STATUS OF RESISTANCE TO INSECTICIDES IN POPULATIONS OF THE ORIENTAL FRUIT MOTH *GRAPHOLITA MOLESTA* (BUSCK) (LEPIDOPTERA: TORTRICIDAE) IN SOUTHERN ONTARIO

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Abstract

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Populations of Oriental fruit moth *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) were assessed for levels of resistance to organophosphorus (OP) and pyrethroid insecticides approximately 10 years after initial assays identified the resistance, and 6-8 years after a resistance management strategy was introduced for use in peach production systems. Resistance to OP insecticides was detected at all three locations tested (Niagara, Norfolk, and Essex). Resistance frequencies had increased at one site (Jordan Station Experimental Farm) that had been monitored closely in 1999; however, frequencies at that site did not increase over the three years reported here. Results also indicated that pyrethroid resistance had declined in the Niagara area, occurred at low levels in the Norfolk area, and was not found in the Essex area. Mechanisms and cross resistances between OP and carbamate insecticides appeared similar to those described in earlier studies. Resistance was associated with elevated general esterase activity and the presence of an acetylcholinesterase which was less sensitive to inhibition than in susceptible populations. Resistance to azinphosmethyl and phosmet was expressed at low levels but high levels of resistance was expressed to the methyl carbamates, carbaryl, or carbofuran. Chlorpyrifos was equally toxic to both susceptible and resistant populations. Resistant populations were more susceptible to acephate. All of these characteristics were similar to the resistance described in previous reports. Chlorpyrifos, which is scheduled to be deregistered in 2006, may be replaced by the ecdysone agonist methoxyfenozide or the neonicotinoid acetamiprid. The data indicated low levels of resistance (1.7 fold at the LC_{50}) for methoxyfenozide associated with OP resistance, but control of the first generation was achieved in both small plot and program trials. Later applications were less effective. Acetamiprid was generally effective throughout the season and was equally toxic to both OP resistant and susceptible populations. In field trials over two seasons, neither of these products was associated with outbreaks of phytophagous mites. However,

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the potential fit of these products into IPM programs for peach will need further assessment.

Introduction

An integrated pest management (IPM) program for peach, introduced to growers in the mid 1970's, was the first widely used IPM program in Ontario. The program relied on the use of pheromone trap catch data to time applications of the organophosphorus insecticides azinphosmethyl and phosmet to control the Oriental fruit moth (OMF), *Grapholita molesta* (Busck) (Lepidoptera: Tortricidae) (Phillips 1973). This program remained effective for approximately 20 years but resistance to these insecticides resulted in up to 45% fruit infestations in 1993 and 1994 (Pree et al. 1998). This was the first documented occurrence of resistance to pesticides in *G. molesta* worldwide. Tests with neonate larvae (the targeted life stage in the field) indicated cross-resistance to most OP insecticides, except acephate and chlorpyrifos. Acephate was more toxic to resistant larvae than to susceptible larvae, and chlorpyrifos was equitoxic to both populations. Resistance was highest (>100 fold) to methyl carbamates, carbaryl, and carbofuran. Cross-resistance to pyrethroids was not observed. Based on these observations and additional small plot tests, growers switched to programs of repeated applications of pyrethroids. Concerns were expressed (Pree et al. 1998) that a program of repeated pyrethroid use might accelerate the development of further resistance. Therefore, an interim resistance management strategy was implemented which consisted of 1-2 applications of chlorpyrifos for the first annual generation, followed by pyrethroids for later generations. From 1996-1999, monitoring of resistance changes in commercial orchards using this program showed that resistance to OP insecticides declined from about 50% to 12%, while pyrethroid resistance was approximately 16% (Kanga et al. 2003). While this program successfully reduced the risk that resistance to pyrethroids might become common, registration of chlorpyrifos was granted only on a temporary and annual basis, and deregistration is scheduled for 2006 (Pest Management Regulatory Agency 2003).

We report on the current status of resistance to organophosphorus (OP) and pyrethroid insecticides in both apple and peach plantings in southern Ontario, provide an update on the mechanisms of OP resistance, and compare both of these findings to previous studies. Further, with the impending removal of chlorpyrifos, we also report on the effectiveness of potential alternative materials and how they might fit into both Integrated Pest Management and resistance management programs for Oriental Fruit Moth populations in tree fruit.

Methods

Oriental fruit moth populations

The population of Oriental fruit moth used as the standard susceptible population was the same colony used in earlier studies (Pree et al. 1998; Kanga et al. 1999) and unless otherwise indicated was maintained on small green apples as described by Pree (1985).

This colony has been maintained unselected with few infusions of field collected insects (none since 1985) for approximately 50 years.

The standard resistant population in these tests was collected from a mixed apple/peach/pear planting near Beamsville, ON in 2002. Initial tests using a standard field resistance monitoring procedure (Kanga et al. 1999) indicated that approximately 75% of the population was resistant to OP and carbamate insecticides. The colony was established on apple from 200-300 larvae. Larvae of this population were selected in each generation with carbaryl at 150 mg/L using a Potter spray tower using procedures described by Pree (1979). Newly hatched larvae were held in Petri dishes on ice and sprayed with 5 ml of a 150 mg/L solution of carbaryl in analytical grade acetone. Sprayed larvae were transferred to apples in a standard rearing container (Pree 1985). Laboratory bioassays with neonate larvae were conducted with the 9^{th}-15^{th} laboratory selected generations.

For tests with methoxyfenozide, which is more active when ingested, we developed subcolonies of each population adapted to an artificial diet. The diet was modified from that of Yokoyama et al. (1987) and initially resulted in some larvae mortality (previously adapted to green apples), but this decreased after 3-4 generations on the diet. Larvae were used for tests after at least 6 generations on the diet. Three or four Oriental fruit moth neonate larvae were placed in plastic cups (souffles) (P100, 25 ml capacity, SOLO Cup Company, Urbana, IL) each containing about 10 ml of diet. Pupae were removed after 3-4 weeks and held in rearing jars (Pree 1985) for adult emergence.

Preparation of artificial diet

The diet consisted of: 3.0 g methyl-*p*-hydroxybenzoate, 1.8 g sorbic acid, 7.0 g *L*-ascorbic acid, 10.5 g fructose, 13.0 g Vanderzant vitamin mix, 17.5 g α-protein (soybean protein), 35.0 g wheat germ, 70.0 g Brewers yeast, 350.0 g ground pinto beans (BioServ, Frenchtown, NJ), and 1500 ml distilled water.

Unless indicated, all ingredients were from ICN Biomedical (Aurora, OH). Dry ingredients were blended for approximately 30 seconds with 1500 ml distilled water until a smooth consistency was obtained. A 1 L media bottle containing 500 ml distilled water and 16 g of agar was autoclaved until the mixture boiled, and the warm agar was thoroughly mixed with the aqueous nutrient mixture. Warm diet mix was transferred to plastic squeeze bottles and dispensed into individual cups. Cups (25 ml capacity) were filled to an approximate depth of 1.5 cm, allowed to cool at room temperature, capped when condensation had disappeared, and stored at room temperature until needed. The quantities listed here provided approximately 400 individual cups of diet.

BIOASSAYS

Determination of resistance frequencies in field population

Resistance frequencies for OP and pyrethroid insecticides in the various orchard populations and locations were monitored as described by Kanga et al. (1999; 2003). Adult males captured in pheromone-baited traps were brought to the laboratory and fed overnight with a 10% sucrose solution. They were then exposed to insecticides in glass vials as described by Kanga and Plapp (1995). For tests with the Niagara populations, one or two moths were placed in each vial and held for 24 hours in a cabinet at 22 ± 2°C, 60% relative humidity (RH), and 16:8 Light:Dark (L:D) cycle. Assays with populations from Norfolk

and Essex were conducted in the test areas on a laboratory bench where temperatures were similar but RH values were lower (about 40%). Adults unable to fly when tossed into the air were considered dead (Kanga et al. 1999). The diagnostic concentrations used in all bioassays were those used by Kanga et al. (2003) but were verified as diagnostic prior to use here. Concentrations used were 0.1 µg/vial for carbofuran (which indicates both OP and carbamate resistance and used because the higher level of resistance results in a better separation of resistant and susceptible populations) (Kanga et al. 2003) and 2.5 µg /vial for cypermethrin (as diagnostic of pyrethroid resistance). Both of these concentrations killed all of a susceptible population. The carbofuran treatment did not affect any of an OP resistant population whereas in tests by Kanga et al. (1999), cypermethrin at 2.5 µg/ vial killed approximately 25% of a pyrethroid resistant population. We did not have a pyrethroid resistant population for comparison. There were 100-250 male moths tested for each compound per generation per site reported. Data from 3-4 days trapping were combined and mean survival rate over the generation trapped is presented.

Contact toxicity tests

Tests with contact insecticides on neonate larvae were similar to those described by Pree et al. (1998). Insecticides, technical or analytical grade, obtained either from the manufacturers or from Chem Services (West Chester, PA), were applied to first instar larvae with a Potter spray tower in 5 ml of analytical grade acetone. After treatment, larvae were held in plastic Petri dishes (Falcon 1006, Becton Dickinson, Lincoln Park, NJ) for 2 hours at 22 ± 2°C and 60% RH. Larvae that were unable to crawl when prodded were considered dead. Mortality data from six concentrations of each insecticide with 10 replications of 10 larvae were used to plot regression lines of concentration vs. mortality. Data were subjected to probit analysis (POLO-PC, Le Ora Software, Berkeley, CA). Resistance ratios were considered significantly different if the 95% confidence limits at the LC_{50} did not overlap.

Insecticide-diet mixtures

For tests with artificial diets, measured amounts of commercial formulations of the test chemicals, methoxyfenozide (Intrepid 240F, DowAgrosciences Canada Inc, Calgary, AB) or acetamiprid (Assail 70WP, Dupont Canada Inc, Mississauga, ON) were diluted in 10 ml of distilled water and added to 390 ml of freshly prepared diet to provide the desired final concentration expressed as mg/l active ingredient (ai). The diet and test chemical were mixed thoroughly in a Waring blender and distributed approximately evenly into 50 SOLO cups (P100, SOLO Cup Company). Two neonate larvae were added to each cup and held in a cabinet at 22 ± 2°C, 60% RH, and a 16:8 L:D regime. Five concentrations plus a water treated control were used for each chemical and each population with 10 replicates of 5 cups each. Tests were set up over at least 2 days with fresh insecticide, diet preparations and newly hatched larvae each day. Mortality was assessed after 4 and 6 weeks when cups were examined for pupae. Cups containing no pupae were rated as negative or dead. Cups which held one or 2 pupae were classed as positive (alive). Data were expressed as the proportion of cups with dead larvae based on 10 replicates of 10 cups each (percent mortality). Mortality in controls (i.e. control cups which produced no pupae) was 4-6%. Concentration:mortality data were analyzed by probit analysis as described for contact toxicity tests above. Differences between responses were considered significantly different

if the 95% confidence limits at the LC_{50} did not overlap.

BIOCHEMICAL ASSAYS

General esterase

Esterase activity in the susceptible and OP-resistant populations was measured using a procedure adapted from Herath et al. (1987) that used α-naphthyl acetate as a substrate. The reaction mixture consisted of 800 µl of α-naphthyl acetate (0.3 mM) in 0.1 M, pH 7.2 phosphate buffer, and 100 µl of insect homogenate. One adult abdomen/ml was homogenized in ice-cold phosphate buffer and the homogenate centrifuged at 10,000 g for 10 minutes at 4°C. The supernatant was used in assays. The reaction was run in a 1.5 ml Eppendorf tube in an Eppendorf Thermomixer at 37°C and 450 rpm. The reaction was stopped after 15 minutes with 80 µl of a solution of 100 mg of Fast Blue B salt in 50 ml of a 5% solution of sodium dodecyl sulfate. The change in absorbance at 450 nm was measured on an Ultraspec 3100 pro spectrophotometer (Biochrom Ltd, Cambridge, UK). There were 15 replications over 12 different days using 112-121 insects for each population. Protein concentrations in tissue homogenates were determined by the method of Bradford (1976). Mean esterase activities were compared using an unpaired t-test (P<0.05) (Sigmastat Version 2.0, SPSS Inc, Chicago, IL).

Acetylcholinesterase assays

Acetylcholinesterase (AChE) activity was measured using acetylthiocholine as a substrate (Ellman et al. 1961). Inhibition of AChE was determined using methods adapted from Moores et al. (1988) and Pree et al. (2003). For assays, moth heads were frozen at –70°C for at least 30 minutes and each head was placed into a 1.5 ml Eppendorf tube; 50 µl of 0.1 M phosphate buffer (pH 7.5) was added, and the head was ground for 10-15 seconds. This homogenate was held on ice until used. The reaction mixture was 25 µl of homogenate, 50 µl of 1 mM 5,5-dithio-bis (2-nitrobenzoic acid) (DTNB) in 0.1 M phosphate buffer (pH 7.5), and 100 µl of 0.1 M phosphate buffer containing Triton X-100 (10 g/l). This mixture was equilibrated for 2 minutes in an Eppendorf tube and the reaction started with the addition of 20 µl of the substrate (1mM acetyl thiocholine iodide). For inhibition tests, 20 µl of 10^{-4} M carbaryl in ethanol was placed in tubes prior to the addition of the reaction mixture and the ethanol evaporated. The rate of change at OD 405, was measured for the initial 10 minutes of the reaction in an Ultraspec 3100 Pro spectrophotometer. For protein determinations, one adult Oriental fruit moth head was ground in 50 µl of 0.1 M phosphate buffer with no Triton X-100 and 10 µl was used in the Bradford (1976) assay for protein with bovine serum albumin as the standard. Activity (and inhibition at 10^{-4} M carbaryl) was determined for males and females of both populations. Data were based on 12-14 replications of 96-101 individuals. Differences between means were identified by an analysis of variance and a Tukey test (P<0.05) (Sigmastat Version 2.0 SPSS, Chicago, IL).

FIELD TESTS OF ALTERNATIVE INSECTICIDES

Trials were conducted at the Jordan Station Experimental Farm of Agriculture and Agri-Food Canada (AAFC), Jordan, ON. For tests in small plots, treatments were replicated 4 times, assigned to 2 tree plots arranged according to a randomized complete block design. Based on pheromone trap catches of male moths in adjacent or nearby plantings,

applications were timed for egg hatch of the first or second generations of Oriental fruit moths using standard methods (Pree et al. 1983) and a phenology model (Rice et al. 1982) that used 7.2°C as a base temperature. Trees were spaced 4.6 x 5.5 m and were 3-6 years old as indicated. Insecticides were diluted to a rate comparable to 3,000 L/ha and trees sprayed to runoff with a truck-mounted sprayer (Rittenhouse Sprayers Ltd., St. Catharines, ON) equipped with a Spraying Systems handgun (Spraying Systems Co., Wheaton, IL) fitted with a D-6 orifice plate. Pressure was set at 2,000 kPa. Nine to thirteen L of spray mix was applied per plot. Plots were assessed 10-19 days post-spray when all twig and fruit damage was removed and counted. Data were analyzed using an analysis of variance and a Tukey test ($P<0.05$).

In larger scale trials, insecticides were applied to approximately 0.50 ha blocks of mature cv. Loring peach trees at the AAFC Jordan Station Experimental Farm in season-long programs. All plots were treated with superior oil (60 L/ha) as a dilute spray (20 L/1000 L water) in April for control of overwintered eggs of European red mite (*Panonychus ulmi* (Koch), Acari: Tetranychidae).

In 2003, we assessed 3 programs. The most widely used commercial program for control of Oriental fruit moth consists of 1.7 kg ai/ha chlorpyrifos (Lorsban 50W, Dow AgroSciences, Calgary, AB), for generation 1, followed by pyrethroids for generations 2 and 3 (program 1). In our tests we used 10 g ai/ha deltamethrin (Decis 5 EC, Bayer CropSciences, Calgary, AB). Program 2 was 360 g ai/ha methoxyfenozide (Intrepid 240F, Dow AgroSciences) for generation 1, followed by deltamethrin for generations 2 and 3. Program 3 was chlorpyrifos for generation 1 followed by methoxyfenozide for generations 2 and 3. Treatments were applied using a Rittenhouse GB Laser P20 sprayer (Rittenhouse Sprayers Ltd., St. Catharines, ON) set to deliver 840 L/ha and were timed using standard procedures (Pree et al. 1983) based on data from pheromone-baited traps placed in test plots or in nearby peach plantings. Insecticides were applied 30 May for generation 1, 16 July and 1 August for generation 2 and 30 August for generation 3 (and as a preharvest treatment). Infested terminals were assessed 17 June and 30 July when all of the terminals on 10% of the trees in each plot were examined for damage by larvae. At harvest, on 8 and 10 September 2003, we examined 10-12 of the ripest fruit on each tree for Oriental fruit moth damage. Further, 20% of these fruit were cut apart and checked for damage not visible from surface assessments. Data from twig damage and fruit assessments did not fit a normal distribution and attempts to transform data were unsuccessful (based on Kolmogorov-Smirnov test with Lilliefors' correction), so were analyzed using a Kruskal-Wallis test (Sigmastat Version 2.0).

Mite populations were assessed 25 August when 3 replicate samples of 100 leaves were collected from each plot. Leaves were examined for numbers of European red mite *Panonychus ulmi* (Koch) (Acari:Tetranychidae), peach silver mite *Aculus cornutus* Banks (Acari: Eriophyidae), and for predaceous mites (Acari: Phytoseidae). For each sample, 20 leaves were examined under a binocular microscope and an additional 80 leaves brushed with a Henderson-McBurnie mite brushing machine (Henderson and McBurnie 1943). Peach silver mite infestations were assessed on 20 leaves/sample. Infestations were rated on a scale of 0-5: 0 = 0 mites/leaf; 1 = 1-10 mites/leaf; 2 = 11-25 mites/leaf; 3 = 26-50 mites/leaf; 4 = 51-100 mites/leaf; 5 = 101+ mites/leaf. After testing for fit in a normal distribution, data were analyzed by analysis of variance with differences between treatment

means identified using a Tukey test ($P<0.05$).

In 2004, 4 programs were assessed: Program 1-chlorpyrifos for generation 1 followed by deltamethrin for generations 2 and 3; Program 2-methoxyfenozide for generation 1, with deltamethrin for generations 2 and 3; Program 3-acetamiprid for generation 1, methoxyfenozide for generations 2 and 3; and Program 4-methoxyfenozide for generation 1, acetamiprid for generations 2 and 3. Rates used were as in 2003 with acetamiprid (Assail 70WP) applied at 168 g ai/ha. Treatments were timed as described by Pree et al. (1983) with applications on 21 May for generation 1, 9 July for generation 2, and 6 and 24 August (preharvest) for generation 3. On 8 June and 28 July, infested terminals were counted and removed on 10% of the trees, selected randomly, in each block. At harvest, 27 and 30 August, we examined 10-12 of the ripest fruit on each tree for damage by Oriental fruit moth larvae. As in 2003, we cut apart 20% of these fruit and checked for damage not visible from surface assessments. Data from assessment of damaged twigs by generation 2 larvae did not fit a normal distribution until transformed (log x +1). Data were analyzed by an analysis of variance and differences between means were separated by a Tukey test ($P<0.05$). Mites were sampled August 25 as described above for 2003. Data were analyzed as described for 2003.

Results

Status of resistance in field populations

The occurrence of resistance to organophosphorus (OP) and carbamate insecticides at the Jordan Station site ranged from 31% in generation 1 in 2004 to 75% in generation 2 in 2003 (Table 1). The diagnostic concentrations used for tests allowed survival of resistant insects only. Over the three seasons at the Jordan Station site, resistance rates were similar in generation 1 (from 31-39%), but were generally higher than reported for the same location in 1999 by Kanga et al. (2003) who found a decline to <20% resistance for OP insecticides. Resistance to OP insecticides was usually highest at the end of each season in generation 3. Resistance to OP insecticides in the populations from the Niagara peninsula were always lowest in the first or overwintered generation and lower than in the third generation of the previous year at the Jordan site where observations were made over 3 years. The Grimsby site, largely planted with apples (no peach) and the Beamsville site, a mixture of peach, apple, and pear, showed similar patterns of increased frequencies of OP resistance over the season. However, OP resistance did not continue to increase over the 3 years of sampling at the Jordan Station site, nor did resistance levels in generation 3.

Control programs for Oriental fruit moth on peach were chlorpyrifos for the first generation followed by up to four applications of a pyrethroid (cypermethrin, deltamethrin, or, lambda cyhalothrin) over the rest of the season. Programs on apple were variable but most included at least one application of azinphosmethyl or phosmet. Resistance to pyrethroids did not increase over the three seasons sampled at the Jordan site and was generally lower than OP resistance at all locations tested in Niagara. Resistance to pyrethroids was similar or lower than reported in previous studies by Kanga et al. (2003).

Sites sampled in Norfolk (Table 2) were all apple and all showed the occurrence of resistance to OP insecticides at frequencies up to 54%. The percent identified as resistant

TABLE 1. Occurrence of resistance in Oriental fruit moth populations with organophosphorus (OP) and pyrethroid insecticides in the Niagara peninsula, ON, 2002-2004. The survival rate is based on 100-120 adult males/ generation for each insecticide. OP resistance determined with carbofuran, pyrethroid resistance with cypermethrin. Diagnostic concentrations used killed all susceptible mortality data indicate percent resistant moths.

	Survival Rate (%) ± SE					
	OP Generation			Pyrethroid Generation		
Site	1	2	3	1	2	3
2002						
Jordan Station	39.0 ± 17.3	53.0 ± 6.0	59.9 ± 7.7	0	1.0 ± 2.0	1.0 ± 1.9
Grimsby	29.3 ± 2.9	35.8 ± 13.2	71.3 ± 4.7	0.9 ± 1.9	2.8 ± 3.5	0
Beamsville	72.2 ± 7.5	74.3 ± 11.6	83.9 ± 4.6	0.8 ± 1.7	3.0 ± 3.8	8.8 ± 6.0
2003						
Jordan Station	34.8 ± 14.0	75.4 ± 5.2	n.a.	0	1.2 ± 13.9	n.a.
2004						
Jordan Station	31.0 ± 6.8	38.0 ± 6.9	53.0 ± 12.8	0	1.0 ± 2.0	0
Vineland	45.0 ± 19.7	61.0 ± 22.0	58.0 ± 10.6	1.3 ± 2.3	1.7 ± 2.9	0

declined over the 3 seasons of the test at the Simcoe site but this was the only location sampled each season and initial samples (2001) were from generation 3 when resistance was generally higher than in generation 1 (Kanga et al. 2003). Pyrethroid resistance was detected at most of the sites.

In Essex county (Table 2), samples were largely from peach plantings and both the Oxley and Varner populations showed OP resistance but not pyrethroid resistance. Whether resistance to pyrethroids declined (despite up to 4 applications/season) and OP resistance increased relative to levels indicated in earlier reports (Kanga et al. 2003) or whether these results are an expression of fluctuations in resistance frequencies and do not necessarily represent trends, is not clear. Most of the sites trapped in these studies had not been previously tested but the Jordan Station site had been extensively tested by Kanga et al. (2003). It seems unlikely that changes in bioassay techniques were responsible for these observed changes because concentration: response regressions were redeveloped for these tests and produced results similar to those used in earlier assays by Kanga et al. (2003). Diagnostic concentrations were the same as in their earlier report. These data may support the argument of Tabashnik et al. (2000) that the frequency of resistance does not necessarily increase each season despite considerable selection pressures. They reported that Bt resistance frequencies in pink bollworm *Pectinophora gossypiella* (Saunders) (Lepidoptera: Gelechiidae) did not increase as expected over three seasons (1997-1999)

TABLE 2. Occurrence of resistance to organophosphorus (OP) and pyrethroid insecticides in Oriental fruit moth populations from Norfolk and Essex counties, ON, 2001-2003. Survival rate was based on the responses of 100-250 males.

Site	Generation	Survival Rate (%) ± SE OP	Pyrethroid
Norfolk County			
2001			
Walsh	3	49.1 ± 4.9	3.9 ± 2.7
Simcoe	3	29.1 ± 9.5	1.1 ± 1.3
Vittoria	3	29.8 ± 5.0	2.6 ± 3.3
2002			
Walsh	1	54.0 ± 16.0	5.0 ± 3.4
Simcoe	1	20.0 ± 9.9	2.4 ± 4.8
Vittoria	1	36.3 ± 20.1	7.1 ± 4.7
2003			
Renton	1	24.1 ± 2.8	0
Simcoe	1	13.7 ± 6.3	0
Essex County			
2003			
Oxley	1	60.0 ± 10.9	0
Varner	1	41.9 ± 14.7	0

despite high levels of selection from Bt cotton.

Laboratory tests

Laboratory bioassays of neonate larvae with the population collected from Beamsville and selected in the laboratory (Table 3) indicated that the characteristics of the resistance levels expressed were similar to those reported for populations collected in 1994 (Pree et al. 1998). Resistance to the OP insecticides azinphosmethyl and phosmet was expressed at low levels, chlorpyrifos was equally toxic to both resistant and susceptible populations, and acephate was more toxic to the resistant population than the susceptible population. Resistance to the methyl carbamates carbaryl and carbofuran was expressed at high levels and could not be quantified. There was no cross resistance to the pyrethroid cypermethrin. All of these observations indicated that the resistance was not different from that determined in the initial report (Pree et al. 1998). Additional tests with the neonicotinoids, imidacloprid, and acetamiprid, indicated these were equally toxic to both susceptible and resistant populations.

TABLE 3. Toxicity of insecticides to susceptible and resistant populations of Oriental fruit moth first instar larvae in 2004 compared to populations tested in 1994. Resistance ratio was calculated from LC_{50} resistant strain divided by LC_{50} susceptible strain. Resistance ratio calculated from LC_{50} resistant strain divided by LC_{50} susceptible strain.

Insecticide	Population (n=700)	Slope ± SE	LC_{50} (mg/L) (95% CL)	χ^2	Resistance Ratio 2004	Resistance Ratio 1994 Pree et al. (1998)
Azinphosmethyl	Susuceptible	4.6 ± 0.46	7.1 (6.6-7.6)	3.5		
	Resistant	4.3 ± 0.33	23.3 (20.5-25.9)	4.2	3.3	3.9
Phosmet	Susuceptible	4.0 ± 0.30	19.1 (17.6-20.6)	0.3		
	Resistant	3.4 ± 0.27	35.4 (32.0-38.7)	3.8	1.9	3.2
Chlorpyrifos	Susuceptible	4.5 ± 0.37	29.5 (25.6-33.1)	8.2		
	Resistant	3.8 ± 0.34	28.6 (24.2-32.6)	6.0	1.0	1.0
Acephate	Susuceptible	2.7 ± 0.24	203.3 (146.6-261.0)	12.4		
	Resistant	2.2 ± 0.21	87.4 (54.2-103.8)	6.8	0.4	0.4
Carbaryl	Susuceptible	4.4 ± 0.32	17.1 (14.5-19.6)	7.8		
	Resistant		>1000		>100	>100
Carbofuran	Susuceptible	2.6 ± 0.31	8.8 (6.0 - 11.4)	7.2		
	Resistant		>1000		>100	>100
Cypermethrin	Susuceptible	3.6 ± 0.32	0.88 (0.62-1.09)	10.5		
	Resistant	2.7 ± 0.20	1.03 (0.93-1.14)	3.5	1.2	1.0
Imidacloprid	Susuceptible	2.6 ± 0.23	2.0 (1.7-2.2)	2.0		
	Resistant	2.7 ± 0.27	2.0 (1.6-2.3)	3.9	1.0	n.a.
Acetamiprid	Susuceptible	3.8 ± 0.28	0.39 (0.29-0.49)	14.5		
	Resistant	3.2 ± 0.32	0.58 (0.47-0.69)	4.7	1.5	n.a.

Tests with insecticides incorporated into an artificial diet indicated that acetamiprid was equally toxic to both susceptible and resistant populations but that methoxyfenozide was slightly more toxic (1.7 fold) to the susceptible population (Table 4). By this procedure, methoxyfenozide was more toxic (to both susceptible and resistant populations) than acetamiprid. Resistance to methoxyfenozide, and to its analog tebufenozide, has been shown in populations of the obliquebanded leafroller *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae) that expressed resistance to OP insecticides (Waldstein et al. 1999; Pree et al. 2003).

Resistance mechanisms

Esterase activity in adult abdomens was higher by a factor of 1.6 in the resistant population (20.0 ± 2.8 μmoles min^{-1} mg^{-1} protein versus 12.5 ± 1.1 for the susceptible population). Differences between resistant and susceptible populations in 1994 (Kanga et al. 1997) were 3.9 fold. AChE activity was higher in both adult males and females of the resistant population (Table 5) but was not different between the sexes for either population. Measurements of AChE inhibition with carbaryl at 10^{-4} M indicated >90% inhibition in both sexes for the susceptible population and <20% inhibition of AChE in the resistant population. In earlier studies Kanga et al. (1997) reported no differences between populations in total AChE activity but did report large differences in inhibition between susceptible and resistant populations. Both elevated general esterases and the presence of an AChE insensitive to inhibition remain likely important factors in this resistance to OP and carbamate insecticides. We did not assay other possible resistance mechanisms. Elevated levels of oxidases and glutathione S-transferases were not shown to be involved in the resistance in earlier studies (Kanga et al. 1997).

Field tests of alternative insecticides

In small plot trials in 2002, methoxyfenozide applied at 360 g ai/ha was as effective as the standard chlorpyrifos or deltamethrin (Table 6). Lower rates of methoxyfenozide were less effective than the standards. Populations of Oriental fruit moth at this site were 40-55%

TABLE 4. Toxicity of insecticides incorporated into diet fed to susceptible and resistant populations of Oriental fruit moth larvae. Resistance ratio was calculated as LC_{50} resistant strain divided by LC_{50} susceptible strain.

Insecticide	Population (n=500)	Slope ± SE	LC_{50} mg/kg	χ^2	Resistance Ratio
methoxyfenozide	Susceptible	4.1 ± 0.41	0.028 (0.024-0.032)	9.3	
	Resistant	3.1 ± 0.23	0.049 (0.042-0.057)	5.8	1.7
acetamiprid	Susceptible	6.9 ± 0.73	0.45 (0.41-0.48)	6.6	
	Resistant	6.7 ± 1.07	0.48 (0.45-0.52)	12.2	1.1

TABLE 5. Inhibition of acetylcholinesterases in susceptible and resistant populations of Oriental fruit moth.

Population	Sex	Mean Rate ± SE 10^{-4} M Carbaryl µmoles/min/mg protein (n = 96-101)	Mean % Inhibition ± SE (n = 95-134 heads)
Susceptible	Male	28.1 ± 2.3 a[1]	91.0 ± 2.7
	Female	29.4 ± 3.8 a	91.7 ± 2.6
Resistant	Male	52.3 ± 4.8 b	17.2 ± 7.4
	Female	42.9 ± 4.3 b	15.8 ± 6.0

[1]Same letters are not significantly different (Tukey test (P<0.05)).

TABLE 6. Control of Oriental fruit moth damage on peach in small field plots, Jordan Station, ON, 2002. OFM Damage/Plot includes damage to twigs and fruit.

Treatment	Formulation	Rate a.i./ha	OFM Damage/Plot
Generation[1]			
chlorpyrifos	Lorsban 50WP	1700 g	2.8 c[2]
methoxyfenozide	Intrepid 2F	120 g	12.85 b
methoxyfenozide	Intrepid 2F	240 g	9.1 bc
methoxyfenozide	Intrepid 2F	360 g	8.5 bc
Control	-		24.0 a
Generation 2[3]			
deltamethrin	Decis 5EC	10 g	19.2 c[2,3]
methoxyfenozide	Intrepid 2F	120 g	127.0 b
methoxyfenozide	Intrepid 2F	240 g	127.0 b
methoxyfenozide	Intrepid 2F	360 g	101.5 bc
Control	-		239.5 a

[1] Applied 3 June 2002, to cv. Loring, Damage assessment 27 June 2002.
[2] Same letters are not significantly different (Tukey test, P<0.05).
[3] Applied 10 and 23 July 2002, to cv. Loring, Damage assessment 2 August 2002.

TABLE 7. Control of Oriental fruit moth damage on peach in small field plots, Jordan Station, ON, 2004. OFM Damage/Plot includes damage to twigs and fruit.

Treatment	Formulation	Rate a.i./ha	Total OFM Damage
Generation[1]			
deltamethrin	Decis EC	10 g	1.0 b[2]
acetamiprid	Assail 70WP	47.2 g	13.8 b
acetamiprid	Assail 70WP	168.8 g	11.5 b
acetamiprid	Assail 70WP	176 g	4.3 b
Control	-		30.3 a
Generation 2[3]			
deltamethrin	Decis 5EC	10 g	1.3 b
acetamiprid	Assail 70WP	47.2 g	3.0 b
acetamiprid	Assail 70WP	168.8 g	1.8 b
acetamiprid	Assail 70WP	172 g	1.5 b
Control	-		9.8 a

[1] Applied 21 May 2004, 124 DD base 7.2 °C after first male capture, cv. Elberta, Damage assessment 9 June 2004.
[2] Same letters are not significantly different (Tukey test, $P<0.05$).
[3] Applied 9 July 2004, 617 DD, base 7.2°C after first male capture, cv. Elberta, Damage assessment 22 July 2004.

resistant to OP insecticides and >5% were resistant to pyrethroids (Table 1). Infestations were higher in tests with the second generation in 2002. In similar tests in 2004 (Table 7), acetamiprid at three different rates was as effective as the standard deltamethrin.

In season long tests of various Oriental fruit moth control programs in 2003 and 2004 (Table 8) all of the programs effectively prevented damage to twigs by first generation larvae. As in most seasons, damage was less in generation 1 than later in the season. In 2003, in generation 2, damage to terminals was higher in methoxyfenozide-treated plots. This did not result in significantly higher damage at harvest although damage to fruit was slightly higher than the grower accepted threshold of 1%. In 2004 (Table 8) in generation 2, damage to terminals was again higher in methoxyfenozide-treated plots than in deltamethrin or acetamiprid-treated plots. At harvest, damage to fruit was higher in plots treated with methoxyfenozide in generations 2 and 3. All other programs had <1% fruit damaged at harvest.

In 2003, populations of European red mite did not reach threshold or action levels (5-10 mites/leaf in July) (Anonymous 2004) under any of the programs tested in 2003 (Table 9). Numbers of European red mites were higher in 2004 but did not exceed the economic threshold. However, in 2003, populations of the peach silver mite and numbers of beneficial mites were higher in all plots treated with deltamethrin. In 2004, numbers of peach silver

TABLE 8. Seasonal control of Oriental fruit moth (OFM) on peach, Jordan Station, ON, 2003-2004.

Program			Mean Damaged twigs/tree (± SE)		% Fruit Damaged At Harvest
	OFM Generation			OFM Generation	
1	2	3	1	2	
2003					
chlorpyrifos	deltamethrin	deltamethrin	1.0 ± 1.5 **a**[1]	1.2 ± 1.7 **a**	0.1 ± 0.3 **a**
chlorpyrifos	methoxyfenozide	methoxyfenozide	1.8 ± 1.8 **a**	9.8 ± 2.2 **b**	1.3 ± 1.7 **a**
methozyfenozide	deltamethrin	deltamethrin	1.7 ± 1.9 **a**	2.3 ± 2.4 **a**	0.7 ± 0.2 **a**
2004					
chlorpyrifos	deltamethrin	deltamethrin	1.9 ± 1.9 **a**[2]	5.0 ± 5.2 **b**	0.6 ± 0.6 **b**
methoxyfenozide	deltamethrin	deltamethrin	3.7 ± 2.2 **a**	3.0 ± 2.4 **b**	0.1 ± 0.2 **b**
methozyfenozide	acetamiprid	acetamiprid	4.4 ± 2.9 **a**	4.0 ± 3.1 **b**	0.5 ± 0.4 **b**
acetamiprid	methoxyfenozide	methoxyfenozide	4.2 ± 2.3 **a**	18.5 ± 11.5 **a**	3.1 ± 2.8 **a**

[1]For 2003, numbers in same column followed by same letter are not significantly different (Kruskal Wallis, $P<0.05$).

[2]For 2004, data were subjected to an ANOVA after log (x+1) transformation and differences between means separated by a Tukey test ($P<0.05$).

TABLE 9. Effects of Oriental fruit moth (OFM) control programs on populations of pest and beneficial mites Jordan Station, ON, 25 August 2003 and 25 August 2004.

Program			European red mites *Panonychus ulmi* (Koch) per 100 leaves Mean ± SE	Beneficial mites Acari: Phytoseidae per 100 leaves Mean ± SE	Peach Silver Mite Mean Rating/Sample
	OFM Generation				
1	2	3			
2003					
chlorpyrifos	deltamethrin	deltamethrin	5.3 **a**	15.3 **a**	4.97 **a**
chlorpyrifos	methoxyfenozide	methoxyfenozide	0 **a**	0.7 **b**	0.22 **b**
methoxyfenozide	deltamethrin	deltamethrin	9.0 **a**	16.3 **a**	4.65 **a**
2004					
chlorpyrifos	deltamethrin	deltamethrin	4.3 **a**	1.3 **a**	0.05 **a**
methoxyfenozide	deltamethrin	deltamethrin	156 **b**	3.3 **a**	0.5 **b**
methoxyfenozide	acetamiprid	acetamiprid	65.7 **ab**	61.3 **b**	1.5 **b**
acetamiprid	methoxyfenozide	methoxyfenozide	5.7 **a**	4.3 **a**	0 **a**

Numbers in the same column for each year followed by same letter were not significantly different (Tukey test, $P<0.05$).

mites did not increase as in 2003 and remained at low numbers in all plots. Beneficial mites were found in all plots but numbers were generally higher where phytophagous mites (either *P.ulmi* or *A. cornutus*) were available as a food source. In 2003, numbers of beneficial mites were higher in plots treated with deltamethrin in generations 2 and 3 of Oriental fruit moth, plots which held large numbers of peach silver mites. In 2004, numbers of beneficial mites were highest in plots treated with acetamiprid in generations 2 and 3.

Discussion

The initial goal of the resistance management strategy for Oriental fruit moth established after the episode of resistance to OP insecticides in 1993-1994 was to maintain susceptibility to pyrethroids, the only effective alternative at that time. That program appears to have been successful. The percentage of the population expressing resistance to pyrethroids declined from levels reported in earlier studies and resistance to pyrethroids was not found at all sites. Resistance to OP insecticides was found at all sites and was at a higher frequency than previously reported at one extensively monitored site. This occurred despite avoidance of the OP insecticides, azinphosmethyl and phosmet, that had previously been used on peach for control of Oriental fruit moth. Chlorpyrifos was equally toxic, as in previous studies, to both resistant and susceptible populations. In the last 10 years, the Oriental fruit moth has become a pest on apples where OP insecticides have continued to be used for other pests and this may be the source of OP resistant insects. Pyrethroids are not used extensively on apples.

The potential impact of the impending removal of chlorpyrifos after 2006 may be ameliorated by the use of either methoxyfenozide or the neonicotinoid acetamiprid. The cross resistance to methoxyfenozide identified here was expressed at low levels (1.7 fold at the LC_{50}) and, at higher rates, this compound effectively controlled Oriental fruit moth in both small plot and program trials against the first generation. Later applications against the second and third generations, especially in the program trials, were less effective. Acetamiprid was effective throughout the season. If methoxyfenozide were reserved as a replacement for chlopyrifos for use against generation 1, this would hold the neonicotinoid acetimiprid and/or pyrethroids or rotations of these two groups for the rest of the season. The use of pyrethroids in IPM programs has often been discouraged because of their impact on beneficial mites and the associated outbreaks of phytophagous mites (Croft 1990). In the program trials reported here, numbers of European red mites did not exceed acceptable thresholds but high populations of peach silver mites were associated with pyrethroid use in 2003. Further evaluation of the impact of these products on beneficial mite populations in peach and apple ecosystems is necessary but Beers et al. (2005) have shown increased populations of phytophagous mites following repeated applications of various neonicotinoids. In any case, the addition of these two new insecticides will provide an opportunity not only to manage or prevent resistance to pyrethroids, but if all three groups of chemicals are utilized, should also delay the development of resistance to these new products. There is also an alternative control program (Trimble et al. 2001) that involves the integration of insecticides for the first generation with mating disruption for later generations. That program would likely provide the best long-term resistance management strategy for

Oriental fruit moth on peach in Ontario.

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