

## Insecticide resistance in the major coleopterous pests of stored grain in southern Queensland

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### Summary

Field strains of *Rhyzopertha dominica*, *Tribolium castaneum* and *Sitophilus oryzae* were collected from central storages, grain merchants and farms. Samples were initially screened with a discriminating dose of insecticide. If this indicated the presence of resistance the insects were subjected to a full dose-mortality bioassay to determine the level of resistance.

Low level resistance to fenitrothion was found in a few populations of *T. castaneum* (x2 to 3) and in many populations of *S. oryzae* (x2 to 4). Low level resistance was found to carbaryl in *R. dominica* (x2 to 4) but there was no resistance to bioresmethrin. Resistance to fenitrothion in *T. castaneum* and *S. oryzae* was not economically significant but as some resistant *R. dominica* will survive in carbaryl treated grain resistance to this chemical will result in an increased proportion of control failures.

Resistant and susceptible reference strains of these three beetle species, reared in the laboratory for several years, were found still to be similar to resistant and susceptible field populations in their response to these insecticides.

### INTRODUCTION

The Australian grain industries rely extensively on the use of residual insecticides (grain protectants) to protect their crop in storage from insect infestation (Champ 1981). In Queensland, an organophosphorothionate, usually fenitrothion, is used to control all beetle pests except multi-resistant strains of the lesser grain borer (*Rhyzopertha dominica* L.). *R. dominica* is controlled by either carbaryl (a carbamate) or bioresmethrin (a pyrethroid). The latter is synergised with piperonyl butoxide.

Although grain protectants are relatively cheap and versatile, their efficacy can be limited by the development of resistance among target insect species. Malathion was introduced in 1960-61 and, because of its great success, by 1964-65 it was used to treat all wheat received into storage (Murray 1979). This compound, however, was replaced in 1976-77 because of malathion-resistance. Also, in that same season, the widespread distribution of multi-resistant strains of *R. dominica* required the introduction of a compound specifically to control *R. dominica*. The incidence of malathion resistance in stored products Coleoptera has been well documented (Champ and Dyte 1976). In relation to current materials, published work includes studies of resistance in the sawtoothed grain beetle (Heather and Wilson 1983; Collins 1985) and extensive cross-resistance studies by Attia (1983).

This study was undertaken to determine the current extent and level of resistance to grain protectants in the rust-red flour beetle, *Tribolium castaneum* (Herbst.), the rice weevil, *Sitophilus oryzae* (L.) and the lesser grain borer, *R. dominica*.

In addition, our laboratory is actively involved in the evaluation of alternative chemicals for the control of stored grain pests (Bengston *et al.* 1983, 1984), hence a second aim was to verify the reliability of test strains in representing field resistance.

### MATERIALS AND METHODS

During the period of this study, May 1983 to May 1984, insect infestations were sampled in 14 central storages belonging to Bulk Grains Queensland (BGQ), 20 grain

merchant premises and 58 farms. These locations, on the Darling Downs and extending to the Maranoa district and central Queensland, represent a cross-section of the grain industry of Queensland. Large numbers of *S. oryzae* and *R. dominica* were obtained by sieving following inspection of likely sites of infestation at each location. *T. castaneum* was collected in large numbers on farms and in grain merchant premises using cardboard flour traps (De Coursey 1931).

Adults collected in the field were held in the laboratory for an oviposition period of one to two weeks. *S. oryzae* and *R. dominica* were cultured in whole wheat (moisture content 12%) while *T. castaneum* was held in a medium of wholemeal flour and yeast (12:1 w/w). The adults were then removed and tested for resistance by exposure to a discriminating dose of insecticide in the FAO impregnated-paper assay (Anon. 1974). This involves exposing test insects to filter-papers impregnated with insecticide in an oil carrier. Technical grade insecticide ( $\geq 93\%$  pure) was dissolved in a mixture of Risella 17 oil (Shell Australia), acetone and petroleum ether (3:1:1) and 0.5 mL of this solution, at the appropriate concentration, was added to filter-papers (70 mm diameter). The volatile solvents were then allowed to evaporate overnight. Test insects were confined for a specific time (*T. castaneum* 5 h; *S. oryzae* 6 h; *R. dominica* 24 h) to the impregnated-papers within 50 mm diameter glass rings. Samples of from 5 to 40 insects were tested. The criterion of response to insecticide was knockdown (KD). This was defined as the inability of the insects to stand and walk. Discriminating doses (DD) were based on the results of at least six replicates of impregnated-paper assays of appropriate insecticide-susceptible laboratory strains. The origins of these susceptible strains have been described by Bengston *et al.* (1975) and Champ and Campbell-Brown (1970). The discriminating doses (Table 1) were as close as practicable to the  $KD_{99.9}$  for *S. oryzae* and *R. dominica* and the  $KD_{99.9} \times 2$  for *T. castaneum*. Twice the  $KD_{99.9}$  was used as the DD for *T. castaneum* as G.G. White (pers. comm. 1983) has found that low level resistance ( $\times 2.5$  at  $KD_{99.9}$ ) to fenitrothion is widespread in this species and the purpose here was to determine additional resistance. If the discriminating dose indicated resistance (i.e. if there were any survivors), the strain was kept in culture and its first generation progeny were exposed to full impregnated-paper assays consisting of three replicates of 40 adult insects exposed to each of a graded series of six doses of insecticide. All impregnated-paper assays were performed at 25°C and 70% relative humidity (r.h.). The results are reported as g/L insecticide in non-volatile solvent.

**Table 1.** Discriminating doses used for screening the major coleopterous pests of stored grain for resistance to insecticides

Species	Strain	Insecticide	Discriminating dose (g/L in non-volatile solvent)
<i>T. castaneum</i> . . . . .	QTC4	fenitrothion	0.08
<i>S. oryzae</i> . . . . .	QSOLS2	fenitrothion	0.02
<i>R. dominica</i> . . . . .	QRD14	bioresmethrin	0.02
	QRD14	carbaryl	0.02

Grain assays involved exposure of adult test insects under standard conditions to grain treated with insecticide. Methods were as described by Hargreaves *et al.* (1982) except that the assays were carried out at 30°C and 55% r.h.

The results of bioassays were analysed by probit analysis (Finney 1971) using a log transformation of dose. Because of variation in regression slopes, resistance factors were calculated simply as the ratios:  $KD_{50}$  resistant strain/ $KD_{50}$  susceptible strain and  $KD_{99.9}$  resistant strain/ $KD_{99.9}$  susceptible strain. Resistance factors of less than 2 were not regarded as significant.

### Reference laboratory strains

The responses of field strains were compared with those of insecticide-resistant strains maintained in this laboratory chiefly for the development of newer grain protectants. These strains — QS056 and CS0231, multi-resistant *S. oryzae*; CTC12, multi-resistant *T. castaneum*; QRD63 and QRD124, multi-resistant *R. dominica* — are believed to be typical of resistant strains currently present in eastern Australia (M. Bengston pers. comm.1984). QS056 and CS0231 were continuously reared in wheat treated with 3 and 6.5 mg/kg fenitrothion respectively. CTC12 was reared in flour treated with 10 mg/kg fenitrothion dust while QRD63 was reared in wheat treated with up to 10 mg/kg carbaryl and QRD124 was reared in wheat with up to 0.5 mg/kg bioresmethrin.

## RESULTS AND DISCUSSION

### *Sitophilus oryzae*

*S. oryzae* were often found on farms and in grain merchant premises but were not found in central storages (Table 2). The discriminating dose test showed that resistant individuals were present in 53% of farm strains and 73% of strains collected at grain merchant premises (Table 2). Full dose-response impregnated-paper assays revealed low resistance factors (<4×) at the  $KD_{50}$ . Of the field strains showing resistance, the response of the two strains with lowest resistance and the two strains with highest resistance to fenitrothion are plotted in Figure 1. The two least resistant strains exhibited a mixed response which was similar to that of the susceptible Q50LS2 at low doses but diverged at higher doses. The most resistant strains were intermediate in response between QS056 and Q50LS2. The resistant field strains were probably heterogeneous for low-level resistance similar to QS056, with a low frequency of resistant individuals in the least resistant strains and a high frequency in the most resistant strains.

Table 2. Suspect resistance in field strains of coleopterous pests of stored grain detected in discriminating dose assays

Species (insecticide)	Source	No. samples tested	No. samples showing suspected resistance
<i>S. oryzae</i> (fenitrothion)	farms	51	27
	private traders	11	8
	central storages	0	0
<i>T. castaneum</i> (fenitrothion)	farms	47	4
	private traders	19	0
	central storages	7	1
<i>R. dominica</i> (bioresmethrin)	farms	42	5
	private traders	13	0
	central storages	8	1
<i>R. dominica</i> (carbaryl)	central storages	6	6

Resistance to fenitrothion of the level seen in CS0231 was not encountered in this survey. However, the existence of this strain which was collected originally from Western Australia, indicates that resistance higher than that now occurring in the field, is possible.

### *Tribolium castaneum*

Only five of the total of 73 strains tested contained resistant individuals as indicated by the discriminating dose test (Table 2). The probit regression lines of the field strains had relatively low slopes and resistance factors at the  $KD_{99.9}$  level ranged from 1.4 to 20.1 while those at the  $KD_{50}$  were 1.9 to 5.2. This is indicative of mixed populations of

susceptible and resistant insects. The two field strains exhibiting highest resistance, QTC214 and QTC244, had resistance factors at the  $KD_{50}$  similar to that of malathion non-specific resistant CTC12 (Champ and Dyte 1976). The responses of QTC214 and QTC249, the strain with lowest resistance of those detected by the discriminating dose test, are plotted in Figure 2. The resistant proportion of QTC249 is clearly indicated by the sharp inflexion in the response line at 90 to 98% knockdown. The response of the reference susceptible strain, QTC4, and that of CTC12 are also shown.

### *Rhyzopertha dominica*

A few field strains of *R. dominica* failed the discriminating dose test with bioresmethrin (Table 2). When these strains were subsequently subjected to a full impregnated-paper bioassay, it was revealed that none had resistance factors greater than 2. These results illustrate the necessity of labelling strains with individuals that survive a discriminating dose as only 'suspected' resistant and then verifying this with a full dose-response assay.

There is no evidence of resistance to bioresmethrin in *R. dominica* in the field. However, in the laboratory, QRD124 has been under selection pressure with bioresmethrin for 13 generations, and its resistance level has increased to 2 to 3 × above that shown by the reference susceptible strain (QRD14) and by the four field strains tested.

Six *R. dominica* strains were collected from grain that had been treated with carbaryl. All of these were classed as suspected resistant by the discriminating dose test (Table 2). Full impregnated-paper assays of these strains showed low-level resistance, 2.1 to 4.5 at the  $KD_{50}$  and 1.1 to 4.1 at the  $KD_{99.9}$  (Table 3). This resistance was at about the same level as that shown by QRD63, the carbaryl-selected laboratory strain.

Table 3. Response of laboratory and field strains of *Rhyzopertha dominica* to carbaryl in impregnated-paper bioassays

Strain	$KD_{50}$ (95% limits) (g/L)	$KD_{99.9}$ (95% limits) (g/L)	Slope (s.e.)	Resistance factor	
				$KD_{50}$ level	$KD_{99.9}$ level
Laboratory					
QRD14* . . . . .	0.004	0.058	3.0	1.0	1.0
QRD63 . . . . .	0.006 (0.005–0.008)	0.163 (0.095–0.354)	2.2 (0.2)	1.6	2.8
Central storages					
QRD201 . . . . .	0.018 (0.016–0.020)	0.236 (0.178–0.334)	2.7 (0.2)	4.5	4.1
QRD204 . . . . .	0.020 (0.008–0.011)	0.147 (0.105–0.233)	2.6 (0.2)	2.5	2.5
QRD125 . . . . .	0.013 (0.011–0.014)	0.111 (0.089–0.146)	3.3 (0.2)	3.2	1.9
QRD126 . . . . .	0.013 (0.012–0.014)	0.126 (0.100–0.168)	3.2 (0.2)	3.4	2.2
QRD127 . . . . .	0.008 (0.006–0.010)	0.064 (0.039–0.173)	3.4 (0.5)	2.1	1.1
QRD128 . . . . .	0.010 (0.009–0.011)	0.078 (0.063–0.103)	3.4 (0.2)	2.5	1.3

\*  $KD_{50}$ ,  $KD_{99.9}$  and slope QRD14 were meaned from seven separate bioassays.

Two field strains, one with low resistance (QRD127) and another with higher resistance (QRD126) were also subjected to bioassay in grain treated with carbaryl (Table 4). The mortality responses of QRD127 and QRD126 were similar to that of QRD63 (about 2 × resistance). Although this resistance is low, it is economically significant. Carbaryl is applied to grain at a dose of 8 mg/kg in eastern Australia for 39 weeks protection from insect infestation. The half-life of carbaryl is 14 weeks (Desmarchelier and Bengston 1979), thus although susceptible insects would be controlled, these bioassays have shown that some resistant *R. dominica*, a major migratory species (Sinclair and Haddrell 1985), would survive in treated grain (Table 4).

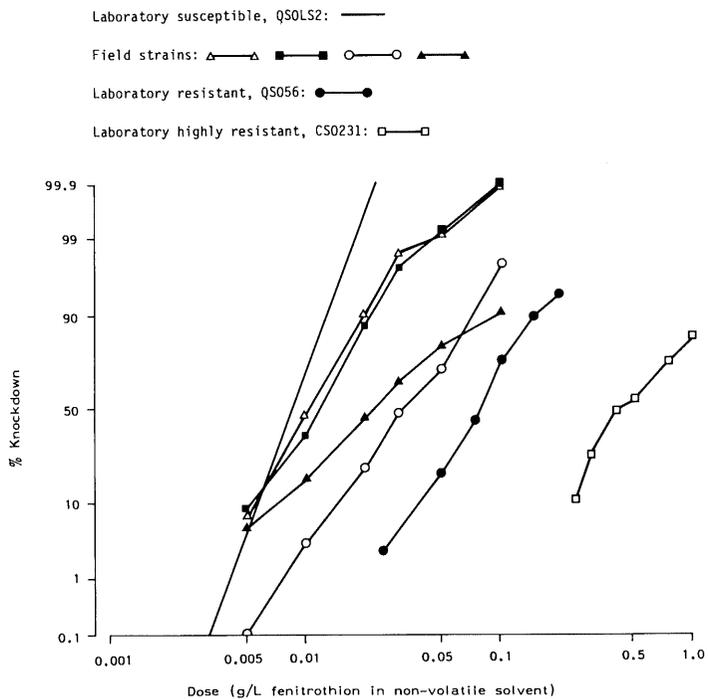


Figure 1. Response to fenitrothion of field and laboratory strains of *Sitophilus oryzae*.

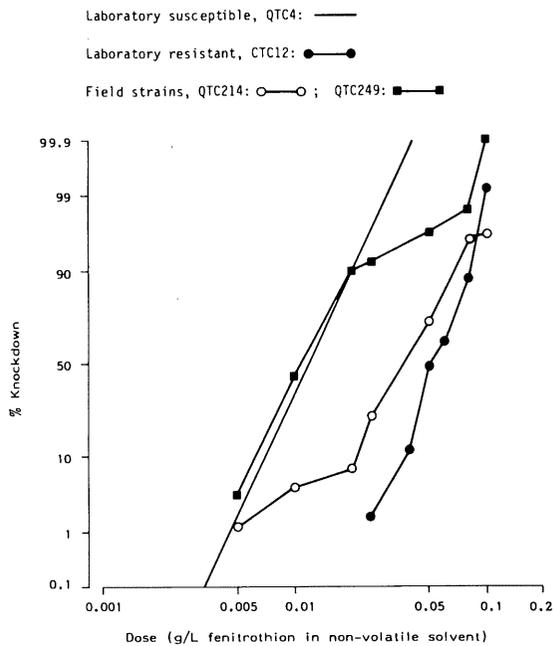


Figure 2. Response to fenitrothion of field and laboratory strains of *Tribolium castaneum*.

**Table 4. Response of laboratory and field strains of *Rhyzopertha dominica* to carbaryl in treated-grain assays**

Strain	LC <sub>50</sub> (95% limits) (mg/kg)	LC <sub>99.9</sub> (95% limits) (mg/kg)	Slope (s.e.)	Resistance factor	
				LC <sub>50</sub> level	LC <sub>99.9</sub> level
Mortality at 3 days					
QRD14 . . . . .	1.2 (1.1-1.3)	29.3 (21.7-42.4)	2.2 (0.1)	1.0	1.0
QRD63 . . . . .	1.9 (1.6-2.2)	28.6 (19.1-50.0)	2.6 (0.2)	1.6	1.0
QRD126 . . . . .	1.7 (1.4-2.2)	117.4 (62.1-202.8)	1.7 (0.1)	1.5	4.0
QRD127 . . . . .	1.9 (1.7-2.2)	47.3 (34.0-71.2)	2.2 (0.1)	1.6	1.6
Mortality at 26 days					
QRD14 . . . . .	0.9 (0.7-1.1)	3.8 (2.4-10.4)	4.9 (0.7)	1.0	1.0
QRD63 . . . . .	0.9 (0.8-1.1)	6.4 (4.2-12.8)	3.7 (0.4)	1.0	1.7
QRD126 . . . . .	1.2 (1.1-1.3)	9.0 (7.1-12.2)	3.5 (0.2)	1.3	2.4
QRD127 . . . . .	1.1 (0.9-1.4)	5.3 (3.3-14.4)	4.7 (0.8)	1.3	1.4

### GENERAL DISCUSSION AND CONCLUSIONS

The results of this survey have shown that low level resistance is present in populations of *S. oryzae* and *T. castaneum* to fenitrothion and in *R. dominica* to carbaryl. The economic significance of this resistance in each species is dependent on the dose of insecticide used to control the pest. In Australia, grain protectants are applied to grain at a rate high enough to give complete protection from insect infestation for up to 9 months. However, at the end of the storage period these doses must not exceed acceptable maximum residue limits (MRL). At present, the dose of fenitrothion applied to grain is high enough to overcome the low resistance levels in *S. oryzae* and *T. castaneum* for nine months and low enough to comply with MRL requirements. In the case of carbaryl the recommended dose of 8 mg/kg will not completely control resistant *R. dominica* especially as the insecticide residues decay (Table 4). Although data suggest complete control of current resistant strains of *R. dominica* in the field could be achieved by increasing the dose of carbaryl, the MRL of 5 mg/kg for this compound means that the grain so treated would not be acceptable in many markets. If the frequency of control failures due to carbaryl resistance in *R. dominica* becomes economically untenable, the best alternative protectant would probably be bioresmethrin. This chemical is already in widespread use and currently there is no sign of resistance to it in the field.

At the population level, resistance can be defined in two ways:

1. As a change in response to an insecticide through selection which increases the upper tolerance limit of the population. It is important to note that changes in LC<sub>50</sub> levels alone without a change in LC<sub>99.9</sub>'s simply represent an increase in the frequency of the more tolerant individuals in the population and do not represent resistance (Champ 1986).
2. A control failure when the recommended dose no longer gives adequate control of insects (Sawicki and Denholm 1984).

These two definitions are interdependent but not necessarily synonymous. A change in response to fenitrothion in some populations of *S. oryzae* and *T. castaneum* has not led to control failure. However, an equally small change in response to carbaryl has resulted in an increase in the frequency of failure to control *R. dominica*.

The absence of resistance to bioresmethrin in *R. dominica* is intriguing, especially in view of the fact that resistance to lindane, and to the cyclodiene and organophosphorus insecticides as well as to carbaryl had been reported in this species (Champ and Dyte 1976; Georgiou and Mellon 1983).

Workers in the field of insecticide resistance have expressed concern that laboratory-based insecticide susceptible populations may not be a true representation of the level of susceptibility expressed by insects in the field. Thus resistance tests using laboratory-reared strains as a reference may produce misleading results as has been demonstrated recently with the housefly (Farnham *et al.* 1984). This survey has provided evidence to verify that our laboratory strains are a good representation of both resistance and susceptibility to the chemicals tested as it occurs in field populations in southern Queensland.

The value of surveys for insecticide resistance firstly lies in their ability to provide early warning of possible control failures and secondly, as a method of evaluating the present and likely future effectiveness of chemical control methods. This information, coupled with the establishment of discriminating doses, also provides the background necessary to confirm resistance as the cause when control failures take place. Usually by the time a control failure is detected in the field resistance has become well-established.

The major advantage of establishing discriminating doses is that a large number of individual insects can be tested. However, for an accurate diagnosis of resistance, strains that fail a discriminating dose test should be submitted to a full bioassay for confirmation (Anon. 1974). A drawback with impregnated-paper assays is that although resistance can be detected this method provides no information on the significance of this resistance in the field. We have found that exposing insects to grain treated with commercially formulated insecticides is essential in establishing the practical importance of various resistances.

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(Accepted for publication 11 July 1986)

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