RESISTANCE OF *PLANOTORTRIX OCTO* (GREENHEADED LEAFROLLER) TO AZINPHOS-METHYL IN HAWKE'S BAY

P.L. LO¹, J.T.S. WALKER¹ and D.M. SUCKLING²

*The Horticulture and Food Research Institute of New Zealand Ltd.,
Hawke's Bay Research Centre, Private Bag 1401, Havelock North, New Zealand
P.O. Box 51, Lincoln, Canterbury, New Zealand*

ABSTRACT

Apple orchardists in Twyford, Hawke's Bay, have reported increased fruit damage by leafrollers for several years. Of the three pest species present in this area, *Planotortrix octo* (greenheaded leafroller), is the main species found on fruit. First instar *P. octo* larvae were screened for resistance by a direct spray test using a Potter tower. Dosage-mortality responses for the offspring of wild-wild pairs from three orchards showed a 7-32 fold resistance to azinphos-methyl compared with larvae from a susceptible laboratory colony. *P. octo* larvae from one of the above and 16 further orchards were sprayed with a single diagnostic dosage (LD₉₉ for the susceptible strain). Resistance was found at 17 of 19 sites covering approximately 1000 ha. A resistance management strategy, using pheromone mating disruption, is being implemented.

Keywords: *Planotortrix octo*, greenheaded leafroller, Tortricidae, resistance, azinphos-methyl

INTRODUCTION

Organophosphate insecticides have been used in Hawke's Bay orchards for over 30 years. Organophosphate resistance in this region has been documented in *Pseudococcus viburni*, formerly *P. affinis* (obscure mealybug) (Charles *et al.* 1993) and *Edwardsiana crataegi* (Froggatt's apple leafhopper) (Charles *et al.* 1994). Insecticide resistance occurs in two species of leafroller (Lepidoptera: Tortricidae) elsewhere in New Zealand. *Epiphyas postvittana* (lightbrown apple moth) near Nelson developed resistance to azinphos-methyl in the 1970s (Suckling *et al.* 1984). This example of resistance appears to be stable because the resistant moths have not spread from their original location (Suckling and Khoo 1993). Wearing (1995) recently showed that *Planotortrix octo* (greenheaded leafroller) in Central Otago were resistant to azinphos-methyl and cross resistant to several other insecticides.

Some apple orchardists in the Twyford area north of Hastings have reported difficulty achieving adequate control of leafrollers during the past few years, despite regular organophosphate insecticide applications. Three species of leafroller, *E. postvittana*, *P. octo* and *Ctenopseustis obliquana* (brownheaded leafroller), are pests of orchards in Hawke's Bay. We suspected that one or more of these species could have developed resistance to azinphos-methyl, which is the predominant organophosphate insecticide used on export apples. The prime candidate was *P. octo* because, compared with the other two species, it occurs in high numbers in pheromone traps and larval collections only in Twyford (Lo and Walker unpubl. data). Our objectives were to confirm whether *P. octo* was resistant to azinphos-methyl and if so, to determine whether resistance within Twyford was widespread or localised.

METHODS

Dosage-mortality tests

In the 1993/94 season, the incidence of leafroller damage on fruit was assessed from three Twyford orchards. Approximately 7000-8000 apples of the cultivar Granny Smith

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were sampled at harvest (mid April to early May) from each property. Orchards A and B received nine insecticide applications, mainly diazinon and azinphos-methyl, while Orchard C received 12 applications mostly of chlorpyrifos. All leafroller larvae found were collected and reared at 20°C in test tubes containing artificial diet. The adult *P. octo* that developed from these larvae were mated with another moth reared from the same orchard. No larvae were found at Orchard B, but larvae were collected from a fourth orchard (D) in 1995 and reared the same way. We tested the dosage-mortality responses of first instar larvae from these three Twyford populations and from a known susceptible colony (that originated from Canterbury) maintained by the HortResearch insect rearing unit in Auckland. The latter population had not been exposed to organophosphates for many generations and was therefore used to indicate the baseline level of susceptibility of *P. octo* to azinphos-methyl.

Neonate larvae were sprayed with 7-9 concentrations of azinphos-methyl (Gusathion M35) plus tap water as the control. The insecticide was suspended in tap water and a dilution series prepared from a stock solution. We tested four replicates from the laboratory colony and 2, 1, and 4 replicates from Orchards A, C, and D respectively, depending on the number of larvae available. For each test 20-30 larvae were placed in a 9 cm diameter Petri dish and sprayed using a Potter Tower following the method of Wearing (1995). The tower was operated at 104 kpa (15 psi) and used 2 ml of solution per test with a 10 second settling time. The dishes were closed for 10 min after spraying and the larvae were then transferred to another dish containing artificial diet. The second dish was then closed, sealed with plastic wrap and held at 20°C for 48 h before assessing larval mortality. Larvae were classed as dead if no sustained movement occurred when touched with a fine brush.

The mortality data were transformed to probits and then regressed against the logarithm of insecticide concentration using POLO-PC (LeOra Software 1987). The analysis also compared the dosage response lines of each population. We obtained a measure of the level of resistance in field populations by dividing their median lethal dose (LD_{50}) by the LD_{50} of the susceptible population (Suckling 1996).

Diagnostic dosage tests

The LD_{99} (25 ppm) for the susceptible population was chosen as a diagnostic dosage to detect resistance in other field populations (Roush and Miller 1986), because this concentration gave a large difference in mortality between the susceptible and resistant populations. In addition to the three orchards above, we screened larvae from a further 16 Twyford orchards spread over approximately 14 km². We used the methods described above except that only a single dose was tested. To determine whether mortality was significantly less than 99% indicating resistance, we performed a one-sided Z test with correction for continuity (Snedecor and Cochran 1967).

The larvae were either the progeny of two field-collected parents (Orchards D-F) or one wild parent mated with a susceptible laboratory moth. The wild-susceptible crosses were obtained in two ways. In 1995, virgin female moths from the susceptible colony were tethered inside Pherocon 1CP traps (Suckling *et al.* 1989). They were placed in Orchards G-L for about a week between 3 April and 6 June, to be mated with wild males. In 1996, male *P. octo* were attracted to pheromone lures and caught in sweep nets on eight orchards (M-T) around Twyford between 15 March and 9 April. They were mated in Petri dishes with one or two virgin females from the susceptible laboratory colony.

RESULTS

Dosage-mortality tests

The dosage-mortality lines for larvae from the HortResearch colony and the three Twyford orchards (A, C, D) differed significantly ($P < 0.001$) in both their LD values and slopes (Fig. 1). This confirmed that *P. octo* at the three orchards are resistant to azinphos-methyl. These three populations produced dosage-mortality lines with similar slopes ($P > 0.05$) but different intercepts ($P < 0.001$). Compared to the susceptible colony, the larvae from the three orchards were respectively 7x, 11x, and 32x more resistant to azinphos-methyl. The flatter response lines of the orchard populations compared with the laboratory strain suggests that the former contain a mixture of resistant and susceptible

moths.

Diagnostic dosage tests

The mortality of first instar larvae from six pairs of wild-wild crosses varied from 16-43% (Table 1). The mortality of these larvae was similar to the mortality calculated at the same concentration for two of the three field populations for which a full dosage-mortality response was established. We tested larvae from 28 pairs of wild-susceptible crosses from 14 orchards. At every site except Orchards L and O, larval mortality was significantly less than 99% for at least one pair of moths (Table 1). Resistance to azinphos-methyl was present at 17 out of 19 sites, spread over approximately 1000 ha.

TABLE 1: Mortality of first instar *P. octo* from individual pairs, 48 h after being sprayed with 25 ppm of azinphos-methyl (LD₉₉ of the susceptible strain).

Orchard	No. Tested	% mortality	Z score ¹
Wild-Wild cross			
D	100	16.0	82.92 ***
	131	27.5	81.83 ***
E	28	42.9	28.91 ***
	72	33.3	55.41 ***
	75	36.0	54.25 ***
	79	39.2	52.82 ***
Wild-Laboratory cross			
G	147	83.7	18.26 ***
H	29	86.2	5.99 ***
	21	95.2	0.64 ns
I	74	93.2	4.39 ***
	56	100.0	
J	81	93.8	4.12 ***
K	142	95.8	3.41 ***
L	223	100.0	
M	108	89.8	9.11 ***
	57	94.7	2.57 **
N	46	73.9	16.36 ***
	28	75.0	11.81 ***
	47	100.0	
O	38	97.4	0.20 ns
	119	97.5	1.21 ns
	93	100.0	
P	29	100.0	
	103	95.1	3.44 ***
Q	19	100.0	
	47	76.6	14.70 ***
	129	96.9	1.96 *
	139	97.8	0.95 ns
R	50	100.0	
	37	78.4	11.78 ***
S	80	38.8	53.60 ***
	61	96.7	1.15 ns
	23	100.0	
T	49	67.3	21.55 ***

¹ Probability of mortality being less than 99%: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

Fruit damage

FIGURE 1: Dosage-mortality responses to azinphos-methyl of first instar *P. octo* from a susceptible laboratory colony and from three orchards in Twyford, Hawke's Bay. Error bars are 95% confidence intervals at LD₅₀ for the laboratory colony and Orchards A and D.

Leafroller larvae damaged 14.0%, 0.4% and 5.8% of the fruit sampled at Orchards A, B and C respectively.

DISCUSSION

We have shown that *P. octo* in Twyford are resistant to azinphos-methyl and that resistance occurs over much of the district. This corroborates the high level of leafroller damage found at Orchards A and C and other reports of field control failure. Leafroller damage under a regular organophosphate insecticide programme would normally be expected to be less than 0.1%. The low level of fruit damage at Orchard B suggests that resistant *P. octo* were absent from the vicinity of this property. This orchard is located on the western edge of Twyford, approximately 1 km from Orchard A.

The Twyford area has a relatively uniform landscape of orchards, mainly apples, with some arable cropping. There are also many large deciduous trees such as oak (*Quercus* sp.) and poplar (*Populus* sp.), and shelter belts such as willow (*Salix* sp.) that are hosts for leafrollers. Data from Orchard F indicates that at least some of these trees harbour resistant moths. The larvae at Orchard F were resistant and their distribution on the orchard showed they came from adjacent amenity trees (Lo unpubl. data). Consequently, Twyford apparently does not have large unsprayed areas that contain susceptible moths, except perhaps along the Ngaruroro River on its northern boundary. There do not appear to be sufficient natural barriers to prevent leafroller populations from mixing throughout the Twyford area and the wider Heretaunga Plains.

In contrast to Twyford, the resistant leafroller populations in Nelson and Central Otago are isolated by extensive tracts of scrub or farmland, which act as refugia of unsprayed leafroller habitat. The large scale immigration of susceptible moths from these areas into orchards is believed to dilute the resistance genes (Suckling and Khoo 1993; Wearing 1995). This mechanism contributes to the relative lack of development and spread of resistance in Nelson and Central Otago despite long histories of intensive organophosphate use.

The 7-32 fold increase in resistance recorded in the centre of Twyford was similar to that estimated for *P. octo* in Central Otago (14-20x) by Wearing (1995). Slight differences in methodology between the two studies, however, make it difficult to

compare the levels of resistance between districts. We tested the dosage-mortality responses of the progeny of wild moths, whereas Wearing (1995) made wild-susceptible crosses that were then selected four times over seven generations to remove susceptible progeny.

Azinphos-methyl resistant *E. postvittana* in Nelson and *P. octo* from Central Otago are also cross-resistant to chlorpyrifos and carbaryl (Suckling and Khoo 1990; Wearing 1995). If the resistance of *P. octo* in Twyford is due to similar mechanisms as in these two cases, then we would expect cross-resistance to also occur in Hawke's Bay. We suspect this is the case because resistance was demonstrated at Orchard C, where chlorpyrifos was the main insecticide used.

A resistance management strategy is being implemented in Twyford to improve leafroller control and to try and prevent or delay the spread of resistance. The primary strategy is mating disruption of *P. octo* with pheromone dispensers. This technique has been used in Twyford on a large scale for the first time in 1996/97. This season has also seen the introduction of Integrated Fruit Production (IFP) guidelines in apples, where the insect growth regulator, tebufenozide, is the primary insecticide for leafroller control. Under IFP, the monitoring of pest populations has resulted in better targeting of and a reduction in insecticide applications. This should help to decrease the selection pressure for resistance.

Further testing is needed to determine whether azinphos-methyl resistant *P. octo* from Twyford also have cross resistance to other insecticides. In future, the replacement of organophosphates with insecticides from alternative groups is likely to provide the best long term solution to this resistance problem.

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