either a private or publicly funded program for development of protectants that has provided feasible alternatives.

**Conclusions**

Choice of appropriate protectants is best made on the basis of the history of chemical use in a particular region. Much can be learned also from the experiences of countries such as Australia where protectants have had extensive use and where episodes of resistance have been documented. Whatever protectant regime is in place, however, it is likely that resistance will be encountered. Therefore a program aimed at minimising the impact of resistance must be in place before resistance occurs. Such a program should include a resistance monitoring function and the ability to study cross-resistance patterns and research pest management tactics. Finally, despite the inherent problems associated with the use of grain protectants, there are some positive steps that can be taken that will prolong the useful life of these materials.

**References**


