

ASSESSMENT OF AZINPHOS-METHYL RESISTANCE IN APPLE LEAFCURLING MIDGE FROM NEW ZEALAND APPLE ORCHARDS

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ABSTRACT

Apple leafcurling midge larvae (*Dasyneura mali*) were collected from commercial and organic orchards from several apple growing districts throughout New Zealand and tested for resistance to azinphos-methyl using petri dish bioassays. High mortality (>90%) occurred at most concentrations tested and there was no difference between the response of those larvae collected from organic or commercially sprayed orchards. The results suggest that resistance to azinphos-methyl is not the cause of recent outbreaks of this pest.

Keywords: apple leafcurling midge, *Dasyneura mali*, resistance, azinphos-methyl

INTRODUCTION

Apple leafcurling midge (ALM), *Dasyneura mali* Kieffer [Diptera: Cecidomyiidae] is found throughout New Zealand and may cause significant damage to leaves, flowers and developing fruitlets of apple (Tomkins *et al.* 1994). Although this species has commonly been regarded as a secondary pest (Scott 1984) and has apparently been suppressed by the application of organophosphate insecticides for control of other insect pests, eg. leafroller and codling moth, in recent years its incidence in commercial apple orchards in Auckland, Waikato, Hawkes Bay and Nelson districts has increased noticeably (June 1994). Such outbreaks appear not to have occurred in other South Island districts, eg. Marlborough, Canterbury and Otago.

Although no proven explanation for these recent outbreaks has yet been forwarded, it has been suggested that ALM may have developed resistance to organophosphate insecticides that have been used in most commercial apple orchards for more than two decades. This is not an unreasonable suggestion because organophosphate resistance in other secondary pests, eg. mealy bug (Charles *et al.* 1993) and Froggatt's apple leafhopper (Charles *et al.* 1994) has recently been detected in some North Island apple growing districts.

Field control failure is often the first sign of resistance development, although it cannot be assumed that resistance has developed until the response of the suspected resistant population to the commonly used insecticide has been compared with that of a known susceptible population. These responses are typically determined using laboratory bioassays and appropriate statistical comparisons of the results, eg. comparison of LC₅₀ values (Robertson and Preisler 1991).

The main objective of this study was to compare the responses to azinphos-methyl of selected ALM populations from the main apple growing districts of New Zealand using a laboratory bioassay. Azinphos-methyl was used in the bioassays because it has had the longest period of use of any organophosphate insecticide in New Zealand apple orchards.

METHODS AND MATERIALS

ALM were collected from commercial (standard spray programme) and, when possible, organic apple orchards (no chemical spray programme) in the Canterbury, Nelson, Hawkes Bay and Waikato districts. Most leaf samples were taken during

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December 1994 from apple blocks with high levels of ALM infestation. Collected leaves were returned to the laboratory and mature (orange) larvae were extracted from the leaf rolls.

Bioassay method

Larvae were exposed to residues of azinphos-methyl (50% WP) on No.2 filter paper in 9 cm diameter petri dishes. Six concentrations (serially diluted 1:1 from the highest concentration) of azinphos-methyl were prepared in water and ranged from 0.0039 g/litre to 0.125 g/litre. This concentration range was chosen because previous tests (ENZA, unpubl. report) had shown that high mortality occurred at concentrations above 0.125 g/litre. It was therefore predicted that mortality would range between 5-95% for the concentration range used.

Two ml of each concentration was pipetted onto the filter paper in each petri dish. A water-only control dish was established in a similar manner. Three replicates of each concentration and control were prepared for each property from which larvae were collected. Twenty larvae were placed in each dish within a short time of treatment with the insecticide suspension or water while filter papers were still damp. A small plug of agar (2% w/v) was placed on the internal surface of the lid of each petri dish to help maintain humidity.

All petri dishes were stored in the dark for 24 h at ca. 20°C after which mortality was assessed. Larvae were scored dead if no movement was observed within 30 seconds exposure to bright light. Mean percentage mortalities for each concentration were calculated.

Modified bioassay

Because the results of the above tests did not produce mortality responses between 5-95% (see Table 1), and certain other practical difficulties occurred, eg. larval escape or entrapment, a modified bioassay based on the method commonly employed for spider mites (Bowie *et al.* 1988) was used to test the responses of one population from Canterbury (Lincoln) and two from commercial orchards in Nelson.

Five cm diameter Falcon petri dishes with tight fitting lids were treated with a range of aqueous suspensions of azinphos-methyl concentrations (0.00035-0.0125 g 50% WP/litre) in a Potter precision spraying tower. The internal surfaces of each base and lid pair were sprayed with 2 ml of azinphos-methyl suspension or water (control) at 55 kPa allowing a 10 second settling period. The spray deposits in the dishes were allowed to dry (ca. 30 minutes at 20°C) before larvae were transferred. Twenty mature larvae were transferred to each dish and humidity was maintained with an agar plug. Replication and mortality assessment were as for the previous bioassays (unless otherwise specified in the Results section).

Concentration-mortality data for each replicate for each population were pooled and analyzed using the log-probit analysis program POLO (Russell *et al.* 1977) to estimate the LC₅₀ and 95% confidence interval, and the slope (\pm SE) of the regression. The program also performed Chi-square tests for goodness-of-fit of the data to the probit model and parallelism of the regressions.

RESULTS

The mean percentage larval mortalities for each population tested with azinphos-methyl from the Nelson, Waikato and Hawkes Bay districts are shown in Table 1. Data are presented only for the highest and lowest concentrations because the percentage mortalities recorded for the intervening concentrations were similar. No tests were conducted with larvae from Canterbury because ALM populations were extremely low during December.

Table 1 shows that high mortality (>90%) occurred at all concentrations of azinphos-methyl tested, and in many instances 100% mortality was recorded. Untreated control group mortality ranged between 0-32%, although it was usually lower than 15%. In those instances where a greater mean control group mortality was calculated it was invariably due to higher mortality occurring in one replicate. Table 1 also shows that the results obtained from commercial and organic orchards (Nelson and Hawkes Bay districts only) were similar.

TABLE 1: Percentage mortality of mature apple leafcurling midge larvae from different apple growing districts exposed to azinphos-methyl in Petri dish bioassays.

District	Concentration g/litre ¹	Percentage mortality (replicates pooled)			
		Orchard 1	Orchard 2	Orchard 3	Orchard 4 ²
Hawkes Bay	0.125	100	100	100	100
	0.0039	91.2	98.3	96.7	100
	Untreated	3.3	5.0	10.0	1.7
Nelson	0.125	98.2	100	98.3	100 ³
	0.0039	95.0	100	100	100
	Untreated	12.1	0	5.7	15.0
Waikato	0.125	100	100	100	- ⁴
	0.0039	100	100	96.7	-
	Untreated	32.0	22.0	11.7	-

¹ g ai azinphos-methyl (50% WP formulation)

² organic orchard

³ two replicates only

⁴ not tested

The results of the log-probit analysis for each population tested by the modified bioassay are shown in Table 2. The LC₅₀ values for each population are similar, although data from the second Nelson population were not adequately described by the probit model (i.e. Chi² value exceeds table value for 3 d.f.) and therefore the LC₅₀ value for this population may not be reliably estimated. The 95% confidence intervals for the Lincoln and Nelson 1 populations overlap indicating that no significant difference exists between the LC₅₀ values. The test of the hypothesis that the slopes of the regressions were equal was rejected.

TABLE 2: Log-probit analyses of the responses of mature apple leafcurling midge larvae to azinphos-methyl in the modified bioassay.

Population	LC ₅₀ ¹	95% CI	Slope	(SE)	Chi ²	d.f.
Lincoln	0.0030	0.002-0.004	1.83	0.26	1.29	4
Nelson 1	0.0025	0.001-0.0045	3.02	0.56	3.62	3
Nelson 2 ²	0.0035	not estimated	1.64	0.21	21.7	3

¹ grams 50% WP per litre

² two replicates only

DISCUSSION

The results presented in Table 1 suggest that resistance to azinphos-methyl has not developed in the ALM populations tested. Two reasons in particular support this conclusion, viz., that high mortality occurred at the lowest concentration tested, and there was no difference between the results of tests with larvae from commercial and organic orchards. It is also worthwhile noting that the lowest concentration tested in these bioassays (0.0039 g/litre) was 128-fold lower than the recommended 50 g/100 litre application rate for azinphos-methyl. Although these bioassays have demonstrated that the ALM populations tested are very susceptible to azinphos-methyl, caution is suggested when trying to use these laboratory results to predict field efficacy (Robertson and Preisler 1991).

The results of the modified bioassay (Table 2) also strongly indicate that the ALM populations from Nelson and Canterbury were not resistant to azinphos-methyl as the

LC₅₀ values estimated for each population were similar. The Lincoln population was regarded as being susceptible because it was collected from a research orchard at Lincoln University that had received very few organophosphate sprays in the past, whereas the Nelson populations were from commercial orchards receiving recommended organophosphorus insecticide programmes.

No cases of ALM resistance to insecticides have been reported in the literature (CAB data base, 1987 to date), and resistance is apparently rare in other species belonging to the Family Cecidomyiidae. Georghiou and Lagunes-Tejeda (1991) reported only two cases of resistance in the genus *Dasyneura* (*D. pyri* to azinphos-methyl and *D. tetensi* to endosulfan), and *Aphidoletes aphidomyza* to azinphos-methyl. Unfortunately, information about these cases of resistance is very limited. However, the potential for resistance development in ALM clearly exists as the species is widespread, produces many offspring and generations per year and is under intensive insecticide selection pressure throughout much of the growing season. Furthermore, the selection pressure from organophosphate insecticides has increased in many commercial apple orchards in recent years through the inclusion of insecticides like diazinon into the spray programme specifically for ALM control (Smith and Chapman 1995).

In conclusion, the results of this study suggest that ALM has not developed resistance to azinphos-methyl and, therefore, is unlikely to be the cause of the recent outbreaks of this pest that have been experienced in certain apple growing districts of New Zealand. Because many orchardists are now applying specific insecticides in an attempt to control this pest, further evaluation of the responses of ALM to other organophosphorus insecticides (eg. diazinon) to establish reliable base line susceptibility data is recommended.

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