SUSCEPTIBILITY OF TWO STRAINS OF AMERICAN SERPENTINE LEAFMINER (*LIRIOMYZA TRIFOLII* (BURGESS)) TO REGISTERED AND REDUCED RISK INSECTICIDES IN ONTARIO

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American serpentine leafminer, *Liriomyza trifolii* (Burgess), is a pest of floriculture greenhouses in Ontario. Growers rely on chemicals to provide acceptable pest control and, consequently, this leafminer has developed resistance to many insecticides. Our objectives were to determine if the American serpentine leafminer in Ontario has developed resistance to registered insecticides and to evaluate effectiveness of reduced risk insecticides with potential for inclusion in management programs.

Two leafminer cultures were established – one collected from greenhouses in the Niagara region, the other being an insecticide susceptible strain never exposed to any of the test insecticides. Dosage-mortality curves were constructed using a leaf dip bioassay in which newly infested bean leaves were dipped in formulated insecticide solutions and larvae were allowed to develop until adults emerged. At the LC_{50}, the Ontario strain was resistant to abamectin (17.5x) and cyromazine (10.2x) and showed low levels of resistance to spinosad (2.8x) and chlorantraniliprole (3.0x) – such low resistance levels also could be due to natural variation in the strains. A comparison of LC_{95} to application rates showed that the amount of insecticide required to kill 95% of the Ontario strain would be much higher than the recommended rate for cyromazine, just within the rate for abamectin, and lower that suggested rates for spinosad and chlorantraniliprole. While the LC_{95}s for spinosad and chlorantraniliprole were lower than suggested application rates. Nevertheless, the low level resistance shown by the Ontario strain suggests that these 2 insecticides may have the potential to develop higher levels of resistance over time.

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Introduction

In 2006, Ontario produced about 14 million potted and 21 million cut chrysanthemums (*Dendranthema grandiflora* Tzvelev), and 3 million potted and 30 million cut gerberas (*Gerbera jamesonii* H. Bolus ex J. D. Hook) (Statistics Canada 2006). The American serpentine leafminer (*Liriomyza trifolii* (Burgess)) attacks both crops causing aesthetic damage to leaves when larvae feed on leaf mesophyll producing serpentine mines. It was reported to be a major pest in Ontario floriculture greenhouses in the late 1970s (Broadbent 1982) but has been kept under control by insecticides. Floriculture producers often rely on heavy application of chemicals to control pests because consumers demand a high quality product free from aesthetic damage (Jones et al. 1986; Parrella 1987; Gullino and Wardlow 1990). There are only a few insecticides registered for American serpentine leafminer control in Canada (Chaput 2000). Abamectin and cyromazine are registered systemic insecticides acting on leafminer larvae. Permethrin can be used against leafminer adults but resistance to permethrin may be present in the population (Keil and Parrella 1990; Murphy 2004). Unfortunately, the short generation time and high reproductive rate of this leafminer combined with excessive insecticide use have caused it to develop resistance to many insecticides (Jones et al. 1986; Parrella 1987; Keil and Parrella 1990). In 2004, growers in Ontario reported difficulty in controlling it with registered insecticides (G. D. Murphy, pers. comm., 2005), suggesting that it was becoming resistant to both cyromazine and abamectin. In a survey of Ontario chrysanthemum and gerbera growers Conroy et al. (2007) found that 22% of respondents had observed failure to effectively control the leafminer with insecticides. Reduced risk insecticides, such as spinosad and chlorantraniliprole, need to be tested for control of this leafminer and compatibility with integrated pest management programs.

The objectives of this study were to determine if the American serpentine leafminer has developed resistance to currently registered insecticides in Ontario since control failures have been observed and to evaluate the effectiveness of reduced risk insecticides with potential for inclusion in management programs.

Materials and Methods

American Serpentine Leafminer Culture

Two American serpentine leafminer colonies were established. A reference strain was obtained from Dr. Scott Ferguson (Syngenta Crop Protection, Florida). This strain had been reared in the laboratory for over 20 years and had never been exposed to insecticides currently registered for leafminer control. The second strain was collected from chrysanthemum and gerbera greenhouses in the Niagara region of Ontario in 2006-2007. Resistance may have developed as a result of the history of insecticide use in affected greenhouses, or more likely given the synchronicity of problems in a number of greenhouses, it was imported along with plant material from the United States where resistance to currently registered insecticides has been documented (Ferguson 2004; G. D. Murphy, pers. comm., 2005). Both strains were kept in plexi-glass cages in environmental chambers at 26 ± 2ºC, 50-70% RH, and 18:6 L:D. The strains were kept in separate buildings following proper quarantine protocols. Insects were reared on green beans (cv. Provider®, Veseys Inc.) grown in ProMix®, and held in growth chambers at 25 ± 2ºC and 18:6 L:D. A 10 cm filter paper covered in dilute honey was supplied for carbohydrate. Greenhouse collected leafminers were added to the Ontario strain culture every 1-2 months to maintain similar genetic diversity to field populations.

Leaf Dip Bioassay

Bioassay methods were modified from Ferguson (2004). Four to 6 bean plants at the 2-4 leaf stage were exposed to leafminer colonies for 1-3 h depending on colony fitness (number of adults). Plants were removed from the colony and placed in environmental chambers (26 ± 5ºC, 30-50% RH, and 18:6 L:D) for 2-3 days or until small mines appeared on the leaves. Mines on each leaf were counted and marked with a permanent marker. Leaves were removed and placed in flower picks filled with deionized water on a metal drying rack on a cafeteria tray lined with paper towel. Seven to15 mines per leaf were used per replication for both strains. The number of mines per leaf was difficult to control due to the varying number of adults in each colony for both strains at any given time. A higher number of mines (> 20/leaf) decreased overall survivorship.

Cyromazine (Citation® 75 WP, Syngenta Crop Protection Canada), abamectin (Agri-Mek®/Avid® 1.9 % EC, Syngenta Crop Protection Canada), spinosad (Success® 480 SC, Dow AgroSciences) and chlorantraniliprole (Altacor™ 35% WG, DuPont Canada) were tested. *Note: Altacor™ 35 WG (DuPont Canada) was the only formulation available at the onset of this project. Coragen™ 20 SC (DuPont Canada) is now the formulation appropriate for greenhouse use.* The formulated insecticides were mixed with deionized water. Super Spreader® (United Agri Products Canada Inc.) wetting agent (0.05 ml) was added to each concentration to enhance wetting of the leaves (Ferguson 2004). Deionized water mixed with 0.05 ml wetting agent was used as the control. One or 2 infested leaves with a total of at least 7 mines were dipped into a beaker containing 200 ml of the insecticide solution for 5 s and then placed on a rack in a fumehood to dry for 1 h. Leaves were then removed from the flower picks and placed in 10 cm plastic Petri® dishes lined with filter paper. Petri dishes were sealed with parafilm. Post-treatment containers were kept in an environmental chamber at 26 ± 5ºC, 30-50% RH, and 18:6 L:D for 7 days until pupation. Pupae were then counted, the leaves were removed and the pupae were returned to the Petri dishes in the chamber for 2-4 weeks until all adults had eclosed and died. Emerged adults were then counted. Preliminary screening tests were done to determine a range of concentrations (15-95% mortality) appropriate for construction of a dosage mortality curve. Tests were replicated 4 times.

Data Analysis

Abbott's formula (Abbott 1925) was used to correct for control mortality (< 15%). Data were analyzed using SAS version 9.1 (SAS institute, Cary, NC) with a type 1 error rate of $\alpha = 0.05$. The probit procedure was used to determine LC_{50} and LC_{95} values by log transforming the data to fit the probit scale. A Chi-square goodness of fit test was used to test the significance of the probit regression and determine the fiducial limits. The difference between the 2 LC_{50} values was deemed significant if there was no overlap of the 95% fiducial limits. Resistance ratios were calculated by dividing the resistant Ontario strain LC_{50} by the susceptible strain LC_{50}.

Results and Discussion

Cyromazine, abamectin and spinosad resistance have been documented in Florida (Ferguson 2004) but only anecdotal evidence has been reported from Ontario since 2004 (G. D. Murphy, pers. comm., 2005). Defining "resistance", particularly at low levels can be arbitrary. Comparison of fiducial limits, as suggested by Robertson et al. (2007) indicates, that as the limits of the LD_{50} of the insecticide susceptible and Ontario leafminer strains did not overlap, the latter was resistant to abamectin (17.5x) and cyromazine (10.2x) and also showed low levels of resistance to spinosad (2.8x) and chlorantraniliprole (3.0x) (Table 1) - these low levels of "resistance" also could be due to natural variation in the Ontario population (Robertson et al. 2007) or to enhanced susceptibility of the reference strain reared for many years under controlled laboratory conditions (ffrench-Constant and Rousch 1990).

Resistance also can be defined as failure of an insecticide to control an insect pest in the field (Ball 1981). The survey of the Ontario chrysanthemum and gerbera growers discovered that 22% of respondents observed failure to control American serpentine leafminer with registered insecticides (Conroy et al. 2007). Comparing the LC_{95} to the recommended field rates (Table 2) shows that the amount of insecticide needed to kill 95% of the Ontario strain would be much higher (4-5x) than that recommended for cyromazine. The recommended rate for abamectin was close to the LC_{95} suggesting that resistance in Ontario greenhouses may not be present. However, since the population was not gathered from a single source it is possible that resistance varies between greenhouses and could be higher in some, causing control failures. A low level of insecticide resistance was observed with spinosad (Table 1). It is registered in the United States for leafminer control and thus it is not surprising that the Ontario strain, possibly imported from the United States on infested propagation material would show decreased susceptibility to this insecticide. However, the recommended application rate would appear to be adequate to provide effective leafminer control (Table 2).

Chlorantraniliprole is a new insecticide with a unique mode of action. Comparing the application rate to the LC_{95} suggests that the amount needed to provide 95% control also is lower than the suggested application rate (Table 2). Nevertheless, the low level of insecticide resistance shown by the Ontario strain to this product suggests that it may have the potential to develop a higher level of resistance (Table 1). If chlorantraniliprole or spinosad is registered for American serpentine leafminer control, it should be in the context of an integrated pest management program minimizing use in order to delay resistance development.

Conclusions

These results stress the importance of developing a multifaceted integrated pest management program for American serpentine leafminer control in Ontario. Insecticides alone will not control it without rapid development of insecticide resistance. An IPM

TABLE I. LC_{50} and level of resistance of 2 American serpentine leafminer strains (Ontario = O; Susceptible = S) exposed to formulated insecticides, either registered (abamectin, cyromazine) or reduced risk (chlorantraniliprole, spinosad), using a leaf dip bioassay.

Insecticide	Strain	N	Slope	Pearson $\chi 2$	LC_{50}[1]	95 % Fiducial Limits	Resistance Ratio O/S
Abamectin	O	249	1.52	0.262	1.05	0.72 - 1.40	17.5
	S	318	1.94	0.219	0.06	0.05 - 0.07	
Cyromazine	O	343	1.40	0.453	33.6	26.0 - 44.0	10.2
	S	220	3.13	0.087	3.28	2.24 - 4.41	
Chlorantraniliprole	O	289	1.99	0.292	0.63	0.53 – 0.76	3.0
	S	295	3.10	0.169	0.21	0.19 – 0.24	
Spinosad	O	192	2.14	0.673	2.82	2.01 - 3.67	2.8
	S	177	2.31	0.121	1.00	0.81 - 1.28	

[1] Concentrations expressed as ppm.

TABLE 2. LC_{95} for the Ontario American serpentine leafminer strain exposed to formulated insecticides using a leaf dip bioassay compared to recommended application rates.

Insecticide	LC_{95}[1]	95 % Fiducial Limits	Recommended Application Rates[1]
Cyromazine	506	301 – 1051	141
Abamectin	12.7	7.9 – 27.8	11
Spinosad	16.5	12.0 – 26.5	24
Chlorantraniliprole	4.3	2.6 – 11.0	9

[1] Concentrations and rates are expressed as ppm.

program that decreases the number of insecticide applications, rotates products with different modes of action and incorporates biological control would be more effective.

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