Assessment of Resistance Risk in Obliquebanded Leafroller (Lepidoptera: Tortricidae) to the Reduced-Risk Insecticides Chlorantraniliprole and Spinetoram

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J. Econ. Entomol. 103(4): 1378–1385 (2010); DOI: 10.1603/EC09354

ABSTRACT Obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), is a major pest of pome fruit in Washington. The use of broad-spectrum insecticides for decades has led to the development of insecticide resistance in *C. rosaceana*. Recently registered insecticides with novel modes of action, such as chlorantraniliprole and spinetoram, have provided effective *C. rosaceana* control, but resistance remains a threat. The risk of insecticide resistance development in a pest can be assessed by artificial selection in the laboratory. Subsequently, this information can be valuable in developing strategies to retain susceptibility in the field. A laboratory population of *C. rosaceana* was selected after repeated exposure to chlorantraniliprole and spinetoram to determine the risk of resistance evolution. After six generations of selection, 6.58- and 3.64-fold increases in LC_{50} were recorded for chlorantraniliprole and spinetoram, respectively. The realized heritability (h^2) of resistance was estimated as 0.17 for chlorantraniliprole and 0.18 for spinetoram by using threshold trait analysis. The rates of resistance development were compared using the response quotient (Q), which was estimated as 0.11 and 0.07 for chlorantraniliprole and spinetoram, respectively. Projected rates of resistance evolution indicated that if $h^2 = 0.2$ and 80% of the population was killed at each generation, then a 10-fold increase in LC_{50} would be expected in less than six generations for chlorantraniliprole and 10 generations for spinetoram. These results indicate that the risk of resistance development in *C. rosaceana* exists to both of these insecticides but that resistance development in *C. rosaceana* would be slower against spinetoram than chlorantraniliprole.

KEY WORDS obliquebanded leafroller, chlorantraniliprole, spinetoram, resistance, heritability

The obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), is one of the most destructive lepidopteran pests of pome fruit in Washington (Brunner 1999). The use of broad-spectrum organophosphorus insecticides (OPs) against *C. rosaceana* and a wide spectrum of other tree fruit pests for more than four decades has led to the development of insecticide resistance in *C. rosaceana* (Brunner 1996). Insecticide resistance against OPs and cross-resistance to other groups of chemicals have been documented in *C. rosaceana* (Reissig et al. 1986, Lawson et al. 1997, Waldstein et al. 1999, Ahmad et al. 2002, Smirle et al. 2002, Dunley et al. 2006), in some cases to newly developed insecticides even before they had been used in the field (Sauphanor et al. 1998, Dunley and Welter 2000). The evolution of insecticide resistance, and increased public concern over health and environmental effects of broad-spectrum insecticides, has led to a greater priority in the development of reduced-risk insecticides (USEPA 1997). Some reduced-risk insecticides with novel chemistries such as chlorantraniliprole and spinetoram have recently been registered as OP alternatives for use in tree fruit production.

Chlorantraniliprole is a member of a new class of insecticides, the anthranilic diamides. Anthranilic diamides selectively bind to the ryanodine receptors (RyRs) in insect muscles, resulting in an uncontrolled release of internal calcium stores from the sarcoplasmic reticulum (Lahm et al. 2005, Cordova et al. 2006), causing impaired regulation of muscle contraction leading to feeding cessation, lethargy, paralysis, and death in target organisms. Anthranilic diamides have very low vertebrate toxicity due to a >500-fold differential selectivity toward insect over mammalian RyRs (Cordova et al. 2006). Spinetoram is a recently developed member of the spinosyns class of insecticides. They primarily activate the nicotinic acetylcholine receptors by acting on a unique site (Salgado 1998, Salgado et al. 1998). Both chlorantraniliprole and spinetoram have shown high efficacy against *C. rosaceana* (Hull et al. 2009; J.F.B., unpublished data).

The integration of these highly selective insecticides into pest management programs is critical for

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successful production of tree fruit on a sustainable basis. However, the development of pest resistance is a continual threat, especially to recently introduced insecticides. Resistance management strategies are often implemented after resistance has been detected in field populations, reducing their value in managing resistance. Therefore, the assessment of resistance risk in a pest such as *C. rosaceana* before resistance occurs in the field would provide valuable information supporting a proactive implementation of strategies to manage and maintain susceptibility in field populations and thus to delay the development of resistance.

There are several techniques available to assess resistance risk for an insecticide, including selecting for resistance in the laboratory (National Research Council 1986, Brown and Payne 1988). Data from selection experiments can be analyzed using quantitative genetic models that consider resistance as a continuous variable and estimate heritability of resistance (Firko and Hayes 1990). Estimation of heritability (narrow sense), the proportion of phenotypic variation accounted for by additive genetic variation (Falconer and Mackay 1996), provides a standardized way to quantify and summarize results from selection experiments (Tabashnik 1992) and can therefore be used to predict rate and direction of the genetic change associated with resistance (Firko and Hayes 1990).

Assessing risk of insecticide resistance development in *C. rosaceana* to newly introduced insecticides is an important factor to managing resistance against these chemicals and thus sustaining the efficacy of these products for as long as possible. In this study, we assessed the risk of resistance development in *C. rosaceana* to chlorantraniliprole and spinetoram using truncated selection whereby successive generations of a susceptible population are selected and maintained in the laboratory.

Materials and Methods

Insects. A laboratory colony (LAB) of *C. rosaceana* was established in 1990 from larvae collected from apple orchards in Mattawa, WA. This colony has been reared continuously since their collection on a pinto bean diet following the method of Shorey and Hale (1965) under constant conditions of 23 ± 2°C, 70% RH, and a photoperiod of 16:8 [L:D] h, and without exposure to insecticides.

Insecticides. The insecticides tested were chlorantraniliprole (Rynaxypyr/Altacor 35 WG [active ingredient: 35%]), E.I. du Pont Co., Wilmington, DE; and spinetoram (Delegate 25 WG [active ingredient: 25%]), Dow AgroSciences, Indianapolis, IN.

Bioassays. Toxicity of chlorantraniliprole and spinetoram to neonate *C. rosaceana* larvae was determined using a diet incorporation bioassay. Diet used in the bioassay was a dry premix of a *Heliothis* diet (Stonefly Heliothis Diet [item 38 V 0600], Ward's Natural Science, Rochester, NY). Insecticide incorporated diet was prepared by mixing insecticide dilution (insecticide and water), water, vinegar, and dry diet premix at a ratio of 10:61:4:25 to produce 100 g of final product. Vinegar was used in the diet as recommended by the manufacturer. A stock solution of each insecticide was prepared by diluting it at 10× the highest concentration to be used in the bioassay. Serial dilutions were then prepared from the stock solution at 10× each of the target concentrations to be used in the bioassay. A treatment solution was prepared by combining 61 g of water to 4 g of vinegar and then with 10 g of the appropriate 10× insecticide dilution. The treatment solution was then added to 25 g of dry diet premix to complete an insecticide incorporated diet of known concentration. An untreated control was prepared by combining water only (75 g) with the dry diet premix. Enough insecticide incorporated diet was prepared prior at the start of the bioassays, so that all tests were conducted using the same diet mixtures, and new diet was prepared for each replication.

A small portion of insecticide incorporated diet (≈8.0 cm^3) was added to a plastic 50- by 9-mm petri dish (BD Biosciences, Franklin Lakes, NJ). The diet was pushed firmly along the edges of the dish, and scored with a pin so that *C. rosaceana* neonates could readily colonize the diet. Petri dishes were chosen randomly, and five 1-d-old *C. rosaceana* larvae were transferred into each dish by using a camel's-hair brush. Six to 10 dishes were prepared for each treatment (30–50 larvae per treatment) depending on the availability of neonate larvae. The dishes were placed in growth chambers at constant conditions of 23 ± 2°C, 70% RH, and a photoperiod of 16:8 (L:D) h. Larval mortality in each bioassay was evaluated at 7 d. Larvae were recorded as dead if they did not move when probed with camel's-hair brush. To ensure that offspring of many females were assayed, larvae emerging from any given egg mass were systematically distributed among various concentrations so that a maximum of five to10 larvae per egg mass were exposed to any one concentration.

Selection for Resistance. Based on results from initial bioassays, cohorts of larvae from the laboratory colony were selected with chlorantraniliprole (RYN) or spinetoram (SPIN) for six consecutive generations, whereas another cohort treated in the same way but without exposure to insecticides served as a control (LAB). In the first selection, neonate larvae were exposed to LC_{70} of the baseline established for the LAB population. After 4 d of exposure, surviving larvae were transferred to untreated pinto bean diet and reared in the laboratory under conditions described above. The concentration of chlorantraniliprole and spinetoram used to select each subsequent generation was the ≈LC_{70} based on the results of bioassays from the previous generation. The number of neonate larvae used for each generation varied (1,000–2,000) depending on availability. In total, six rounds of selection (generations) were conducted with each insecticide. Based on availability, a subset of the progeny of the *C. rosaceana* surviving each selection was exposed to a range of concentrations using diet incorporation bioassay to determine the effect of selection on the susceptibility of the selected populations. Each time a bioassay was conducted on larvae from the

Table 1. Effect of chlorantraniliprole contaminated diet on *C. rosaceana* neonate larvae from chlorantraniliprole-selected population (RYN) and unselected susceptible laboratory population (LAB)

Selected generation	Pop	Larvae assayed (n)	Slope (± SE)	χ^2	LC_{50} (ppm) (95% FL)[a]	LC_{90} (ppm) (95% FL)[a]	LCR-LC_{50}[b] (95% CL)[c]
1	RYN	450	1.02 (0.39)	20.74	0.16 (0.07–0.32)	2.94 (1.41–8.37)	2.2 (1.02–4.65)*
	LAB	450	1.08 (0.10)	25.07	0.08 (0.03–0.15)	1.17 (0.56–3.42)	
3	RYN	350	1.72 (0.17)	17.10	0.26 (0.20–0.34)	1.46 (1.00–2.43)	3.1 (2.12–4.43)*
	LAB	350	2.24 (0.28)	6.62	0.08 (0.06–0.11)	0.32 (0.23–0.49)	
5	RYN	210	1.19 (0.17)	10.31	0.77 (0.31–1.48)	9.26 (4.40–33.02)	6.6 (3.27–13.24)*
	LAB	210	1.90 (0.28)	3.01	0.12 (0.08–0.16)	0.55 (0.36–1.09)	
6	RYN	180	1.88 (0.36)	7.71	1.03 (0.50–1.66)	4.93 (2.88–14.19)	6.6 (3.68–11.79)*
	LAB	180	1.59 (0.19)	11.83	0.16 (0.11–0.23)	1.00 (0.59–2.17)	

* Lethal concentration ratio was significant ($\alpha = 0.05$) (Robertson et al. 2007).
[a] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[b] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[c] 95% CL estimated using lethal concentration ratio significance test (Robertson et al. 2007).

selected colonies, a bioassay also was conducted on larvae from the LAB colony. There were larvae available for bioassays only at generations 1, 3, 5, and 6 for the RYN colony and at generations 1, 2, 4, and 6 for the SPIN colony to conduct the concentration response bioassays.

Data Analysis. Lethal concentration values (LC_{50} and LC_{90}) and their corresponding 95% fiducial limits (FL) were estimated using POLO (LeOra Software 1987), and lethal concentration ratios (LCRs) at LC_{50} values and their corresponding 95% confidence limits (CL) were calculated using lethal concentration ratio significance test (Robertson et al. 2007). A laboratory population (LAB) that was not selected with any of the insecticides but otherwise treated the same way served as the reference susceptible population for comparison purposes and was assigned a ratio of 1.0. Lethal concentrations of the LAB population before and after selection were considered significantly different if the 95% CL of their corresponding LCR did not include the value of 1.0.

Estimation of Realized Heritability. Realized heritability (h^2) of resistance to chlorantraniliprole and spinetoram in *C. rosaceana* was estimated using a threshold trait analysis method (Tabashnik 1992, Tabashnik and McGaughey 1994), where resistance was considered a threshold trait with an underlying continuous variable called tolerance (Falconer and Mackay 1996):

$$h^2 = R/S \quad [1]$$

where R is the response to selection and S is the selection differential (Falconer and Mackay 1996).

Response to selection (R), the difference in mean phenotype between the offspring of the selected parents and the whole parental generation before selection was estimated as follows:

$$R = [\log(\text{final } LC_{50}) - \log(\text{initial } LC_{50})]/n \quad [2]$$

where final LC_{50} is the LC_{50} of offspring of the *C. rosaceana* surviving after n generations of selection, initial LC_{50} is the LC_{50} of LAB population after n generations without selection, and R is the average response to selection per generation.

The selection differential (S), the difference in mean phenotype between the selected parents and the entire parental generation (Hartl 1988), was estimated as follows:

$$S = i\sigma_p \quad [3]$$

where i is the intensity of selection and σ_p is the phenotypic standard deviation. The intensity of selection (i) was estimated from p, the percentage of population with values above the selection threshold (i.e., the percentage of treated larvae surviving selection), by using appendix A of Falconer and Mackay (1996), which is based on the properties of normal distribution.

The phenotypic standard deviation was estimated as the reciprocal of mean of the estimated slopes of the probit regression lines from the LAB population after n generations without selection (initial slope) and the offspring of the RYN or SPIN populations after n generations of selection (final slope):

$$\sigma_p = [1/2\ (\text{initial slope} + \text{final slope})]^{-1}. \quad [4]$$

We also used the regression method proposed by Tanaka and Noppun (1989) for estimating narrow-sense heritability (h^2), where h^2 is the regression of breeding values (R) on phenotypic values (S) and is equivalent to the ratio of additive genetic variance (V_A) to total phenotypic variance (V_P): $h^2 = V_A/V_P$ (Falconer and Mackay 1996). The values of parameters R and S were determined at every generation of selection and h^2 was estimated as the regression coefficient of cumulative response to selection on cumulative selection differential.

To determine whether the genetic parameters such as R, S, or h^2 changed during the course selection, we calculated each of the parameters for the first and second halves of each experiment separately. The split between the two parts was as close to half as allowed by the data.

The response to selection (R) can be estimated as the product of heritability (h^2) and selection differential (S) (Falconer and Mackay 1996):

$$R = h^2 S. \quad [5]$$

Table 2. Effect of spinetoram contaminated diet on *C. rosaceana* neonate larvae from chlorantraniliprole-selected population (RYN) and unselected susceptible laboratory population (LAB)

Selected generation	Pop	Larvae assayed (n)	Slope (± SE)	χ^2	LC_{50} (ppm) (95% FL)[a]	LC_{90} (ppm) (95% FL)[a]	LCR-LC_{50}[b] (95% CL)[c]
1	SPIN	450	2.56 (0.37)	4.18	0.10 (0.07–0.12)	0.31 (0.23–0.48)	1.26 (0.86–1.85)
	LAB	450	4.00 (0.90)	3.62	0.08 (0.06–0.10)	0.16 (0.13–0.26)	
2	SPIN	350	2.53 (0.33)	3.96	0.12 (0.09–0.15)	0.39 (0.29–0.59)	2.3 (1.59–3.26)*
	LAB	350	1.75 (0.16)	7.61	0.05 (0.02–0.19)	0.29 (0.12–2.10)	
4	SPIN	350	3.63 (0.58)	2.98	0.17 (0.14–0.20)	0.38 (0.30–0.56)	3.5 (2.37–5.09)*
	LAB	350	2.72 (0.55)	6.22	0.05 (0.02–0.07)	0.14 (0.10–0.39)	
6	SPIN	210	3.01 (0.48)	2.52	0.22 (0.17–0.29)	0.59 (0.43–1.02)	3.64 (2.42–5.46)*
	LAB	210	1.97 (0.24)	7.59	0.06 (0.04–0.11)	0.27 (0.15–0.87)	

* Lethal concentration ratio was significant ($\alpha = 0.05$) (Robertson et al. 2007).
[a] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[b] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[c] 95% CL estimated using lethal concentration ratio significance test (Robertson et al. 2007).

Based on the response of *C. rosaceana* to selection in laboratory, predictions about the risk of resistance development were made under varying conditions of heritability and slope at different selection intensities in terms of number of generations required for a 10-fold increase in LC_{50} (G), which is the reciprocal of R (Tabashnik 1992):

$$G = R^{-1}. \quad [6]$$

For any particular value of S, the rate of resistance development will be directly proportional to h^2 (equation 5 and inversely proportional to slope (equation 4). S can be constant across insecticides for a particular intensity of selection only if the slope of the probit regression lines (and thus σ_p) is constant across insecticides (equations 3 and 4), but slope is not necessarily constant across insecticides. Thus, response quotient (Q) was used to compare the rates of resistance development against chlorantraniliprole and spinetoram, which can be defined as R divided by i (Tabashnik and McGaughey 1994):

$$Q = R/i. \quad [7]$$

The value of Q enables comparing the rates of resistance evolution among different insecticides without reference to slope, and thus allows us to evaluate the durability of an insecticide against a particular pest population.

Results

Susceptibility of RYN population to chlorantraniliprole significantly decreased as a result of selection with chlorantraniliprole (Table 1). The first round of selection resulted in 2.2-fold increase in LC_{50} of the RYN population compared with that of the unselected LAB population. LC_{50} of the RYN population continued to increase as a result of each of the subsequent rounds of selection leading to LCR of 6.6 at F_5. However, there was no additional increase in LC_{50} as a result of sixth round of selection.

Similarly, a significant decrease in the susceptibility of SPIN population was observed as a result of selection with spinetoram (Table 2). There was a 1.26-fold increase in LC_{50} of the SPIN population as a result of first round of selection compared with the unselected LAB population. After six rounds of selection, LC_{50} of the SPIN population was increased to 3.64-fold compared with the unselected LAB population.

The overall mean estimate of h^2 of chlorantraniliprole resistance using threshold trait analysis in *C. rosaceana* was 0.17, with mean response to R of 0.19 and an overall mean S of 1.12 (Table 3). The h^2 of spinetoram resistance was 0.18 with an R of 0.13 and an S of 0.70 (Table 4). However, the values of heritability of chlorantraniliprole and spinetoram resistance using the regression method were estimated as 0.13 (R^2 = 0.96) (Fig. 1) and 0.18 (R^2 = 0.96) (Fig. 2), respectively.

The estimates of h^2 of chlorantraniliprole resistance were higher for the first half of the selection experiment (mean = 0.28) than the second half (mean = 0.11) (Fig. 3a). Although the S was higher in the second half of the selection experiment (mean = 1.41) than in the first half (mean = 0.82), R was higher in the first half of the selection experiment (mean = 0.24) than in the second half (mean = 0.15) (Table 3).

Table 3. Estimation of response to selection (R) and selection differential (S) of chlorantraniliprole-selected population of *C. rosaceana*

Selected generation	Estimation of response to selection			Estimation of selection differential				
	Initial LC_{50} (95% FL)	Final LC_{50} (95% FL)	R	i	Initial slope (± SE)	Final slope (± SE)	σ_p	S
1	0.08 (0.03–0.15)	0.16 (0.07–0.32)	0.30	1.159	1.08 (0.10)	1.02 (0.39)	0.952	1.10
3	0.08 (0.06–0.11)	0.26 (0.20–0.34)	0.17	1.097	2.24 (0.28)	1.72 (0.17)	0.505	0.55
5	0.12 (0.08–0.16)	0.77 (0.31–1.48)	0.16	2.421	1.90 (0.28)	1.19 (0.17)	0.647	1.57
6	0.16 (0.11–0.23)	1.03 (0.50–1.66)	0.14	2.154	1.59 (0.19)	1.88 (0.36)	0.576	1.24

Table 4. Estimation of response to selection (R) and selection differential (S) of spinetoram-selected population of *C. rosaceana*

Selected generation	Estimation of response to selection			Estimation of selection differential				
	Initial LC_{50} (95% FL)	Final LC_{50} (95% FL)	R	i	Initial slope (± SE)	Final slope (± SE)	σ_p	S
1	0.08 (0.06–0.10)	0.10 (0.07–0.12)	0.10	1.400	4.00 (0.90)	2.56 (0.37)	0.305	0.43
2	0.05 (0.02–0.19)	0.12 (0.09–0.15)	0.19	1.858	1.75 (0.16)	2.53 (0.33)	0.467	0.87
4	0.05 (0.02–0.07)	0.17 (0.14–0.20)	0.13	1.627	2.72 (0.55)	3.63 (0.58)	0.315	0.51
6	0.06 (0.04–0.11)	0.22 (0.17–0.29)	0.09	2.459	1.97 (0.24)	3.01 (0.48)	0.402	0.99

Likewise, the estimates of h^2 of spinetoram resistance were higher for the first half of the selection experiment (mean = 0.22) than those for the second half (mean = 0.15) (Fig. 3b). Although S was higher in the second half of the selection experiment (mean = 0.75) than that in the first half (mean = 0.65), R was higher in the first half of the selection experiment (mean = 0.14) than that in the second half (mean = 0.11) (Table 4). These results indicate that the proportion of total phenotypic variation for resistance against chlorantraniliprole attributable to additive genetic variation in resistance declined during the selection experiment. This also was the case for spinetoram.

The mean values of Q for resistance against chlorantraniliprole and spinetoram were 0.11 and 0.07, respectively. These results indicate that resistance evolution would be slower against spinetoram than that against chlorantraniliprole; thus, spinetoram would be more durable than chlorantraniliprole against this particular population of *C. rosaceana.*

Discussion

Insect populations maintained in the laboratory for several years without being exposed to any insecticides are likely to have less genetic variation than field populations (Keiding 1986, Tanaka and Noppun 1989, Firku and Hayes 1990). It took only six generations of selection of a susceptible laboratory strain of *C. rosaceana* with chlorantraniliprole and spinetoram to produce a 6.6- and 3.6-fold increase in LC_{50}, respectively. The increase in levels of tolerance indicates that resistance could result in the field situations where selection pressures can be much higher than in the laboratory and populations are likely to be more heterogeneous.

Estimation of h^2 based on the method proposed by Tabashnik (1992) provides a standardized means to quantify the results of selection experiments by incorporating estimates of the strength of selection as well as the rate of resistance development. It also places the results of selection experiments in the broader context of the empirical and theoretical literature of evolutionary biology (Mousseau and Roff 1987, Falconer and Mackay 1996). In this study, R declined for both chlorantraniliprole and spinetoram as the selection progressed whereas S did not, leading to significantly higher h^2 in the first half of the selection experiment compared with the second half. These results are in agreement with those of Tabashnik (1992) where substantial additive genetic variation was present initially (i.e., alleles for resistance were not rare) and then declined as selection proceeded.

There was no change observed in the LCR as selection for chlorantraniliprole resistance proceeded from F_5 to F_6 and only a slight increase in the LCR as selection for spinetoram resistance proceeded from generations F_4 to F_6. These results point out that most of the variation in susceptibility of the LAB population to chlorantraniliprole and spinetoram had been exhausted. Likewise, a decrease in h^2 of chlorantraniliprole after three generations and spinetoram after four generations of selection indicates that most of the additive genetic variation in susceptibility of that particular LAB population to chlorantraniliprole and spinetoram had been exhausted. In addition, high mortalities observed after the fifth round of selection for both chlorantraniliprole and spinetoram resistances indicate the occurrence of bottlenecks that might have contributed to the reduced variation in the se-

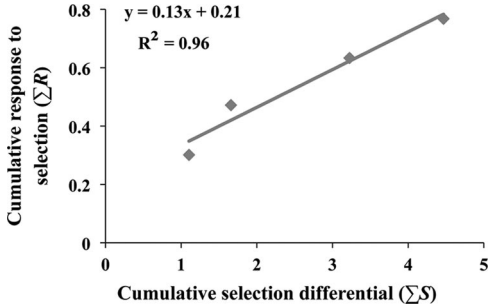

Fig. 1. Regression of cumulative response to chlorantraniliprole selection on cumulative selection differential in *C. rosaceana.*

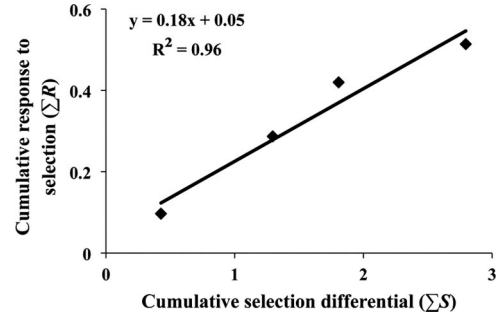

Fig. 2. Regression of cumulative response to spinetoram selection on cumulative selection differential in *C. rosaceana.*

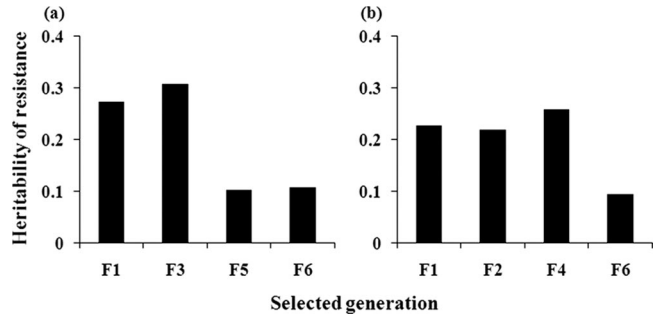

Fig. 3. Heritability in the narrow sense (h^2) of resistance to chlorantraniliprole (a) and spinetoram (b) in a laboratory population of *C. rosaceana* selected for resistance in the laboratory.

lected populations (Falconer and Mackay 1996, Saccheri et al. 2001).

The estimated values of h^2 of chlorantraniliprole resistance from our selection experiment using two different methods, 0.17 (Tabashnik 1992) and 0.13 (Tanaka and Noppun 1989) are in close agreement, and those of spinetoram resistance, 0.18 (Tabashnik 1992) and 0.18 (Tanaka and Noppun 1989), are the same. These results indicate that ≈13–17% of the total variation in chlorantraniliprole susceptibility and ≈18% of that in spinetoram susceptibility of the LAB population was caused by additive genetic variation. In a summary of 13 previously reported studies, estimates of h^2 to various insecticides for other insect pests ranged from 0.05 to 0.85, with a mean of 0.29 (Omer et al. 1993). Thus, our estimates of h^2 of resistance to chlorantraniliprole (0.13 and 0.17) and spinetoram (0.18) in *C. rosaceana* were lower than the mean for other reported cases.

Like any laboratory studies, the estimation of h^2 based on selections (Tanaka and Noppun 1989, Tabashnik 1992) is not free from limitations including technical problems in estimating parameters and uncertainty about extrapolation of experimental results to the field populations. Falconer and Mackay (1996) discussed technical difficulties generally encountered in estimating h^2 from selection experiments, whereas Tanaka and Noppun (1989) and Tabashnik (1992) examined the specific problems in the context of estimating h^2 of insecticide resistance. A detailed analysis of the factors that may introduce bias into the estimates of S (selection differential) was provided by Rosenheim (1991). One of these factors, the unequal selection of males and females, was minimized by selecting neonates without regard to sex. Efforts were made in bioassays to minimize the individual differences in exposure, but we do not know the extent of bias in estimation of S introduced by unequal treatment of individual neonates (which can overestimate S), and sublethal effects (which can underestimate S). Chlorantraniliprole caused mating disruption in codling moth when adult moths were exposed to its residues (Knight and Flexner 2007), but sublethal effects of larval exposure are largely unknown for chlorantraniliprole and spinetoram because these are novel insecticides and were only recently registered (2008) for use in orchards.

Despite recognized difficulties in extrapolating laboratory results to the field, we used estimates of realized h^2 and slope of probit lines in conjunction with varying selection intensities to project the rates of resistance development (Figs. 4 and 5). The projected rate of resistance evolution is directly proportional to h^2 and selection intensity (see equation 5[rsqb; Fig. 4). For example, assuming a slope of 1.6 (the average slope observed for chlorantraniliprole in this study) ($\sigma_p = 0.625$), h^2 of 0.17, and a selection mortality of 80% at each generation, then $R = 0.12$ and LC_{50} increases 10-fold in eight generations. However, in the same would happen in 12 generations if selection mortality is only 50%. Moreover, in a population with h^2 of 0.07 and 50% of the population selected at each generation ($R = 0.04$), a 10-fold increase in LC_{50} is ex-

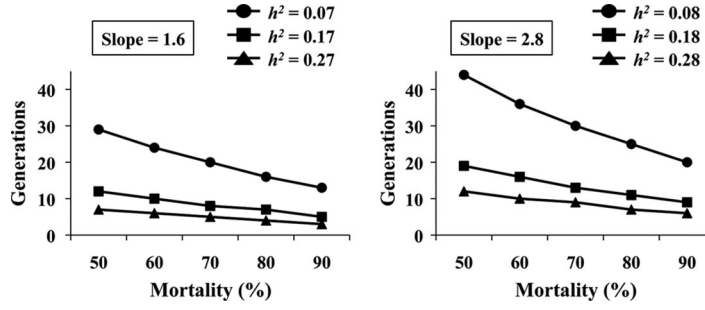

Fig. 4. Effect of heritability (h^2) on number of generations of *C. rosaceana* required for a 10-fold increase in LC_{50} of chlorantraniliprole (slope = 1.6) and spinetoram (slope = 2.8) at different selection intensities (i).

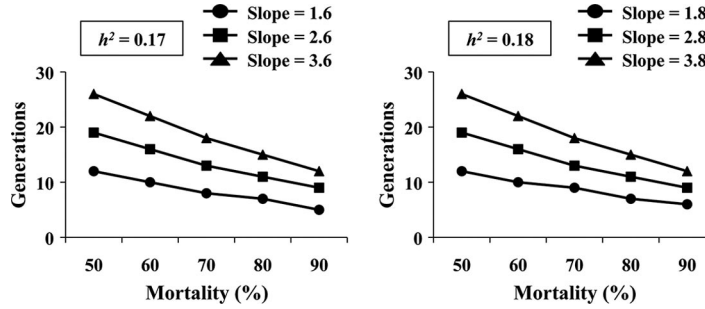

Fig. 5. Effect of slope on number of generations of *C. rosaceana* required for a 10-fold increase in LC_{50} of chlorantraniliprole ($h^2 = 0.17$) and spinetoram ($h^2 = 0.18$) at different selection intensities (i).

pected in 29 generations. If $h^2 = 0.27$ and 90% of the population is killed in each generation ($R = 0.30$), only three generations are needed to increase LC_{50} by a factor of 10. Similarly, assuming a slope of 2.8 (the average slope recorded for spinetoram in this study) ($\sigma_p = 0.357$), h^2 of 0.18, and selection mortality of 80% at each generation, then $R = 0.09$ and LC_{50} increases 10-fold in 11 generations. However, the same would happen in 19 generations if selection mortality is only 50% ($R = 0.05$). Moreover, if the population had an h^2 of 0.08 and 50% mortality at each generation, then $R =$ 0.02 and a 10-fold increase in LC_{50} would take >43 generations. If $h^2 = 0.28$ and 90% of the population is killed in each generation ($R = 0.18$), only less than six generations would be needed to increase LC_{50} by a factor of 10.

However, the projected rate of resistance evolution is inversely proportional the slope of the probit line (Fig. 5). For example, assuming that $h^2 = 0.17$ (the heritability of chlorantraniliprole resistance observed in this study) and selection mortality = 70%, a 10-fold increase in LC_{50} would occur in only eight generations at a slope of 1.6, whereas it would take >18 generations for the same to happen at a slope of 3.6. Similarly, assuming that $h^2 = 0.18$ (the heritability of spinetoram resistance observed in this study) and selection mortality = 80%, it would take seven generations for a 10-fold increase in LC_{50} at a slope of 1.8, whereas the same would happen in >15 generations at a slope of 3.8.

However, predictions must be interpreted cautiously because they are based on estimates of h^2 of a laboratory reared population. The h^2 of resistance to a particular insecticide can vary between conspecific populations as well as within a population through time because of changes in allele frequencies, environmental variation, or both. Nonetheless, the predictions based on the equation ($G = R^{-1}$) from quantitative genetic theory by using a laboratory population provide information that could be valuable in developing strategies to manage resistance (Via 1986, Firku and Hayes 1990, Tabashnik 1992) even before the occurrence of resistance in the field populations.

Relatively quick response of a laboratory population selected with chlorantraniliprole and spinetoram suggests that a risk for resistance development in *C. rosaceana* to both insecticides exists. The higher value of response quotient (Q) for chlorantraniliprole (0.11) compared with that for spinetoram (0.07) suggests that resistance to chlorantraniliprole could evolve faster than to spinetoram in *C. rosaceana.* Our findings serve as an early warning for the growers and pest managers and point out that implementation of resistance management strategies should occur when these chemistries are registered for use.

Although insecticide resistance management in *C. rosaceana* in tree fruit orchards is a challenge for growers and pest managers, especially at the time when broad-spectrum insecticides such as OPs are being phased out, a wide range of newer insecticides with different modes of action is available to control this pest. Significant variation in response of the field-collected populations to the two novel insecticides, chlorantraniliprole and spinetoram, has already been documented before their first field application (Sial et al. 2010). These insecticides must be used wisely in the framework of a well thought out resistance management program. However, resistance management strategies can only be successful if no cross-resistance occurs between different insecticides used in a resistance management program (Georghiou 1983). Therefore, further studies are required to explore the biochemical and molecular basis of mechanisms conferring resistance to chlorantraniliprole and spinetoram so that the insecticides that would not be affected by the same detoxification mechanisms could be incorporated into a pest management program in a manner that would minimize selection for resistance.

Acknowledgments

We thank Mike Doerr and Kathleen Pierre for assistance in maintaining a colony of *C. rosaceana* in the laboratory and Jay A. Rosenheim (University of California–Davis) and John E. Dunley (Tree Fruit Research and Extension Center, Washington State University) for comments on the manuscript. This study was sponsored by the Washington Tree Fruit Research Commission, the Washington State Commission on Pesticide Registration, and Western Sustainable Agriculture Research and Education (project GW10-003).

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Received 16 October 2009; accepted 6 April 2010.