

MANAGEMENT OF RESISTANCE TO INSECTICIDES IN THE OBLIQUEBANDED
LEAFROLLER, *CHORISTONEURA ROSACEANA* (HARRIS), (LEPIDOPTERA:
TORTRICIDAE) IN ONTARIO ORCHARDS

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Abstract

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We assessed the potential for the management of resistance to organophosphorus (OP) and pyrethroid insecticides in three field populations of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera:Tortricidae), under two control programs. We compared resistance in a grower-devised pest management program and in a more extensively modified program. The former program was a grower selected or devised program that removed all OP and carbamate applications against OBLR, but used OP and pyrethroid insecticides against the other apple pests. The latter program largely substituted *Bacillus thuringiensis* Berliner-derived preparations or spinosad for azinphosmethyl (an OP) against resistant OBLR populations and minimized applications of OP and carbamates, using pyrethroids preferentially against the other apple pests. Generally, resistance declined or did not increase when selection was reduced, i.e., OP insecticides were not used for leafroller control but the decline was faster under programs that avoided the use of OPs, carbamates or pyrethroids. Resistance was lost from some populations in 2-3 generations, similar to declines observed in laboratory studies. Results also indicate that pesticide applications for other pests in the apple system are important in the selection or maintenance of resistance in leafroller populations.

Introduction

Populations of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae)(OBLR) resistant to the organophosphorus (OP) insecticide azinphosmethyl have been identified from all of the major apple production areas of Ontario. (Pree *et al.* 2001). Cross resistance to phosmet and carbaryl (a carbamate) and increased tolerance to pyrethroids and methomyl were also reported (Pree *et al.* 2001). The data also indicated that resistance was unstable, i.e., partially resistant or mixed populations tended to revert quickly towards susceptibility in the laboratory. Reported here are results of studies to assess the stability and reversion of resistance in field populations at three separate sites. Two programs were assessed: a grower-selected or -devised program that removed all OP and carbamate applications against

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OBLR, but used OP and pyrethroid insecticides against other apple pests (Table 1). The second was a more extensive insecticide resistance management program (IRM) that generally substituted *Bacillus thuringiensis* Berliner (Bt) preparations or spinosad for azinphosmethyl against OP resistant populations for OBLR and minimized applications of OP and carbamates using pyrethroids preferentially against other apple pests. This second program was designed to avoid selection of OP or pyrethroid resistance in OBLR populations that might occur when these pesticides were applied to the system for control of other insects. We anticipated that OP, carbamate, and pyrethroid resistance in OBLR would decrease more quickly in those plots where OP, carbamate and pyrethroid applications would be minimized.

Materials and Methods

Establishment of field plots and protocols for treatments

To assess the potential for management of resistance in OBLR to OP insecticides, two plots (1.8-6 ha) were established in spring 2000 at each of three Ontario apple orchards (Grimsby in the Niagara production area, and Brighton and Grafton in Northumberland) with one of the plots receiving the grower-devised program.

At Grimsby (43°11'N, 79°33'W), the plots were cv Red Delicious. The grower-devised program was applied to 1.8 ha consisting of 24 rows of 53 2.2m tall trees spaced 4.8 x 3m. The plot was bordered on the north by tart cherries, by additional apple plantings on the east and west, and by pastureland on the south. The IRM program was applied to 5.7 ha, consisting of 51 rows of 125 trees 2.2m tall spaced 3 x 4.8m. Data were from 27 rows of Red Delicious; other rows of cv. McIntosh and Empire were not sampled. Plots were separated by about 500m, largely planted to tart cherries. The IRM plot was adjacent to additional apple plantings on the north and west sides, which were treated with grower-devised programs. Both plots were sprayed by the grower with a Turbomist sprayer calibrated to deliver 760 L/ha.

At Brighton (44° N 1'N, 77° 43'W), the plots were McIntosh. The grower-devised program was applied to 3.6 ha consisting of 30 rows of 25 3m tall trees spaced 5.5 x 2.4m (this plot comprised about half of a ca. 6 ha planting of McIntosh with 5 rows of Empire in the centre). The plot was bordered by woods on the north and west, by a grassed field on the east and by an additional grower-sprayed orchard on the south. The IRM program was applied to 6 ha of which we used ca 3.8 ha consisting of 13 rows of 45 3m tall McIntosh trees spaced 6.1 x 2.4m, with the rest of the plot planted to cv Paulared and McIntosh. The plot was ca 500m north of the woods and was largely surrounded by wood lots. Both plots were sprayed with an FMC NW 430 sprayer at 660 L/ha.

At Grafton (44° 0'N, 77° 57'W) plots were cv Idared. The grower-devised program was applied to 2.7 ha consisting of 46 rows of 64 2.8m trees spaced 4.3 x 2.1m in of which we used 1.2 ha planted to Idared. The rest of the plot was planted to McIntosh with four rows of cv Cortland adjacent to the Idared planted portion. This plot was bordered by a highway with additional apple plantings on the north and by grassed fields on the other three sides. The IRM program was applied to 2.1 ha which was part of a larger planting of ca. 6.5 ha of cv. McIntosh, Cortland and Idared. The 2.1 ha plot consisted of 21 rows of 64 3.2m trees spaced 5.5x 2.8m. This plot was 750m northeast bordering the additional apple plantings, and was located in the northeast quadrant of the 6.5 ha and bordered the rest of the planting on the west and south sides. The north and east sides were adjacent to pasture. The grower applied his own preferred program to the rest of the planting. Both plots were sprayed with an FMC 250 sprayer at 440 L/ha.

TABLE I. Insect control programs applied by growers 2000-2002.

	OBLR		STLM	CM	OFM	MB	APHIDS/LH	PC	AM/CM	THINNING
	Spring	Summer								
Grimsby - Grower -devised - Red Delicious										
2000	cypermethrin	-	-	-	phosmet	diazinon	-	-	-	-
2001	cypermethrin	-	-	-	phosmet	-	-	-	-	-
2002	cypermethrin	-	-	-	cypermethrin	-	pirimicarb/diazinon	-	-	-
Grimsby - IRM - Red Delicious										
2000	Bt (3)1	Bt (2)	-	tebufenozide	-	-	pirimicarb	-	-	Accel* hand
2001	Bt (2)	Bt	-	tebufenozide	-	imidacloprid	-	-	-	-
2002	Bt	Bt	-	-	cypermethrin(2)	-	pirimicarb	-	-	-
Brighton - Grower -devised - McIntosh										
2000	Bt	Bt	-	phosmet	-	-	-	phosmet	phosmet	carbaryl
2001	deltamethrin	spinosad	-	phosmet	-	-	-	phosmet	phosmet	carbaryl
2002	deltamethrin	Bt/spinosad	-	-	-	-	-	phosmet	phosmet	carbaryl/ NAA ²
Brighton - IRM - McIntosh										
2000	Bt	Bt	-	tebufenozide	-	-	imidacloprid (border)	phosmet (border)	phosmet	hand
2001	Bt	spinosad	-	tebufenozide	-	-	imidacloprid	phosmet (border)	phosmet (border)	hand
2002	Bt	spinosad	-	-	-	-	-	-	phosmet (border)2	hand
Grafton-Grower-devised-Ida Red										
2000	deltamethrin/Bt	Bt	deltamethrin	phosalone(2) phosmet(2)	-	-	-	azinphosmethyl	phosmet(3)	carbaryl
2001	Bt	spinosad	deltamethrin	phosmet(2)	-	-	imidacloprid (border)	azinphosmethyl	phosmet	carbaryl
2002	deltamethrin	Bt	-	tebufenozide	-	diazinon	phosalone(2)	-	phosmet(3)	carbaryl

Growers were advised of the occurrence of resistance in OBLR populations at their sites and all elected to use alternative insecticides to azinphosmethyl in their programs. The IRM had a more extensively modified program with no applications of OP, carbamate, or pyrethroid insecticides (Table I). Where alternatives were unavailable or were unacceptable to the grower, border sprays of OP or pyrethroid insecticides (applied to 1-2 outside rows) were used as deemed necessary by the grower. Border sprays were not used in the grower-devised plots. Rates used were those recommended in the provincial fruit production guide (Anonymous 2002).

Insecticides for control of overwintered (spring) OBLR were applied about petal fall (early June). For control of the first summer generation of OBLR, (late June-July), sprays were timed according to established degree day models (Solymar 1999) using five pheromone (OBLR lure, Trece, Salinas CA) baited traps (Pherocon II, Trece) at each site to obtain the date of the first sustained moth captures. Traps were separated by at least 30m and examined twice weekly as described in the protocol outlined by Solymar (1999). First applications of insecticides were delayed until 240-260 degree days Celsius (DDC) (base 6.1°C). Applications were repeated if emergence of moths was extended. Insecticides were applied for other pests as identified as necessary by the growers but trap catch data from codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) and apple maggot *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae) traps as well as weekly scouting for the occurrence of mullein plant bugs, *Campylomma verbasci* (Meyer) (Hemiptera: Miridae), aphids, *Aphis pomi* (DeGeer) and *Dysaphis plantaginea* (Passerini) (Homoptera: Aphididae) etc. were provided to support spray timing and/or needs for control treatments (Table I). Sampling procedures, traps, appropriate timings, etc., were as outlined by Solymar (1999).

Monitoring of resistance

OBLR larvae (200-400) of the overwintered (spring) generation were collected from each plot at each site in spring of each year of the study (2000-2002) just after bloom and prior to any insecticide treatments, and placed on an artificial diet (Pree *et al.* 2001). Moths developing from these collections were reared and larvae produced from mass crosses were assayed for resistance using a Potter spray tower and analytical grade samples of insecticides dissolved in acetone (Pree *et al.* 2001). Concentrations used as diagnostic were 40 mg/kg for azinphosmethyl and 2.5 mg/kg for cypermethrin (a pyrethroid). At these diagnostic concentrations, all susceptible individuals were killed; the 40 mg/kg concentration of azinphosmethyl killed ca. 15% of a laboratory selected OP-resistant population used as a reference or standard resistant population in earlier studies (Pree *et al.* 2001) and the 2.5 mg/kg solution of cypermethrin killed ca. 50% of a similarly selected pyrethroid-resistant population. For tests, we used 10 replications of 10 larvae at each diagnostic concentration. Tests were conducted on at least two days and larvae were from at least 6 egg masses.

Because the inequality of variances prevented transformation of these data to fit a normal distribution, the percentage of resistant individuals in each plot in each year was compared using the Mann-Whitney rank sum test (Zar 1974) ($P < 0.05$, SigmaStat, Version 2, SPSS Inc., Chicago IL). Comparisons were made separately between treatments (grower-devised vs. IRM) and for changes in population responses between years for each treatment.

Efficacy

The effectiveness of the programs in controlling OBLR was determined approximately one week prior to harvest by assessment of fruit for larval feeding damage. Sample trees (5% of the

trees in each plot) were selected randomly, and 50 fruit were examined on each tree. Ladders were used on larger trees and adjacent trees were sampled where less than 50 fruit were available. Percentage fruit damage from each plot at each site was compared with the Mann-Whitney rank sum test (SigmaStat). Overall means for fruit damage at the three sites in the grower-devised and IRM plots were compared using the same test.

Results and Discussion

Insect control programs at the three sites varied widely, with the grower's experience dictating the need for the various insecticides applied for pests other than leafrollers (Table I). The sites at Brighton and Grafton had more insecticides applied than the Grimsby plots. Growers were advised of the presence of OP resistance in OBLR populations in both plots at their sites and all avoided the use of OPs for control of OBLR. Therefore, any differences or reductions in resistance observed between the two programs were likely associated with resistance selection caused by OP (or pyrethroid) use for other pests. The number of treatments of OP insecticides applied for control of other insects or carbaryl (a carbamate insecticide) for thinning (removal of excess small fruit) in mid-late June varied from one-two at Grimsby to four-five at Brighton, and to four-nine at Grafton (Table I).

In 2000 at Grimsby in the grower-devised plot, for OBLR the grower made a single application of cypermethrin (Table I). In the IRM plot, he made three applications of Bt, as Foray 48BA, for control of the spring generation and two more for the first summer generation. The OBLR populations in the grower-devised plot were reduced to levels that prevented further samples in that plot in the subsequent years of the test. There was no significant difference for the OP resistance and the pyrethroid susceptibility between the populations of the two plots in 2000 (Tables II and III). However, the OP resistance significantly decreased from 2000 to 2002 in the IRM plots, and the pyrethroid susceptibility did not change over that same period.

In 2000-2002 at Brighton in the grower-devised plot, for OBLR the grower made a combination of Bt, spinosad and deltamethrin applications (Table I). In the IRM plot, he made applications of Bt and spinosad. There was no significant difference in the OP resistance between the populations in 2000 and 2002, however, in 2001 the OP resistance in the grower-devised plot was significantly greater than in the IRM plot (Table II). OP resistance significantly decreased after two years of treatments in the grower-devised plot from 2000 to 2002, whereas, in the IRM plot OP resistance significantly decreased after one year (Table II). Pyrethroid susceptibility was significantly greater in the grower-devised plots than in the IRM plots in 2000 and 2001, however, in 2002 there were no significant difference between the plots (Table III). Pyrethroid susceptibility significantly increased after two years in the grower-devised plot from 2000 to 2002, whereas in the IRM plots there was no change in susceptibility (Table III).

In 2000 at Grafton, the OBLR population (or at least a substantial portion of the population) was exposed to a prebloom pyrethroid treatment applied to control spotted tentiform leafminer (*Phyllonorycter blancardella* F.). Therefore, except at Grafton, estimates of the percentage resistance in populations (Tables II and III) were established early in 2000 prior to any insecticide use. Changes in resistance are measurements of the impact of control programs used in 2000-2002.

In 2000-2002 at Grafton in the grower-devised plot, the grower made a combination of Bt, spinosad and deltamethrin applications (Table I) for OBLR. In the IRM plot, he made applications of Bt and spinosad. There was no significant difference in the OP resistance between the populations

in 2002, however, in 2000 the OP resistance in the IRM plot was significantly greater than in the grower-devised plot and the reverse was true in 2001 (Table II). OP resistance in 2000 was significantly lower than in 2001 and 2002 in the grower-devised plot, whereas in the IRM plot the reverse was true (Table II). Pyrethroid susceptibility was significantly greater in the grower-devised plots than in the IRM plots in 2001 and 2002, however, in 2000 there were no significant difference between the plots (Table III). Pyrethroid susceptibility was variable in the same way in both plots from 2000 to 2002; the OBLR populations were significantly more resistant to pyrethroids in 2001 relative to 2000 and 2002 (Table III). Application of a pyrethroid (cypermethrin) to a similar population at the Grimsby site reduced populations to levels, which prevented further samples for resistance testing. In this case, numbers of OBLR surviving in this plot were high enough to allow collection of a sample adequate (>200 larvae) for tests of resistance frequencies.

At both Grimsby and Brighton, in populations sampled in spring 2000, prior to the establishment of the two programs, the grower-devised and IRM had similar resistance levels (Table II). At both sites, the populations in both plots were largely resistant to OP insecticides (71-87% survival) but were susceptible to pyrethroids (0-19% survival). At Brighton, OP and pyrethroid resistance was not detected after one year (as measured in 2001) in the IRM plot and after two years in the grower-devised program plot (as measured in 2002). Both of these plots were adjacent to large wood lots on at least two sides and migration of susceptible moths from these areas may have influenced the rate of changes in the susceptibility of these populations. OBLR has an extremely wide host range and larvae could develop on many rosaceous or nonrosaceous species (Chapman and Lienk 1971). Both treatment groups at Brighton and the IRM group from Grimsby remained susceptible to pyrethroids (Table III).

At Grafton, OP resistance was initially higher in the IRM plot than in the grower-devised plot (84% vs. 45%). Under the grower's program, OP resistance increased from 45% to 91%, probably associated with the extensive OP program used for other insects. While OP resistance declined somewhat in the IRM plot in the first year of the test (from 84% to 64%), the decline was not continued in the second season. This plot received approximately nine border sprays of OP insecticides in the second season and these may have affected any further decline of this resistance. The percentage of the population exhibiting pyrethroid resistance was unchanged over the two seasons in both plots, even though after one year OBLR populations were more resistant (Table III).

The decrease in damage to apples in Grimsby and Brighton by the OBLR from 2000 to 2002 (Table IV) was parallel to the trend of decreasing resistance of OBLR to both OP and pyrethroids (Tables II and III). However, damage declined in plots with either treatment over the three years of the study. This was probably associated with the large initial variation in infestations between sites in 2000. However, at Grafton in spite of the trend of decreasing damage to apples over the same period (Table IV), there was no similar parallel decrease in OP and pyrethroid resistance (Tables II and III).

Overall, OP resistance declined, or, at least, did not increase in plots with a IRM strategy that avoided the use of OP and pyrethroid insecticides both for OBLR and for other pests. OP resistance also declined at Brighton under the grower-devised program that avoided the use of OP or carbamate insecticides for OBLR control. Resistance was lost more rapidly in the IRM plot at Brighton. It is likely that the extensive use of border sprays of OP insecticides (up to six were applied to the IRM plot) at Grafton delayed or reduced the decline in the numbers of

TABLE II. Management of resistance to organophosphorus insecticides in populations of oblique-banded leafroller 2000-2002.

Population	Program	Percentage Survival at Diagnostic Concentrations		
		Azinphosmethyl		
		2000	2001	2002
Grimsby	Grower	80 a ¹	-	-
	IRM	83 aA ²	43B	22 C
Brighton	Grower	87 aA	52 aB	0 aC
	IRM	71 aA	0 bB	0 aB
Grafton	Grower	45 aB	88 aA	91 aA
	IRM	84 bA	64 bB	68 aB

¹ Numbers in same column within each site followed by same lower case letter are not significantly different by Mann-Whitney rank sum test P<0.05.

² Numbers in same row for within each plot at each site followed by same capital letter are not significantly different by Mann-Whitney rank sum test P<0.05.

TABLE III. Management of resistance to pyrethroid insecticides in populations of oblique-banded leafroller 2000-2002.

Population	Program	Percentage Survival at Diagnostic Concentrations		
		Cypermethrin		
		2000	2001	2002
Grimsby	Grower	7 a ¹	-	-
	IRM	9 aA ²	3 A	2 A
Brighton	Grower	19 aA	16 aA	0 aB
	IRM	0 bA	0 bA	1 aA
Grafton	Grower	56 aB	84 aA	57 aB
	IRM	41 aB	66 bA	38 bB

¹ Numbers in same column within each site followed by same lower case letter are not significantly different by Mann-Whitney rank sum test P<0.05.

² Numbers in same row within each plot followed by same capital letter are not significantly different by Mann-Whitney rank sum test P<0.05.

TABLE IV. Damage to apples by the oblique-banded leafrollers. 2000-2002.

		Percent Apple Damage		
		2000	2001	2002
Grimsby				
	Grower	1.2 ± 1.9 ¹ a	1.4 ± 2.2 a	0.09 ± 0.4 a
	IRM	14.7 ± 9.6 b	5.5 ± 5.8 b	0.9 ± 1.7 a
Brighton				
	Grower	35.2 ± 22.4 a	4.4 ± 2.2 a	4.0 ± 3.3 a
	IRM	9.8 ± 9.5 b	6.3 ± 4.2 a	8.0 ± 4.4 b
Grafton				
	Grower	4.6 ± 5.0 a	3.1 ± 3.7 a	2.8 ± 2.9 a
	IRM	15.4 ± 8.2 b	2.6 ± 2.4 a	0.9 ± 2.0 b
Mean ± SE				
	Grower	13.7 ± 18.7 ² A	3.0 ± 1.5 B	2.3 ± 2.0 C
	IRM	13.3 ± 3.1 A	4.8 ± 1.9 B	3.3 ± 4.1 C

¹ Numbers in same column for each site followed by same lower case letter are not significantly different by Mann-Whitney rank sum test $P < 0.05$, comparisons are within each site.

² Numbers in same column for overall mean for each year followed by same capital letter are not significantly different by Mann-Whitney rank sum test $P < 0.05$.

resistant larvae in those populations. In laboratory studies, Pree *et al.* (2001) found that resistance declined from 88% to about 12% in the laboratory over three generations when selection was not applied to a newly colonized population. Smirle *et al.* (1998) reported similar declines in resistance in laboratory populations from Quebec. In these field studies, we found similar rapid losses in resistance when selection pressures were removed or minimized. Similar rates of loss were observed with a pyrethroid -selected laboratory colony (Pree, unpublished data).

The data generally indicate that resistance in the OBLR can be managed and that programs which use azinphosmethyl in a rotation with Bt preparations and/or spinosad, compounds that do not exhibit cross resistance to OP or pyrethroids (Pree unpublished, Ahmad *et al.* 2002), may be sustainable. The data also indicate that pesticides applied against pests other than OBLR are important in the selection of OBLR populations for resistance and must be considered as part of any resistance management strategy. However, as the data indicate, resistance management strategies that consider these aspects can be successful and can result in rapid loss of resistance.

A rotational program that incorporates azinphosmethyl is about 50\$/ha/yr cheaper than a program that relies on a rotation of Bt and spinosad. However, a number of the products used as replacements for OP and pyrethroid insecticides (e.g., tebufenozide, imidacloprid etc.) are costly and may make the modified program more expensive overall. The exact cost is likely to be highly variable and dependant upon the pests identified as problems in the various orchards.

However, the value of continued efficacy and potential sustainability of the program to the producer must also be considered. This program may lose some feasibility if the use of

azinphosmethyl is severely reduced or curtailed, but the broad range of compounds involved in the resistance suggests that similar programs with other compounds may be feasible. Carrière *et al.* (1996) have shown a positive correlation between resistance to azinphosmethyl and resistance to cypermethrin in populations from Quebec but our field studies identified two populations (Grimsby and Brighton) that were resistant only to OP insecticides and not to pyrethroids.

However, the population from Grafton was resistant to both groups of insecticides. These observations and those of Ahmad *et al.* (2002) and Pree *et al.* (2002) suggest that resistance in the OBLR, to these different insecticides, is a variable system and that the importance of the various resistance mechanisms may vary with the different types of insecticides used in control programs. Despite the broad-spectrum resistance to many insecticides associated with OP or pyrethroid resistance in the OBLR, a potentially sustainable resistance management strategy involving the rotational use of three groups (Bt, spinosyn, and OP) of insecticides is feasible.

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