INSECTICIDE RESISTANCE IN SOME AUSTRALIAN POPULATIONS OF *ORYZAEPHILUS SURINAMENSIS*, THE SAWTOOTHED GRAIN BEETLE

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Abstract

Infestations of *Oryzaephilus surinamensis* detected during storage of commercial grain bulks in New South Wales in 1984 were assessed for resistance to fenitrothion, malathion and chlorpyrifos-methyl by survival of adults on impregnated papers. Levels of fenitrothion resistance in 6 of 20 populations examined were so high that more than half the insects survived exposure to 20 times the discriminating concentration (DC). Mixed function oxidase (MFO) activity (aldrin epoxidase) in these populations ranged between about 7 and 14 nmol dieldrin produced per min per mg protein, 35 to 70 times as much as the mean susceptible figure. Cytochrome P450 levels were 1.25 to 2.5 nmol per mg protein, about 8 to 17 times the susceptible level. Other populations with lower fenitrothion resistance as determined by response to the DC, 5xDC and 20xDC had intermediate levels of both MFO and cytochrome P450 suggesting that these factors could be related to the resistance. Malathion resistance was present in all fenitrothion resistant populations and in one population susceptible to fenitrothion. Chlorpyrifos-methyl resistance at low levels appeared not to be related to the level of fenitrothion resistance. The significance of fenitrothion resistance in *O. surinamensis* is discussed in relation to previous and current usage of the three insecticides to protect silo bulks.

Introduction

Grain protectants have been used widely in New South Wales bulk handling silos for more than 20 years. By the early 1970s several species of grain insect had developed specific malathion resistance and *Rhyzopertha dominica* had also developed a general organophosphate (OP) resistance (Greening *et al.*, 1975). Malathion was then replaced by fenitrothion mixed with bioresmethrin, and good control of all species was generally achieved.

In the early 1980s a few populations of *Oryzaephilus surinamensis*, saw-toothed grain beetle, were reported resistant to fenitrothion in eastern Australian states (Attia, 1983; Heather and Wilson, 1983). Insects from these infestations were characterised by much higher

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resistance factors to fenitrothion than to malathion or other OPs (Attia and Frecker, 1984). Resistance in adults of one strain was shown to be associated with greatly increased mixed function oxidase (MFO) activity compared with a field susceptible strain (Rose and Wallbank, 1986). Levels of cytochrome P450 in this strain were also increased. This paper examines the resistance status and levels of cytochrome P450 and MFO activity of populations collected from 20 different grain bulks during the 1984 storage year.

Materials and Methods

Insects. *O. surinamensis* adults were collected during routine inspections of grain stored in N.S.W. bulk handling authority storages during 1984. Numbers sampled varied from a few individuals to over 100 and usually occurred with other grain insects. Progeny were reared on rolled oats/kibbled wheat/yeast (8:8:1) at 25-27°C, 50-60% RH, and were tested as adults normally 2-3 weeks after peak emergence.

Insecticide resistance testing. Technical grade fenitrothion (97% w/w, Wellcome Aust.), malathion (95% w/w, Cyanamid) or chlorpyrifos-methyl (99% w/w, Dow Chemical) was dissolved in hexane:acetone:Ondina 17 oil (3:1:1) and applied to filter papers according to the FAO method (Anon., 1974). Chlorpyrifos-methyl is a registered alternative grain protectant. Batches of 40 insects were confined to papers at 25°C and assessed for knockdown after 5 h. Resistance was indicated when insects survived discriminating concentrations (DCs) of 0.5%, 1.0% and 2.5% in oil deposit for the 3 insecticides respectively (from data in Heather and Wilson (1983) and Champ and Dyte (1976)). Resistance levels were considered "low" when <5% insects survived 5xDC for fenitrothion, 2xDC for chlorpyrifos-methyl, or the DC for malathion, and were considered "high" for fenitrothion when >50% insects survived 20xDC. Response to fenitrothion was also assessed with a graded series of concentrations in the range 0.025% to 50%, and was analysed by a maximum likelihood probit programme after Finney (1971).

Enzyme assays. Microsomes were prepared from 2 batches of approximately 900 adults from each population. The methods used in the preparation of microsomes and in the assays of aldrin epoxidase and cytochrome P450 have been reported (Rose and Wallbank, 1986).

Results

Seventeen of the 20 populations tested showed some resistance to fenitrothion. The responses ranged from few low resistant individuals to most individuals being highly resistant (Table I). Although populations were derived from individual field infestations, most were heterogeneous in their response and were characterised by wide fiducial limits and poor X^2 values for goodness-of-fit of probit lines. They were therefore grouped for comparison of resistance according to their survival at the DC, 5xDC and 20xDC (Table I) rather than on the basis of LC50 values. The range of response was generally consistent with two types of

resistance; one of low order with a resistance factor approximately 9 times that of susceptibles, and another where individuals were little affected by fenitrothion in the test conditions. Most populations contained mixtures of these types of individuals, together in some cases with susceptibles. Resistance in populations in culture usually remained stable for at least six generations without insecticide pressure.

TABLE I. Response of 20 field populations of *O. surinamensis* to fenitrothion.

Survival (%) at DC	5xDC	20xDC	Number of pop'lns	Response of representative population Pop'ln	LC50[a]	Slope[b]
Susceptible:						
0	0	0	3	NOS432	0.11(0.10-0.12)	6.2NS
Low resistance:						
60-100	0-5	0	3	NOS446	0.98(0.88-1.07)	7.7NS
Moderate resistance (i):						
40-90	5-25	5-10	5	NOS429	1.10(0.59-1.69)	1.1*
Moderate resistance (ii):						
100	40-60	10-40	3	NOS416	14 (5.6->100)	1.0**
High resistance:						
85-100	60-90	50-80	6	NOS418	20 (7.7->100)	1.1**

a 95% fiducial limits in brackets
b Heterogeneity X^2 values significant at *P<0.05;**P<0.01; NS not significant

TABLE II. OP-resistance and levels of cytochrome P450 and aldrin epoxidase in 20 field populations of *O. surinamensis*

Resistance status[a] FEN[b]	MAL[b]	CHL[b]	Pop'lns tested	Aldrin epoxidase[c] range	Cytochrome P450[d] range
Susceptible	SLS	SSL	3	0.05-0.35	0.14-0.15
Low	RRR	LLL	3	2.6-4.1	0.30-0.61
Moderate i	RRRLR	LSLSL	5	3.9-6.2	0.35-1.36
Moderate ii	RRR	LSL	3	5.0-6.8	0.92-1.31
High	RRRRRR	LSRLLS	6	6.7-14.2	1.25-2.54

a Determined from DCs (see text): S susceptible, L low resistant, R resistant
b FEN fenitrothion, MAL malathion, CHL chlorpyrifos-methyl
c nmol dieldrin/min/mg protein
d nmol/mg protein

Aldrin epoxidase activity was high in all fenitrothion-resistant populations (Table II). Even the least resistant population, where no insects survived 5xDC, contained 14 times the aldrin epoxidase activity of the susceptible mean. Increasingly resistant population groups showed consistent increases in activity, reaching a maximum in one population of 75 times that of the susceptible mean. Cytochrome P450 levels followed a similar trend with increasing fenitrothion resistance but the differences were less marked, particularly with low resistance, and there was considerable overlap between groups.

Resistance to malathion occurred in all instances where resistance to fenitrothion was found, and also occurred in one population susceptible to fenitrothion (Table II). Resistance to chlorpyrifos-methyl at the 2xDC level was found in only one population. Although this population had high levels of cytochrome P450 and aldrin epoxidase, and high fenitrothion resistance, two other populations with similar levels were susceptible to chlorpyrifos-methyl.

Discussion

The correlation between fenitrothion resistance, increased aldrin epoxidase and cytochrome P450 suggests that MFOs are involved in one aspect of the resistance. MFOs are known to be implicated in many reactions including the detoxification of OP oxons by metabolism (Matsumura, 1976). Levels of both aldrin epoxidase and cytochrome P450 in most moderately resistant and highly resistant populations exceeded the levels determined for strain NOS25 collected in N.S.W. during 1982 (Rose and Wallbank, 1986), but no further general increase has been found in the 1985 populations tested to date (unpublished). The levels are considerably higher than found for several other insects including the Rutgers resistant strain of *Musca domestica* (Moldenke and Terriere, 1981) and a resistant strain of *Tribolium castaneum* (Cohen, 1982).

Esterases as well as MFOs may be implicated in the resistance judging by the synergism results of Attia and Frecker (1984). Hydrolytic esterase activity is greater in some resistant populations than in susceptibles and there is some variation of response with different substrates (unpublished results). Certain Victorian resistant populations have also shown correlations with esterase levels (Wegescanyi, personal communication).

Fenitrothion resistance in *O. surinamensis* in eastern Australia became noticeable some years after the introduction of fenitrothion by bulk handling authorities on wheat, but it may also be related to prior malathion resistance in this species. In the few samples of *O. surinamensis* tested in N.S.W. before 1980, malathion resistance was suppressed by triphenyl phosphate, suggesting specific carboxyesterase-type resistance only (Attia, 1983). Malathion resistance was not suppressed in later infestations tested or in virtually all *O. surinamensis* sampled during the FAO global survey of pesticide susceptibility, indicating carboxyesterases were unlikely to be a major factor in the resistance (Champ and Dyte, 1976). Two of the FAO strains

showed fenitrothion cross-resistance (Dyte *et al.*, 1976) at high levels similar to those attained by the N.S.W. populations reported here. It is not clear whether the resistance has developed primarily to malathion or fenitrothion since both insecticides were in widespread use in sequence. Carboxyesterases may also have been involved initially when malathion was widely used, but it is unlikely that they are of importance now with the current small usage of malathion as a grain protectant.

The emergence of fenitrothion resistance in *O. surinamensis* was the first major change in status of grain insects in eastern Australia since organophosphate resistance developed in *R. dominica* about 10 years previously. The infestations were, however, usually controlled with re-treatments of fenitrothion or chlorpyrifos-methyl within the maximum residue limits allowed. In at least one case a resistant infestation appeared to die out naturally. Despite resistance *O. surinamensis* has not reached the importance of *R. dominica* as a stored wheat pest in Australian conditions, presumably because it does not colonise whole grains. Widespread abandonment of fenitrothion in favour of other protectants such as those tested by Bengston *et al.*(1980) has not proved to be necessary to date.

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