

**RESISTANCE OF *PLANOTORTRIX OCTO* TO  
ORGANOPHOSPHATE INSECTICIDES  
IN DUMBARTON, CENTRAL OTAGO**

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**ABSTRACT**

Failure of organophosphate (OP) insecticides to control leafroller in apple orchards is reported from Dumbarton, Central Otago. A laboratory colony of greenheaded leafroller, *Planotortrix octo*, (OCTO) was established from the larvae of tethered virgin female moths which mated with wild males at Dumbarton. Part of the colony was selected four times over seven generations with azinphos-methyl at 100-300 ppm. The resistance of this strain to azinphos-methyl was determined, in comparison with a known susceptible strain of OCTO, using a direct spray test of first instar larvae in a Potter Tower. The resistance (relative potency) to azinphos-methyl was estimated at 14x in generation 7, with high cross-resistance to chlorpyrifos (12x), and carbaryl (8x). These results indicate the dosage mortality responses of the resistant component of OCTO populations at Dumbarton. The research identified that a discriminating dose was 200 ppm ai azinphos-methyl.

**Keywords:** leafroller, organophosphate resistance, azinphos-methyl, chlorpyrifos, carbaryl

**INTRODUCTION**

Research in Central Otago apple orchards from 1990-94 has shown that the greenheaded leafroller, *Planotortrix octo* (OCTO), is the leafroller species primarily responsible for crop damage in the Otago region (Wearing 1995a). Particularly high pheromone trap catches at Dumbarton have been associated with higher levels of crop damage than in surrounding districts (Wearing 1991). Apple blocks within an area of about 70 ha were being increasingly affected, but there was no evidence of spread beyond the affected orchards. While this research confirmed for the first time anecdotal evidence of a Dumbarton leafroller problem which goes back many years and identified OCTO as the likely cause, it did not explain why the OCTO population was so high and/or the field control failure of the organophosphate (OP) spray programme. Growers in Dumbarton were spraying a mean of six or more post-bloom insecticide applications per season, which was as great, or greater than, in other districts. One possible explanation of the fruit damage at Dumbarton would be the presence of a strain of OCTO resistant to OP's. Preliminary dosage-mortality tests conducted with azinphos-methyl in 1991-92 indicated an approximate 3-fold resistance at LC<sub>50</sub> compared to a susceptible (SxS) strain, with a discriminating dose of 100 ppm. Further larvae from Dumbarton were tested with this discriminating dose in 1992 and about 16% of 183 larvae survived, indicating the presence of resistant individuals (Wearing and Suckling 1993).

These results prompted the expansion of research on mating disruption of OCTO in Dumbarton as a component of insecticide resistance management, and this has given outstanding results (Wearing 1995b). Resistance tests were continued from 1993 with the objectives of determining more precisely the level of resistance in the Dumbarton population and its spectrum of cross-resistance to other chemicals used for its control.

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## METHODS

For the dosage-mortality assessments and discriminating dose selections, first instar larvae were subjected to a direct spray test. Azinphos-methyl (Gusathion 50WP) was used for all selections, and dosage-mortality tests were carried out with this product, with carbaryl (Sevin 80WP) and with chlorpyrifos (Lorsban 50WP). For the dosage-mortality tests, the insecticides were suspended in tap water and groups of larvae were treated with a range of 5-1 l concentrations, with tap water used as a control treatment (about 120 larvae/concentration). From 10 to 30 neonate first instar larvae were placed in a standard 9 cm diameter plastic petri dish and sprayed in a Potter Tower. Each spray used 2 ml of liquid applied at 104 kpa (15 psi) with a 10 second settling time at 15-18°C. The larvae were held in the closed dish for 10 min. after spraying and then transferred to a similar dish containing a thin layer of artificial diet. The dish was closed (non-vented) and sealed with cling-film (Glad-wrap) and two rubber bands to tighten the lid-to-base contact and confine the larvae. The larvae were then held for 48 h at 20°C until mortality assessment. Larvae were considered dead if no movement was detected in response to gentle manipulation with a camel-hair brush.

The HortResearch Insect Rearing Unit in Auckland maintains a colony of OCTO which is known to be susceptible to OP insecticides. First instar larvae from this SxS colony were used to compare their responses to insecticides with those of OCTO from Dumbarton.

Virgin female SxS OCTO were tethered individually (Suckling *et al.* 1990) in Pherocon 1CP 'traps' which were hung in apple trees at Dumbarton. The trees had been sprayed with a standard OP insecticide programme during 1992-93 and the moths were deployed from 20 April to 29 April and recovered 26 April to 5 May. A total of 74 females were used of which 55 (74.3%) mated successfully with wild males. A colony was established on artificial diet using larvae primarily from about 20 females. Other first instar larvae from these females were used to obtain a dosage-mortality line for azinphos-methyl. These larvae were the product of crosses between OP-susceptible females (ex-Auckland) and wild males (resistant or susceptible) in Dumbarton. This colony is referred to as SxD (susceptible x Dumbarton) and the larvae from the first cross (in the field) are SxD1.

Because of the mixed parentage of the SxD colony, the survivors of azinphos-methyl (100 ppm and above) applied to SxD1 in the dosage-mortality tests, were retained to establish a third colony, SxDSe. This "resistant" colony was established for continued selection with azinphos-methyl. SxDSe1 (generation 1) refers to larvae from the first cross (in the field) which survived the first selection of SxD1. As the colony was increased, it was selected four times (Table 1) with a discriminating dose of azinphos-methyl to provide a colony which would indicate more accurately the resistance level of the wild resistant strain at Dumbarton.

Selections for resistance with azinphos-methyl were carried out at concentrations of 100-300 ppm ai using up to 110 larvae per dish. Many did not survive and the quantity of diet was adequate to feed any survivors over the 48 h period. These larvae were transferred to individual tubes of artificial diet for rearing to the next generation.

Dosage-mortality tests with azinphos-methyl were carried out with SxD1 (=SxDSe1) (April 1993), SxDSe3 (October 1993), and both SxD7 and SxDSe7 (June 1994). Tests with chlorpyrifos and carbaryl were carried out with both SxD and SxDSe in generations 4 (December 1993) and 7 (June 1994). The SxS colony was tested on each occasion ((1),(4),(7)) except October 1993.

The mortality data were transformed to probits and analysed using Polo-PC (LeOra Software 1987) which calculates the regression of probit mortality on the logarithm of the concentration of insecticide. Polo-PC was also used to compare the dosage response lines for the different colonies and the different generations of the same colony.

## RESULTS

### Selection programme

Larvae which survived the first dosage-mortality test with azinphos-methyl at

100, 150 and 300 ppm were retained to establish the SxDSe1 colony. This and subsequent selections are summarised in Table 1.

**TABLE 1: Selections for resistance carried out with azinphos-methyl on a colony of *P. octo* obtained by crossing tethered virgin female susceptible moths with wild males in a sprayed orchard at Dumbarton, Central Otago.**

Colony/generation	Rate of azinphos-methyl (ppm ai)	n	Mortality (%) and range
SxD1 → SxDSe1	100-300	478	88.49 (53-100)
SxDSe3	100-300	947	89.97 (52-100)
SxDSe5	100-200	2593	89.22 (62-100)
SxDSe6	200	7000	82.09 (56-100)

The first dosage-mortality test suggested that a discriminating dose of 200 ppm was necessary rather than the earlier estimate of 100 ppm. However, the colony was not of sufficient size until generation 6 to enable use of a selection concentration of 200 ppm only. Most larvae prior to this were selected with 100 or 150 ppm. There was no clear trend of increasing survival of larvae at a given concentration as the selections continued. However, 200 or 300 ppm was always associated with mortality >90% until SxDSe6 which was the first time that selection had been carried out with consecutive generations, and the first time that only 200 ppm was used (ie. not lower concentrations). This selection appears to have had a major impact on resistance because dosage-mortality tests conducted using SxDSe7 gave only 38% mortality at 120 ppm (n = 124), 45% at 200 ppm (n = 91), 78% at 240 ppm (n = 88), and 69% at 400 ppm (n = 96).

#### Dosage-mortality tests

*Azinphos-methyl* The dosage-mortality lines for azinphos-methyl, all of which were adequately described by the log probit model, are summarized in Table 2. In the first test, the flatter slope (b) of the SxD1 line compared to SxS was consistent with a mixed population of susceptible and resistant individuals and crosses between them. Despite this, the LC<sub>50</sub> for SxD1 was significantly greater than that for SxS and the LC<sub>90</sub> was approximately three times greater. These results were consistent with the three-fold separation at LC<sub>50</sub> obtained in the earlier tests using field-collected larvae. The test with SxDSe3 was undertaken after only one selection with azinphos-methyl, with the majority of surviving larvae coming from the 100 ppm selection (Table 1). The resistance level of SxDSe (versus SxS) did not increase from SxDSe1 (2x) to SxDSe3 (2.5x). Neither the slopes nor the LC<sub>50</sub>'s changed significantly. These results supported the view that the discriminating dose needed to be >100 ppm.

A repeat of the SxS dosage-mortality line in June 1994 (SxS(7)), with fewer larvae than in the original test, showed greater susceptibility of the colony to azinphos-methyl (relative potency = 1.7). The slope of the line for SxS(7) was steep and the LC<sub>50</sub> and LC<sub>90</sub> were significantly reduced (P<0.01 and P<0.05, respectively) compared to those of SxS(1). Resistance assessments were therefore made using both dosage-mortality lines. However, the comparison with SxS(7) was considered more valid because it was carried out concurrently with tests on the other colonies.

The SxD7 results (Table 2) showed no significant change in LC<sub>50</sub> or LC<sub>90</sub> from those of SxD1 but there was a significant increase in the slope of the line. This change may have been the first indications that the frequency of resistance genes in the colony was slowly declining, as shown by the falling LC<sub>90</sub> values. Compared to SxS(7), SxD7 was 2.3x resistant at LC<sub>50</sub> and 3.6x resistant at LC<sub>90</sub>. This is similar to the original comparison between SxD1 and SxS(1). The results for SxDSe7 showed that the resistance of this colony had increased as a result of the four selections. There was a 4-fold increase in the LC<sub>50</sub> compared to SxDSe3 and a 5.4-fold increase compared to SxD7, the non-selected colony; the differences at LC<sub>90</sub> were also significant, with 3-

fold and 7-fold increases respectively. The results for SxDSe7 reflected the application of a discriminating dose selection (200 ppm) in generation 6 and gave an indication of the resistance level of resistant individuals in the OCTO populations at Dumbarton. The increased slope of the SxDSe7 line compared to SxDSe3 and SxDSe1 (=SxD1) was a further indication of this. Comparison of the data for SxDSe7 with those for SxS indicated a resistance level (relative potency) ranging from 8x (SxS(1)) to 14.5x (SxS(7)). Some heterogeneity remained in the SxDSe7 colony as shown by results for individual oviposition cages (5-10 female moths). For example, larvae from one cage were 20-fold resistant at LC<sub>50</sub> (using SxS(7)) compared to 10-fold for all remaining cages combined ( $P < 0.01$ ).

**TABLE 2: The responses of first instar larvae of greenheaded leafroller, *P. octo*, from Dumbarton (SxD, SxDSe) to Potter Tower spraying of aqueous solutions of azinphos-methyl (Gusathion 50WP), chlorpyrifos (Lorsban 50WP), or carbaryl (Sevin 80WP) compared to an OP-susceptible colony (SxS). In parenthesis, 95% confidence limits for LC values (ppm ai).**

AZINPHOS-METHYL		
<b>SxS(1)</b> n=957 b=2.74 SEb=0.27	<b>SxD1</b> n=1294 b=1.86 SEb=0.11	<b>SxDSe3</b> n=706 b=1.74 SEb=0.18
10 7.5 (5.0-10.0)	10 7.6 (5.7-9.7)	10 8.4 (2.2-16.4)
50 22.1 (18.3-25.6)	50 37.1 (32.4-41.9)	50 45.5 (27.2-63.3)
90 64.9 (56.1-78.3)	90 180.8 (153.2-220.4)	90 247.3 (169.5-478.9)
<b>SxS(7)</b> n=365 b=4.11 SEb=0.47	<b>SxD7</b> n=362 b=2.53 SEb=0.33	<b>SxDSe7</b> n=974 b=2.07 SEb=0.16
10 7.1 (5.3-8.8)	10 10.4 (2.9-17.6)	10 43.3 (22.3-62.6)
50 14.6 (12.6-16.6)	50 33.3 (20.7-46.0)	50 180.5 (139.3-249.8)
90 29.9 (25.8-36.6)	90 106.6 (72.0-249.6)	90 753.1 (466.0-1857.1)
CHLORPYRIFOS		
<b>SxS(4)</b> n=1582 b=2.15 SEb=0.14	<b>SxD4</b> n=482 b=1.62 SEb=0.20	<b>SxDSe4</b> n=478 b=1.77 SEb=0.20
10 0.8 (0.6-1.0)	10 0.9 (5.7-9.7)	10 1.1 (0.2-2.2)
50 3.1 (2.6-3.5)	50 5.5 (2.8-8.6)	50 5.7 (3.2-8.6)
90 12.1 (10.4-14.5)	90 34.0 (19.7-108.1)	90 29.9 (17.5-96.5)
<b>SxS(7)</b> n=1948 b=2.07 SEb=0.11	<b>SxD7</b> n=722 b=3.41 SEb=0.38	<b>SxDSe7</b> n=1199 b=2.41 SEb=0.30
10 0.9 (0.6-1.3)	10 8.6 (5.4-11.5)	10 13.6 (2.1-23.6)
50 3.7 (3.0-4.5)	50 20.5 (16.7-24.2)	50 46.3 (29.8-62.2)
90 15.4 (12.4-20.3)	90 48.7 (39.9-66.0)	90 157.5 (100.0-704.7)
CARBARYL		
<b>SxS(4)</b> n=639 b=1.43 SEb=0.18	<b>SxD4</b> n=494 b=1.74 SEb=0.16	<b>SxDSe4</b> n=478 b=1.44 SEb=0.19
10 10.9 (2.3-21.5)	10 25.3 (8.3-46.8)	10 57.6 (8.9-132.1)
50 85.3 (57.0-125.7)	50 138.2 (85.7-203.0)	50 595.4 (326.9-1119.9)
90 669.6 (344.6-2965.0)	90 755.6 (472.0-1646.9)	90 6158.6 (2591.9-43889.1)
<b>SxS(7)</b> n=1002 b=1.24 SEb=0.13	<b>SxD7</b> n=482 b=1.56 SEb=0.18	<b>SxDSe7</b> n=719 b=2.46 SEb=0.22
10 11.5 (0.9-28.4)	10 34.8 (7.8-70.4)	10 294.6 (173.2-415.6)
50 124.6 (68.9-234.0)	50 231.1 (136.3-367.4)	50 977.2 (762.5-1220.2)
90 1345.8 (528.4-20356.6)	90 1534.2 (819.1-5554.0)	90 3241.3 (2434.5-4940.3)

*Chlorpyrifos* The dosage-mortality lines for chlorpyrifos, all of which were adequately described by the log probit model, are summarised in Table 2. The results were similar to those obtained with azinphos-methyl. After two selections with azinphos-methyl (using mainly 100 ppm ai) there was no significant difference between the dosage-mortality line for chlorpyrifos in SxDSe4 compared to that for SxD4. The flatter slope in both cases compared to the SxS results again suggested a colony containing a mixture of resistant and susceptible individuals. The data for SxD4 and SxDSe4 were combined and compared with SxS. The relative potency was 1.97, with a significant difference at both LC<sub>50</sub> (1.83-fold) and LC<sub>90</sub> (2.64-fold).

By the seventh generation, two further selections of the SxDSe strain were associated with significant increases in the LC<sub>50</sub> and LC<sub>90</sub> values and an increase in the slope, as occurred with azinphos-methyl. These changes resulted in an overall 8-fold difference in resistance between SxDSe4 and SxDSe7. The relationship between SxDSe7 and SxS changed accordingly with an increase in resistance from about 2-fold (SxDSe4) to 12-fold (SxDSe7). The latter is a similar level of resistance to that for azinphos-methyl. Whereas there was no significant difference between the responses of SxD4 and SxDSe4 to chlorpyrifos, there was a significant difference in generation 7 at LC<sub>50</sub> (2.3x) and LC<sub>90</sub> (3.2x).

*Carbaryl* The dosage-mortality lines for carbaryl, all of which were adequately described by the log probit model, are summarised in Table 2. There was a difference between the responses of the SxD4 and SxDSe4 to carbaryl after only two selections with azinphos-methyl. There was a 4.3-fold difference at LC<sub>50</sub> and an 8-fold difference at LC<sub>90</sub>. Compared to SxS, SxDSe4 was 8-fold resistant to carbaryl and the slopes of the dosage-mortality lines were parallel. The line for SxD4 was also significantly different from that of SxS but the difference was only 1.6x at LC<sub>50</sub>. Continued selection of SxDSe from generations 4 to 7 increased the slope of the dosage-mortality line ( $P < 0.01$ ) for carbaryl. There was only a 1.6x increase in LC<sub>50</sub> of SxDSe7 compared to SxDSe4 and this was not statistically significant; the LC<sub>90</sub> in SxDSe7 declined as its confidence limits reduced but this change was also non-significant. There was no significant difference in the responses of larvae from SxD4 and SxD7. All these results indicated that resistance to carbaryl had not been increased by the further selections with azinphos-methyl. Comparison of SxDSe7 with SxS indicated an 8-fold resistance at LC<sub>50</sub> which is similar to the 8x resistance of SxDSe4.

## DISCUSSION AND CONCLUSIONS

These experiments have confirmed the presence of an OP-resistant strain of *P. octo* at Dumbarton. The resistance to azinphos-methyl of this component of the population is estimated at 14x. However, tests with larvae from separate groups of selected females indicate that resistance could be as high as 20x. There is high cross-resistance to chlorpyrifos (12x), and a lower cross-resistance to carbaryl (8x). With these levels of resistance present within the population at Dumbarton, it is probable that high immigration of susceptible OCTO into the orchards is the main mechanism that has limited spread of the problem and has enabled the organophosphate sprays to continue to provide some control of this pest. The resistance levels are sufficient to explain the field control failure of the OP spray programme at Dumbarton and the damage experienced by the growers using all three chemicals, including programmes based on chlorpyrifos alone. This case of OP-resistance differs in this respect from that in lightbrown apple moth at Nelson, which showed low cross-resistance to chlorpyrifos (Suckling and Khoo 1990). However, like the Nelson case, the integration of mating disruption, OP-sprays, and immigrant susceptibles offers an effective long term management programme for the growers (see Wearing 1995b).

The experiments showed that a discriminating dose of 200 ppm ai azinphos-methyl is required to identify resistance to this chemical. The data are also consistent with the use of 100 ppm ai azinphos-methyl to discriminate individuals resistant to carbaryl.

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