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Recent changes in resistance to grain protectants in eastern Australia

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Abstract. A widespread survey of grain storages in eastern Australia was undertaken to assess the extent and severity of resistance to grain protectants and fumigants. Insects were collected from many types of premises and screened for resistance to a number of registered protectants by standard assays. Strong resistance to methoprene increased markedly in *Rhyzopertha dominica* in northern NSW, rising from 2% of samples in 1997 to about 26% in 2002. Some resistant strains have been able to breed in grain treated with methoprene at up to four times the normal rate, with only limited suppression. A similar trend was found in Queensland. In the same period, *Sitophilus oryzae* with a high level of resistance to chlorpyrifos-methyl emerged at several sites in NSW and Queensland, but no new sites have been detected since 2001. These surveys have successfully provided the grain industry with early warning of resistance developments so that resistance can be managed and losses minimised. Despite the continuous emergence of new resistances, grain protectants have remained an effective pest management tool.

Introduction

Resistance to grain protectants in Australia was first detected in 1968 when *Tribolium castaneum* (Herbst), the rust-red flour beetle, from a peanut storage was diagnosed with resistance to malathion (Champ and Campbell-Brown 1970). Other resistances were reported over the next two decades, notably to dichlorvos and other organophosphates (OPs) in *Rhyzopertha dominica* (F.), the lesser grain borer, and to fenitrothion in *Oryzaephilus surinamensis* (L.), the sawtoothed grain beetle (Greening et al. 1974; Heather and Wilson 1983). Much of the wheat harvest in eastern Australia was treated with grain protectants at this time—at first with malathion but subsequently with a mixture of synergised bioresmethrin and either fenitrothion or chlorpyrifos-methyl or pirimiphos-methyl (Bengston et al. 1980; Desmarchelier et al. 1987). These products were widely used for 15–20 years in eastern Australia—the pyrethroid component being effective against *R. dominica*, and the OP component against most other species.

A national resistance survey was established in 1996 to assess the extent and severity of resistance that had emerged to fumigants and protectants (Collins et al. 2003). This survey integrated previous surveys undertaken independently by various government bodies. Significant pyrethroid resistance had emerged in *R. dominica* in southern Queensland in 1990 (Collins et al. 1993), and subsequently appeared at several other sites north of a latitude of about 30°S (Wallbank et al. 2002). The insect growth regulator methoprene was utilised as an alternative and found to be highly effective against *R. dominica*, as well as most other species except *Sitophilus oryzae* (L.), rice weevil. Nevertheless, there were limited alternative options should resistance to methoprene develop in *R. dominica* or to OPs in other species.

This paper details resistance testing for *R. dominica* and *S. oryzae* collected from a wide range of premises in eastern Australia over the last 10 years.

Materials and methods

Sample collection

Insect infestations were sampled from farm storages of many types, central bulk storages, flourmills, stockfeed producers, grain merchants, feedlots and similar premises. The survey extended generally south from central Queensland through grain-growing areas of NSW and Victoria to Ceduna, SA. Farm storages ranged from small holding bins to large, well-constructed silo complexes of several thousand tonnes capacity. Bulk storages and commercial premises included bunkers, a range of horizontal storages to 80,000 t capacity, and vertical concrete silos up to 2500 t per cell. Grain was taken from the bulk surface or at silo outlets, and was sieved to separate any adult insects. Infestations within

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the central storage system were usually collected from probe traps (Agrisense-BCS Ltd, UK) placed continuously in the bulk, or from grain during bin turning or out-loading. Warehouses, flourmills and other holding areas were monitored with traps of corrugated cardboard baited with flour.

Rearing conditions

The species of interest in this paper are the whole-grain feeders *R. dominica* and *S. oryzae*. Insects were identified and reared to about 400 insects of F_1 or later generations in whole wheat conditioned to 12.5% moisture content. Small numbers of field parents were usually not removed before the emergence of progeny. The rearing conditions were maintained at approximately 26°C and 60% relative humidity (RH).

Resistance testing

Resistance was assessed by challenging protectant-treated grain (Collins et al. 1993) with adult *R. dominica* and *S. oryzae* when sufficient numbers were available for testing. For initial screening, 50 adults were placed in two replicates of 100 g treated grain and allowed to oviposit for 10 days. Parents were then removed and assessed for mortality, and the resulting progeny, if any, were allowed to develop for 8 weeks before counting. The level of mortality and progeny suppression was compared with that from a single replicate of untreated grain. This procedure was part of a wider resistance screen and was intended to identify obvious cases of resistance for more detailed examination of threat status. Our surveys reported here cover methoprene resistance in *R. dominica*, and resistance to fenitrothion, chlorpyrifos-methyl and pirimiphos-methyl in *S. oryzae* from 1996 to 2002 in eastern and southern mainland Australia.

The concentrations of insecticide that were used to indicate resistance were based on the lowest rate of the registered label of the insecticide (methoprene), or on 80% of this rate (OPs). Label rates claim to provide at least three months control of susceptible strains; hence failure at this rate would give prima facie evidence of resistance. This method was chosen instead of formal discriminating doses based on 99% (or other level) of control since our monitoring program was designed to detect commercially significant resistances. Strains with low resistance levels may not have been detected. The 80% lowering of concentration was adopted to allow for losses in spray drift or bounce-back, and is a typical figure for normal spray applications. Wheat (2–8 kg) was gently tumbled in a rotating stainless-steel bowl while calculated amounts of diluted formulations were slowly added to achieve the desired concentration and final moisture content of 12.5%. Grain was allowed to stand overnight before use, or was frozen at –10°C for later use. Higher rates to 4 g/t (methoprene) or 50 g/t (OPs) were used when assessing the level of resistance reached by resistant strains.

Resistance assessment

Since methoprene has little effect on adult insects, resistance to methoprene in *R. dominica* was assessed from the number of adult F_1 progeny produced in treated wheat. Preliminary tests indicated that small numbers of progeny might develop in individual grains that may not have received complete coverage with insecticide. To avoid false positives, a minimum of 10 progeny was set as the criterion for distinguishing resistant strains from those with marginal or negligible resistance (Table 1). This level of control was equivalent to 97% suppression of progeny when compared with the numbers that developed on average in untreated wheat. In the case of *S. oryzae* and OPs, similar criteria were used, although some indication of resistance could be deduced from the number of adult parents surviving after the oviposition period.

Table 1. Criteria for diagnosis of resistance to methoprene in *Rhyzopertha dominica* using grain assays.

Diagnosis	F_1 progeny per replicate	Progeny suppression[a] (%)
Susceptible	0	100
Negligible	1,2	>99
Marginal	3–10	97–99
Resistant	>10	<97
High resistance	>50	<82

[a] Compared with the average number of progeny produced in untreated wheat (330).

Results

R. dominica

About 83% of all strains of *R. dominica* tested were diagnosed susceptible or with negligible resistance to methoprene, and a further 8% were diagnosed with marginal resistance. Resistant strains as defined above were first detected in 1997 and increased in frequency rapidly in 2001 and 2002 (Figure 1). A similar increasing trend was seen with strains in which progeny in treated grain exceeded parent numbers, particularly after 1998. Many of these latter strains were poorly controlled in grain treated with the full label rate of methoprene intended for nine months control, and were still incompletely controlled at 4.0 g/t (Table 2).

S. oryzae

About 94% of all strains of *S. oryzae* tested so far have been susceptible to chlorpyrifos-methyl. Resistance was first detected in 1997, and instances of resistance have been found at a low frequency every year since then (Table 3). The resistant strains generally produced several hundred progeny in treated grain, indicating a serious and immediate failure at these sites. Resistance was also detected to fenitrothion. Ten of the 23 (chlorpyrifos-methyl) and 29 (fenitrothion) resistant strains, respectively, were diagnosed with resistance to both OPs. Resistance to pirimiphos-methyl was less common and at much lower levels. One strain with resistance to all three OPs was controlled (F_1 < 10) by pirimiphos-methyl at 8 g/t, or fenitrothion at 20 g/t, but was not controlled to this level by chlorpyrifos-methyl at any rate tested up to 50 g/t (Figure 2).

Table 2. Response of highly resistant strains of *Rhyzopertha dominica* to methoprene-treated grain over a 5-year period.

Year	Range of suppression (%) of F_1 progeny			
Rate (g/t)	0.5	1.0	2.0	4.0
1998 (*n* = 1)[a]	64	87	91	98
1999–2002 (*n* = 12)	2–70	31–80	63–81	75–94

[a] Only a single highly resistant strain was detected in 1998. In each of the other years, the three strains with highest resistance were used.

Discussion

Methoprene resistance in *R. dominica* has now been found regularly in northern NSW from latitude 32°S (Dubbo area) to central Queensland, compared with only two localised areas previously identified in 2000 (Wallbank et al. 2002). Isolated instances of resistance further south have also now been identified, including one at latitude 35°S in southern NSW. Three instances at flourmills in Sydney and country NSW were most likely from grain that had been sourced from northern areas of NSW.

About 70% of all Queensland infestations where resistance levels were high (i.e. progeny exceeded parent numbers) originated from farm or merchant samples. Grain protectants are widely used in these sectors of the industry, but not in Queensland central storage, where fumigation is normally used. In NSW, about 80% of samples with similar resistance status were from central storages, but in many cases these were sites that were not amenable to fumigation. Consequently, full rates of methoprene and chlorpyrifos-methyl had been applied at receival for many years. About 25% of these sites recorded persistent infestations up to two years following first detection. This pattern suggests that resistance developed at the site of application, or was exacerbated if insects were received in low numbers from the surrounding area. The Queensland situation, where grain with potentially resistant insects was delivered to central

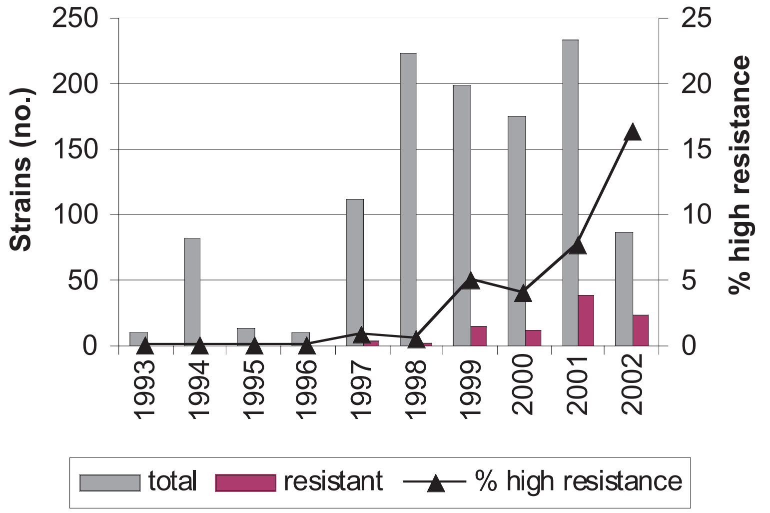

Figure 1. Number of strains of *Rhyzopertha dominica* diagnosed resistant to methoprene (0.5 g/t) or S-methoprene (0.3 g/t) since 1993, and proportion diagnosed with high resistance.

Table 3. Strains of *Sitophilus oryzae* diagnosed with resistance (F1 > 10) to organophosphates.

Year	Chlorpyrifos-methyl (4 g/t)		Fenitrothion (4.8 g/t)		Pirimiphos-methyl (3.2 g/t)	
	number of strains (total)	resistant (%)	number of strains (total)	resistant (%)	number of strains (total)	resistant (%)
1997	3 (32)	9.4	1 (4)	25.0	0 (4)	0.0
1998	2 (104)	1.9	0 (7)	0.0	0 (3)	0.0
1999	6 (96)	6.3	17 (101)	16.8	2 (65)	3.1
2000	6 (68)	8.8	5 (68)	7.4	1 (67)	1.5
2001	5 (58)	8.6	5 (59)	8.5	2 (60)	3.3
2002	1 (23)	4.3	1 (23)	4.3	1 (23)	4.3
All	23 (381)	6.0	29 (262)	11.1	6 (222)	2.7

storage for fumigation rather than treatment with protectants, may have reduced the frequency of resistance in its central system. Approximately similar numbers of borer strains were tested in the two states.

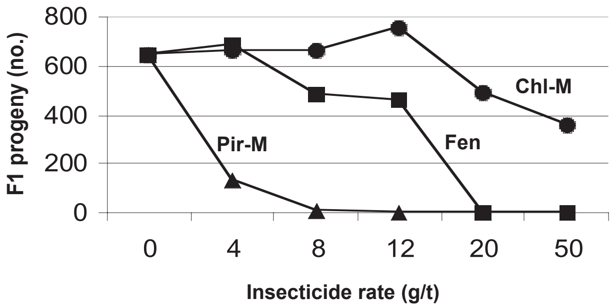

Figure 2. Control of progeny of one strain of *Sitophilus oryzae* with multiple resistance to chlorpyrifos-methyl, fenitrothion, or pirimiphos-methyl at rates up to 50 g/t.

Fewer possibilities for alternative control treatments are now available since synergised bioresmethrin (BRM®) was deregistered in 2002. This pyrethroid had been in use for many years and was generally effective against methoprene-resistant strains. Synergised deltamethrin (K-Obiol®) is registered for use against *R. dominica* on grain but some markets have limited access for treated grain. Pyrethroid resistance is also present throughout the areas where methoprene resistance has been detected, so it is possible that control may not be long-term. However, deltamethrin is intrinsically more potent than bioresmethrin, and may offer a feasible alternative for both types of resistance in the short term (Collins et al. 1993). Natural pyrethrins are also registered for use on grain. Regulatory authorities are currently considering bifenthrin as a component of a protectant mixture, and registration is expected in the near future on some or all cereal grains. Spinosad appears to be particularly promising against a range of eastern Australian strains of *R. dominica* in laboratory and bin trials (Daglish et al., these proceedings; B.E. Wallbank, unpublished results). It is less effective against other species and may need to be mixed with other treatments for full control.

The development of strains of *S. oryzae* with strong resistance to some OPs may have serious consequences for control at sites where grain cannot be fumigated and must be treated with protectants. Current recommendations are for a mixture of a registered OP to be applied together with one of the methoprene formulations. Since methoprene has no effect on *S. oryzae* the grain would virtually be unprotected from attack by OP-resistant strains from the beginning of the storage period. Relatively high numbers of progeny may develop within three months in suitable conditions, and the infestation challenge is likely to be much higher in years of wet harvests or when the moisture content of grain is above average.

The cross-resistance pattern to the three registered OPs appears to be variable, with some strains highly resistant to both fenitrothion and chlorpyrifos-methyl, but susceptible or with marginal resistance to pirimiphos-methyl. This pattern differs from that for *O. surinamensis* in which fenitrothion resistance and pirimiphos-methyl resistance were usually linked, but strong chlorpyrifos-methyl resistance appeared to be separate and did not develop until later (Kotze and Wallbank 1996; Wallbank 1996). It may be possible to identify sites where one particular OP gives better control of *S. oryzae* than either of the others. In one case of triple resistance, grain treated with pirimiphos-methyl at 8 g/t gave almost complete control. This rate is higher than the registered label rate but is within the current maximum residue limit allowed for wheat or sorghum, though not for barley, maize or oats. Further testing of representative strains of *S. oryzae* needs to be done to define whether a feasible rate can be determined that would have widespread effectiveness if approved for use. Testing is also required against synergised deltamethrin, which is currently registered for protection of storage insects including *S. oryzae*.

The use of grain protectants in eastern Australia has declined in the last 20 years from about 80% of the wheat harvest to between 20 and 30%. Although protectants are still used on farms, sometimes widely, their use in central premises is now limited to certain types of storage in NSW and Victoria that are unsuitable to conversion for fumigation. The future effectiveness of remaining protectants, and the potential use of new materials, depends on careful management strategies that minimise the development of resistance. To this end, strategies should avoid exposure of insects to marginal levels of residue caused by poor application techniques. Alternative technologies such as aeration may be used as an adjunct to protectants, and have a double benefit in slowing the rate of decline of residue as well as extending the generation time of insects that may be present. The on-going national resistance survey has shown itself to be invaluable for providing early warning to industry of the emergence of new strains, and hence the ability to plan for changed practices. Although protectant resistance first emerged over 35 years ago, the technology is still in active use and provides an essential part of the process of maintaining grain in good condition during storage.

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