Susceptibility of *Choristoneura rosaceana* (Lepidoptera: Tortricidae) to Two New Reduced-Risk Insecticides

ASHFAQ A. SIAL,[1] JAY F. BRUNNER, AND MICHAEL D. DOERR

Department of Entomology, Washington State University, Tree Fruit Research and Extension Center, 1100 N. Western Avenue, Wenatchee, WA 98801

J. Econ. Entomol. 103(1): 140–146 (2010); DOI: 10.1603/EC09238

ABSTRACT The response of field-collected populations of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), to chlorantraniliprole, spinetoram, spinosad, and azinphosmethyl was assessed using a diet incorporation bioassay. Populations of obliquebanded leafroller were collected from nine orchards in Chelan, Douglas, Grant, and Okanogan counties of Washington. The neonates of the F_1 or F_2 generation were used in all assays. The parameters of probit regression lines were estimated and lethal concentration ratios were calculated for all populations compared with a susceptible laboratory population. Significant variation was detected in response to all four insecticides including chlorantraniliprole and spinetoram, which had never been used in the field. lethal concentration ratios were 3.9–39.7 for azinphosmethyl, 0.5–3.6 for spinosad, 1.2–5.3 for chlorantraniliprole, and 0.5–4.1 for spinetoram. Correlation analysis indicated possibility of cross-resistance between spinosad and spinetoram, which are both members of spinosyn class. The occurrence of low but significant levels of resistance against chlorantraniliprole and spinetoram in field-collected populations of *C. rosaceana* before their first field application indicates that the risk of resistance evolution against these two new reduced-risk insecticides exists. However, it is likely that these low levels of resistance can be managed if the insecticides are used judiciously in conjunction with sound resistance management programs. Implications of these results for developing and implementing resistance management strategies are discussed.

KEY WORDS obliquebanded leafroller, field-collected populations, chlorantraniliprole, spinetoram, insecticide resistance

The obliquebanded leafroller, *Choristoneura rosaceana* (Harris), is a tortricid moth native to North America (Weires and Riedl 1991). It is a major pest of pome fruits, second only to codling moth, C*ydia pomonella* (L.), in Washington (Brunner 1999). In apple, obliquebanded leafroller larvae feed on flower buds, leaves, and developing fruits (Howitt 1993, Ohlendorf 1999). Fruit damage from larval feeding can occur in the spring, during midsummer (the most significant), or just before harvest (Beers et al. 1993). Leafrollers, and the key apple pest, *C. pomonella* (L.), have been controlled using the organophosphate (OP) insecticides for over four decades. However, reports of decreasing efficacy of OP insecticides against leafrollers have been attributed to the development of insecticide resistance (Brunner 1996). Insecticide resistance to OPs and cross-resistance to other groups of chemicals have been documented in obliquebanded leafroller (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 even before the new insecticides had been used in the field (Sauphanor et al. 1998, Dunley and Welter 2000). In addition, the implementation of Food Quality Protection Act of 1996 (FQPA) (U.S. Environmental Protection Agency [USEPA] 1996) has increased restrictions on the use of broad-spectrum insecticides, especially the OPs.

The development of insecticide resistance as well as regulatory actions such as FQPA have led to a greater priority in the development of reduced-risk insecticides as OP alternatives (USEPA 1997). Chlorantraniliprole and spinetoram are the reduced-risk insecticides recently registered as OP alternatives. Chlorantraniliprole is an anthranilic diamide, which belongs to insecticide resistance action committee (IRAC) mode of action class 28 (Insecticide Resistance Action Committee [IRAC] 2009). Anthranilic diamides selectively bind to the ryanodine receptors (RyR) in insect muscles resulting in an uncontrolled release of calcium from internal stores in 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 of target organisms. Anthranilic diamides have very low vertebrate toxicity because of a >500-

[1] E-mail: ashfaqsial@wsu.edu.

fold differential selectivity toward insect over mammalian RyR (Cordova et al. 2006). Spinetoram is a recently developed spinosyn, which belongs to IRAC mode of action class 5 (IRAC 2009). Spinosyns primarily activate the nicotinic acetylcholine receptors by acting on a unique site (Salgado 1998, Salgado et al. 1998). Both chlorantraniliprole and spinetoram have a high degree of efficacy against obliquebanded leafroller in laboratory and field trials (Hull et al. 2009; Brunner, unpublished data).

With the availability of effective OP alternatives, it is critical for growers to incorporate the reduced-risk insecticides into *C. rosaceana* management programs for successful production of tree fruits on a sustainable basis. However, the development of resistance is a continual threat, especially to the novel chemistries such as chlorantraniliprole and spinetoram. The total cost associated with resistance is difficult to assess, but the loss of insecticide effectiveness almost invariably entails the use of increased concentrations and application frequency. Eventually replacement compounds (National Research Council [NRC] 1986) are needed that are more expensive because of increased costs in discovery, development, registration, and manufacturing (Metcalf 1980). Characterizing susceptibility to new insecticides in field populations would be valuable for *C. rosaceana* management programs by providing an early detection of potential problems of resistance and cross-resistance. This would allow growers to change their *C. rosaceana* control strategies and potentially slow the spread of resistance. It could also provide evidence that resistance is not a problem associated with control failures, and encourage growers to address operational factors, for example, sprayer calibration or target coverage, contributing to the lack of control. The objective of this study was to the survey current levels of susceptibility of field-collected populations of *C. rosaceana* to the two new reduced-risk insecticides chlorantraniliprole and spinetoram before their introduction into *C. rosaceana* management programs, and to assess the potential for the occurrence of cross-resistance to the currently used insecticides such as azinphosmethyl and spinosad.

Materials and Method

Insects. *Laboratory Population.* A *C. rosaceana* laboratory colony (LAB) was established by collecting larvae from apple orchards in Mattawa, WA, in 1990. This colony has been reared continuously because their collection on a pinto bean diet following the method of Shorey and Hale (1965) under constant conditions of temperature (23 ± 2°C), relative humidity (RH, 70%), and photoperiod (16L:8D).

Field Populations. In 2007, field populations were collected from the overwintering (spring brood) larvae at three locations JON (Quincy, Grant County, WA), PTH (Mattawa, Grant County, WA), and JAR (Brewster, Okanogan County, WA). In 2008, field populations were collected from the overwintering brood at four locations, STM (Stemilt Hill, Wenatchee, Chelan County, WA), KMP and CLK (Chelan, Chelan County, WA), and WEB (Quincy, Grant County, WA); and from summer brood larvae at two locations, GRF (Crane, Douglas County, WA) and ROB (Brewster, Okanogan County, WA). The field populations were collected as third to fifth instars, returned to the laboratory, transferred to 96 ml plastic portion cups (#S-300, Prairie Packaging Inc., Bedford Park, IL) containing artificial pinto bean diet (Shorey and Hale 1965), and reared to the adult stage. The neonate larvae of the first laboratory generation, and in some cases the second laboratory generation, were used in bioassays.

Insecticides. Materials used in these experiments were chlorantraniliprole (DPX E2Y45–410) 35 WG (Altacor/Rynaxypyr), EPA Est. No. 352-DE-002, E.I. du Pont de Nemours & Co., Agricultural Products Department, Wilmington, DE; spinetoram (XDE-175) 25 WG (Delegate), EPA Est. No. 62719-IN-1, Dow AgroSciences LLC, Indianapolis, IN; spinosad 2 SC (Success), EPA Reg. No. 62719–292, Dow AgroSciences LLC, Indianapolis, IN; and azinphosmethyl 50W (Guthion), EPA Reg. No. 3125–301, Bayer Crop Sciences, KS City, MO.

Bioassays. Toxicity of each insecticide to neonate larvae of *C. rosaceana* was determined using a diet incorporation bioassay. A dry premix of a Heliothis diet (Stonefly Heliothis Diet (#38 V 0600), Ward's Natural Science, Rochester, NY) was used in the bioassay. A stock solution was prepared by diluting the test insecticide at 10× the highest concentration to be used in the bioassay. Then serial dilutions were prepared from the stock solution, each at 10× the target concentration to be used in the bioassay. For each concentration, a treatment solution was prepared by weighing 61 g of water adding 4 g of vinegar and then 10 g of the 10× insecticide dilution. This treatment solution was then added to 25 g of dry diet premix to complete the insecticide incorporated diet. An untreated control was prepared by using water and vinegar plus the dry diet premix. For chlorantraniliprole, five concentrations (0.1–10 ppm) were used in 2007 whereas six concentrations (0.01–3 ppm) were used in 2008. For bioassays performed in 2008, six concentrations were used for spinetoram (0.003–1 ppm) and spinosad (0.3–10 ppm), and seven concentrations (1–1000 ppm) were used for azinphosmethyl. Enough insecticide incorporated diet was prepared before the start of the bioassays, so that all tests were run on the same diet mixtures.

A small portion of insecticide incorporated diet (≈8.0 cm^3) was added to a plastic petri dish (Falcon 1006, 50 by 9 mm, Becton Dickinson Labware, Becton Dickinson and Company, 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 using a camel hairbrush. Six to 10 dishes were prepared for each treatment (30–50 larvae/treatment) depending on the availability of neonate larvae. The dishes were placed in growth

Table 1. Results of probit analyses for chlorantraniliprole using diet incorporation bioassays on *Choristoneura rosaceana* neonate larvae from a laboratory as well as field-collected populations in 2007

Population	*n*	Slope (± SEM)	χ^2	*P* value	LC_{50} (ppm) (95% FL)[a]	LC_{90} (ppm) (95% FL)[a]	LCR-LC_{50}[b] (95% CL)[d]	LCR-LC_{90}[c] (95% CL)[d]
Spring brood								
LAB	250	2.61 (0.39)	1.08	0.78	0.33 (0.24–0.77)	1.03 (0.77–1.60)		
PTH	250	3.06 (0.70)	0.18	0.98	0.39 (0.19–0.57)	1.03 (0.71–2.10)	1.18 (0.88–1.59)	1.00 (0.59–1.68)
JAR	250	5.12 (0.99)	0.75	0.86	1.21 (1.01–1.44)	2.16 (1.76–3.19)	3.07 (2.19–4.29)*	2.09 (1.35–3.25)*
JON	250	2.91 (0.37)	4.97	0.17	1.39 (1.02–1.85)	3.83 (2.74–6.58)	4.18 (2.91–5.99)*	3.71 (2.30–5.99)*

n, no. of larvae assayed.
[a] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[b] LCR-LC_{50}, lethal concn ratio at LC_{50} = LC_{50} (field pop)/LC_{50} (LAB pop).
[c] LCR-LC_{90}, lethal concn ratio at LC_{90} = LC_{90} (field pop)/LC_{90} (LAB pop).
[d] 95% CL estimated using lethal concn ratio significance test (Robertson et al. 2007).
* LC_{50} or LC_{90} of field collected pop significantly different ($\alpha = 0.05$) from that of the LAB pop (Robertson et al. 2007).

chambers at constant conditions of temperature (23 ± 2°C), RH (70%), and photoperiod (16L:8D).

In 2007, larval mortality was evaluated after 4 d. Larvae were recorded as dead if they did not move when probed with a camel hairbrush. In bioassays associated with other studies we found that a high percentage of the larvae treated with chlorantraniliprole that were recorded alive in 2007 bioassays using the criteria described above for assessing larval mortality, were actually moribund and died after a few days. To incorporate this new knowledge and improve the quality of our bioassay techniques for chlorantraniliprole, we revised the criteria of assessing larval mortality in 2008 bioassays. In these bioassays larval mortality in all populations, including the LAB colony, was evaluated after 7 d, and moribund larvae were recorded as dead. 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 5–10 larvae per egg mass were treated at any one concentration.

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 (LCR) at LC_{50} and LC_{90} values and their corresponding 95% CL were calculated using LCR significance test (Robertson et al. 2007). The laboratory colony (LAB) served as the reference susceptible population for comparison purposes and was assigned a ratio of 1.0. The lethal concentration (LC_{50} and LC_{90}) values of the field-collected populations were considered significantly different from those of the LAB population if the 95% CL of their corresponding LCR values did not include the value of 1.0 ($\alpha = 0.05$). Pearson's Product Moment Correlation (Pearson's correlation) was used to detect the occurrence of cross-resistance between the chemicals tested in this study.

Results

Larvae of *C. rosaceana* collected from different orchards in Washington State in 2007 showed varying susceptibility to chlorantraniliprole (Table 1). The mortality for populations from PTH, JAR, and JON indicated a good fit to a probit model (Pearson's χ^2 test; $P > 0.05$). Larvae from PTH were the most susceptible of populations tested in 2007, with LC_{50} and LC_{90} of 0.39 and 1.03 ppm, respectively. Based on the LCR significance test, both JAR and JON populations were less susceptible to chlorantraniliprole than the LAB colony with 3.07- and 4.18-fold higher LC_{50} values, respectively. The slope values suggested that the JAR population had relatively less variation in susceptibility to chlorantraniliprole as compared with the JON population.

In 2008, *C. rosaceana* field-collected larvae showed varying degree of susceptibility to chlorantraniliprole (Table 2). Populations from STM, KMP, WEB, and CLK (spring brood) and from GRF and ROB (summer brood) indicated good fit to a probit model (Pearson's χ^2 test; $P > 0.05$). The STM and CLK populations were the most susceptible to chlorantraniliprole. Based on the LCR significance test, all field-collected populations evaluated in 2008 were significantly less susceptible than the LAB colony with 1.70- to 5.26-fold higher LC_{50} values. However, LC_{90} of the STM and CLK populations were not significantly different from the LAB colony. The WEB and CLK populations had relatively less variation in their susceptibility to chlorantraniliprole, as indicated by the high slope values. The LC_{50} values of the LAB colony observed in 2008 were less than those recorded in 2007. This was due primarily to the change in the criteria for assessment of larval mortality as explained in materials and methods section, and not to an inherent change in the susceptibility of the LAB colony.

In 2008, the larvae of field-collected populations of *C. rosaceana* showed significant variation in their susceptibility to spinetoram (Table 3). Populations from STM, WEB, and CLK (spring brood), and GRF and ROB (summer brood) indicated good fit to a probit model (Pearson's χ^2 test; $P > 0.05$). Larvae from STM and WEB were the most susceptible to spinetoram. Based on the LCR significance test the KMP, CLK, GRF, and ROB populations were significantly less susceptible than the LAB colony with 1.70- to 4.05-fold higher LC_{50} values while the STM and WEB populations were significantly more susceptible than the LAB colony with LCRs of 0.50 and 0.40 at LC_{50}, respectively. However, LC_{90} values of the STM and WEB populations were not significantly different from that

Table 2. Results of probit analyses for chlorantraniliprole using diet incorporation bioassays on *Choristoneura rosaceana* neonate larvae from a laboratory as well as field-collected populations in 2008

Population	*n*	Slope (± SEM)	χ^2	*P* value	LC_{50} (ppm) (95% FL)	LC_{90} (ppm) (95% FL)[a]	LCR-LC_{50}[b] (95% CL)[d]	LCR-LC_{90}[c] (95% CL)[d]
Spring brood								
LAB	180	2.65 (0.57)	0.25	0.99	0.11 (0.07–0.16)	0.35 (0.24–0.69)		
STM	180	2.55 (0.45)	1.34	0.85	0.19 (0.13–0.26)	0.61 (0.42–1.13)	1.70 (1.02–2.79)*	1.76 (0.92–3.38)
KMP	180	1.46 (0.31)	4.68	0.32	0.27 (0.10–0.47)	2.00 (1.05–8.72)	2.40 (1.19–4.64)*	5.79 (2.44–13.77)*
WEB	180	3.84 (0.84)	0.84	0.93	0.57 (0.40–0.75)	1.23 (0.92–2.12)	5.03 (3.11–8.13)*	3.56 (1.97–6.44)*
CLK	180	3.52 (0.92)	1.60	0.81	0.19 (0.12–0.26)	0.44 (0.32–0.91)	1.70 (1.02–2.78)*	1.27 (0.68–2.39)
First summer brood								
LAB	180	2.20 (0.44)	3.44	0.49	0.15 (0.08–0.22)	0.57 (0.33–1.30)		
GRF	180	1.94 (0.47)	0.43	0.98	0.79 (0.43–1.20)	3.60 (2.14–11.97)	5.26 (2.91–9.50)*	6.29 (2.47–16.04)*
ROB	180	2.54 (0.39)	6.76	0.15	0.71 (0.52–0.96)	2.28 (1.58–4.10)	4.76 (2.88–7.87)*	3.98 (2.02–7.84)*

n, no. of larvae assayed.
[a] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[b] LCR-LC_{50}, lethal concn ratio at LC_{50} = LC_{50} (field pop)/LC_{50} (LAB pop).
[c] LCR-LC_{90}, lethal concn ratio at LC_{90} = LC_{90} (field pop)/LC_{90} (LAB pop).
[d] 95% CL estimated using lethal concn ratio significance test (Robertson et al. 2007).
* LC_{50} or LC_{90} of field collected pop significantly different ($\alpha = 0.05$) from that of the LAB pop (Robertson et al. 2007).

of the LAB colony. The KMP population had relatively more variation in its susceptibility to spinetoram, as indicated by low slope value, whereas the CLK and ROB populations had relatively less variation as indicated by high slope values.

The larvae of *C. rosaceana* populations collected in 2008 showed significant variation in their susceptibility to spinosad (Table 4). Populations from STM and KMP (spring brood), and GRF (summer brood) indicated good fit to a probit model (Pearson's χ^2 test; $P > 0.05$). Based on the LCR significance test, LCRs at LC_{50} for four out of five populations were significant ($\alpha = 0.05$). The KMP, GRF, and ROB populations were significantly less susceptible than the LAB colony with 2.4- to 3.6-fold higher LC_{50} values while the STM population was significantly more susceptible than the LAB colony with an LCR of 0.50. The susceptibility of larvae from the WEB population to spinosad was statistically similar to that of the LAB colony. However, LC_{90} values of the KMP and GRF populations were significantly higher than that of the LAB colony. The STM and KMP populations had relatively more variation in their susceptibility to spinosad, as indicated by low slope values, whereas the GRF and ROB populations had relatively less variation in their response to spinosad, as indicated by high slope values.

The larvae of the field-collected populations (2008) of *C. rosaceana* showed significant variation in their susceptibility to azinphosmethyl (Table 5). Populations from KMP and CLK (spring brood), and GRF and ROB (summer brood) indicated good fit to a probit model (Pearson's χ^2 test; $P > 0.05$). Based on LCR significance test, the neonate larvae from all of the five field-collected populations were significantly less susceptible ($\alpha = 0.05$) than those from the LAB colony with 3.9- to 39.7-fold higher LC_{50} values. The WEB, CLK, and KMP populations were more heterogeneous in their response to azinphosmethyl, as indicated by low slope values.

There was significant positive correlation between the tolerances of field-collected populations of *C. rosaceana* to spinosad and spinetoram at LC_{50} with Pearson's correlation coefficient (r) = 0.92 (df = 3; P =

Table 3. Results of probit analyses for spinetoram using diet incorporation bioassays on *Choristoneura rosaceana* neonate larvae from a laboratory as well as field-collected populations in 2008

Population	*n*	Slope (± SEM)	χ^2	*P* value	LC_{50} (ppm) (95% FL)[a]	LC_{90} (ppm) (95% FL)[a]	LCR-LC_{50}[b] (95% CL)[d]	LCR-LC_{90}[c] (95% CL)[d]
Spring brood								
LAB	180	3.82 (0.82)	1.56	0.82	0.09 (0.07–0.12)	0.20 (0.15–0.34)		
STM	180	2.25 (0.55)	2.73	0.60	0.04 (0.02–0.07)	0.16 (0.11–0.37)	0.50 (0.27–0.82)*	0.80 (0.43–1.52)
KMP	180	1.38 (0.19)	15.04	0.01	0.23 (0.11–0.61)	1.92 (0.68–2.50)	2.41 (1.44–4.02)*	9.49 (3.83–23.53)*
WEB	180	1.97 (0.50)	4.40	0.36	0.04 (0.01–0.06)	0.16 (0.12–0.40)	0.40 (0.20–0.71)*	0.78 (0.40–1.53)
CLK	180	3.23 (0.88)	2.86	0.58	0.16 (0.09–0.25)	0.39 (0.28–0.90)	1.70 (1.09–2.64)*	1.95 (1.09–3.49)*
First summer brood								
LAB	180	3.05 (0.64)	1.14	0.89	0.02 (0.01–0.03)	0.05 (0.04–0.10)		
GRF	180	2.97 (0.59)	2.28	0.68	0.06 (0.04–0.09)	0.16 (0.11–0.33)	3.00 (1.86–4.84)*	3.08 (1.66–5.70)*
ROB	180	3.27 (0.39)	0.97	0.91	0.08 (0.06–0.11)	0.20 (0.15–0.37)	4.05 (2.57–6.39)*	3.80 (2.10–6.89)*

n, no. of larvae assayed.
[a] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[b] LCR-LC_{50}, lethal concn ratio at LC_{50} = LC_{50} (field pop)/LC_{50} (LAB pop).
[c] LCR-LC_{90}, lethal concn ratio at LC_{90} = LC_{90} (field pop)/LC_{90} (LAB pop).
[d] 95% CL estimated using lethal concn ratio significance test (Robertson et al. 2007).
* LC_{50} or LC_{90} of field collected pop significantly different ($\alpha = 0.05$) from that of the LAB pop (Robertson et al. 2007).

Table 4. Results of probit analyses for spinosad using diet incorporation bioassays on *Choristoneura rosaceana* neonate larvae from a laboratory as well as field-collected populations in 2008

Population	*n*	Slope (± SEM)	χ^2	*P* value	LC_{50} (ppm) (95% FL)[a]	LC_{90} (ppm) (95% FL)[a]	LCR-LC_{50}[b] (95% CL)[d]	LCR-LC_{90}[c] (95% CL)[d]
Spring brood								
LAB	180	2.43 (0.33)	13.45	0.01	0.26 (0.16–0.43)	0.87 (0.50–2.63)		
STM	180	1.78 (0.25)	5.59	0.23	0.13 (0.08–0.19)	0.68 (0.41–1.66)	0.50 (0.32–0.78)*	0.78 (0.38–1.60)
KMP	180	1.86 (0.22)	5.90	0.21	0.61 (0.28–1.45)	2.96 (1.28–21.60)	2.36 (1.52–3.66)*	3.41 (1.71–6.83)*
WEB	180	2.09 (0.27)	14.07	0.01	0.25 (0.17–0.36)	1.01 (0.63–2.17)	0.95 (0.62–1.45)	1.16 (0.59–2.28)
First summer brood								
LAB	180	1.78 (0.24)	15.02	0.01	0.16 (0.11–0.24)	0.84 (0.50–1.96)		
GRF	180	3.75 (1.02)	5.08	0.28	0.58 (0.33–0.78)	1.27 (0.93–2.49)	3.61 (2.22–5.89)*	1.52 (1.08–2.77)*
ROB	180	3.46 (0.89)	1.45	0.84	0.49 (0.30–0.66)	1.16 (0.84–2.31)	3.09 (1.91–5.00)*	1.39 (0.70–2.75)

n, no. of larvae assayed.
[a] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[b] LCR-LC_{50}, lethal concn ratio at LC_{50} = LC_{50} (field pop)/LC_{50} (LAB pop).
[c] LCR-LC_{90}, lethal concn ratio at LC_{90} = LC_{90} (field pop)/LC_{90} (LAB pop).
[d] 95% CL estimated using lethal concn ratio significance test (Robertson et al. 2007).
* LC_{50} or LC_{90} of field collected pop significantly different ($\alpha = 0.05$) from that of the LAB pop (Robertson et al. 2007).

0.026) indicating the possibility of cross-resistance between the two chemicals (Fig. 1). There was no correlation among tolerances of *C. rosaceana* to any of the other insecticides tested. The correlation coefficient (Pearson's *r*) for chlorantraniliprole and azinphosmethyl was 0.54 (df = 3; $P = 0.35$), for chlorantraniliprole and spinosad was 0.45 (df = 3; $P = 0.44$), for chlorantraniliprole and spinetoram was 0.20 (df = 4; $P = 0.71$), for azinphosmethyl and spinosad was −0.69 (df = 2; $P = 0.31$), and for azinphosmethyl and spinetoram was −0.81 (df = 3; $P = 0.09$) across the field-collected populations.

Discussion

The occurrence of insecticide resistance is a major risk to the sustainability of integrated pest management (IPM) programs for *C. rosaceana.* Effective resistance management strategies could slow the development of resistance only if implemented in a timely manner. The information on existing levels of resistance and cross-resistance to different classes of insecticides is an important factor in developing a successful resistance management program for *C. rosaceana.* All of the populations of *C. rosaceana* tested in this study were resistant to azinphosmethyl, which is consistent with the results of Waldstein et al. (1999), Pree et al. (2001), Ahmad et al. (2002), Smirle et al. (2002), and Dunley et al. (2006). The prevalence of azinphosmethyl resistance can be attributed to the decades of its use in *C. pomonella management* programs, which incidentally exposed *C. rosaceana* to azinphosmethyl.

Implementation of FQPA has increased pressure for fundamental change in IPM strategies leading to the development of reduced-risk OP alternatives including chlorantraniliprole, spinosad, and spinetoram. Chlorantraniliprole, a member of a novel class of insecticides, the anthranilic diamides, was registered for use on tree fruit in 2008. The field-collected populations tested in this study had never been exposed to chlorantraniliprole. The significant variation in the susceptibility of field-collected *C. rosaceana* populations represents the first documentation of preexisting resistance to chlorantraniliprole and suggests that higher levels of resistance could occur rapidly after its

Table 5. Results of probit analyses for azinphosmethyl using diet incorporation bioassays on *Choristoneura rosaceana* neonate larvae from a laboratory as well as field-collected populations in 2008

Population	*n*	Slope (± SEM)	χ^2	*P* value	LC_{50} (ppm) (95% FL)[a]	LC_{90} (ppm) (95% FL)[a]	LCR-LC_{50}[b] (95% CL)[d]	LCR-LC_{90}[c] (95% CL)[d]
Spring brood								
LAB	210	4.25 (0.88)	8.20	0.15	1.33 (1.04–1.66)	2.66 (2.05–4.42)		
KMP	210	2.11 (0.27)	1.17	0.95	9.92 (7.29–13.52)	40.04 (26.99–72.11)	7.46 (5.13–10.58)*	15.04 (8.40–26.94)*
WEB	180	1.52 (0.18)	17.32	0.004	52.69 (33.85–83.81)	368.33 (202.03–963.10)	39.65 (25.88–60.75)*	138.29 (68.54–279.03)*
CLK	210	1.64 (0.19)	10.81	0.06	15.56 (10.52–23.24)	94.32 (56.26–206.07)	11.71 (7.77–17.65)*	35.42 (18.46–67.97)*
First summer brood								
LAB	210	5.10 (1.14)	0.004	1.00	1.96 (1.44–2.43)	3.49 (2.77–5.31)		
GRF	210	2.78 (0.50)	3.02	0.70	50.55 (32.22–72.61)	146.28 (98.60–289.39)	25.83 (17.03–39.19)*	41.91 (24.79–70.86)*
ROB	210	1.48 (0.25)	4.48	0.48	7.56 (3.46–12.79)	55.66 (31.85–138.84)	3.86 (2.12–7.06)*	15.93 (8.08–31.41)*

n, no. of larvae assayed.
[a] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[b] LCR-LC_{50}, lethal concn ratio at LC_{50} = LC_{50} (field pop)/LC_{50} (LAB pop).
[c] LCR-LC_{90}, lethal concn ratio at LC_{90} = LC_{90} (field pop)/LC_{90} (LAB pop).
[d] 95% CL estimated using lethal concn ratio significance test (Robertson et al. 2007).
* LC_{50} or LC_{90} of field collected population significantly different ($\alpha = 0.05$) from that of the LAB pop (Robertson et al. 2007).

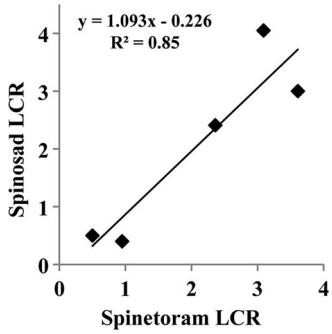

Fig. 1. Regression between spinosad and spinetoram lethal concentration ratios at LC_{50} in populations of *Choristoneura rosaceana* collected from pome fruit orchards in Washington.

use in the field. That there was no significant correlation between chlorantraniliprole and azinphosmethyl resistance in *C. rosaceana* field populations argues against cross-resistance to OPs. The presence of low-level resistance in *C. rosaceana* to an insecticide before its first introduction has been reported before (Waldstein and Reissig 2000, Ahmad et al. 2002, Smirle et al. 2002, Dunley et al. 2006). The *C. rosaceana* is a polyphagous insect with a host range of over 50 plant species including members of family Rosaceae and Cornaceae (Sanderson and Jackson 1909). Its adaptive evolutionary response to insecticides, even novel chemistries, could be attributed to the breadth of compounds that *C. rosaceana* has been exposed to through its diet. All of the *C. rosaceana* populations tested in 2007 and 2008 were collected from conventionally managed orchards except the STM population that came from an organically managed orchard. This population was the most susceptible of all populations tested in 2008. These differences reflect the usage pattern of insecticides under organic and conventional management. IPM strategies can impact the susceptibility of pests to insecticides, regardless of which class they belong to, which has been documented elsewhere (Smirle et al. 2003).

Spinosad was the first spinosyn insecticide registered for use against *C. rosaceana* in 1998. Resistance of *C. rosaceana* populations to spinosad was documented by Dunley et al. (2006) and can be attributed to its extensive use over 6 yr when no other effective control existed. The recent introduction of spinetoram, a chemical in the same class, raised questions about cross-resistance between these two chemicals. Our results showed significant variation in tolerance of the field-collected populations of *C. rosaceana* to spinosad and spinetoram. Moreover, based on LCRs at LC_{50} spinetoram resistance was highly correlated with spinosad resistance, suggesting the possibility of cross-resistance. This was the first study to document the evidence of correlated cross-resistance between spinosad and spinetoram.

Genetic variation provides the basis for evolutionary change. The significant variation in susceptibility of *C. rosaceana* populations to chlorantraniliprole and spinetoram indicates a high risk of resistance evolution in this pest. Our findings establish baseline susceptibility of the field-collected *C. rosaceana* populations to chlorantraniliprole and spinetoram and serves as an early warning for the growers and pest managers, pointing out that implementing a sound resistance management program is essential to the preservation of these reduced-risk insecticides for *C. rosaceana* control on sustainable basis.

At a time when OP insecticide use is being restricted or even phased-out, a number of alternative insecticides with different modes of action have become available for *C. rosaceana* control including chlorantraniliprole and spinetoram. These OP alternative insecticides are highly effective against *C. rosaceana* (Hull et al. 2009; Brunner, unpublished data), exhibit a high degree of worker safety and are environmentally friendly, thereby providing the potential for more sustainable management of *C. rosaceana.* However, these insecticides must be used wisely in the framework of a well-informed resistance management program that reduces selection pressure on *C. rosaceana,* taking into account the potential for cross-resistance between different classes of insecticides (Georghiou 1983, Ffrench-Constant and Roush 1990). Further studies are needed to determine the biochemical and molecular basis of mechanisms conferring *C. rosaceana* resistance to chlorantraniliprole and spinetoram, so that insecticides not affected by the same mechanism could be incorporated into IPM programs.

Acknowledgments

We thank John E. Dunley for his comments on the manuscript and Kathleen Pierre for her assistance in rearing the field-collected populations and maintaining a colony of obliquebanded leafroller in the laboratory. This study was sponsored by the Washington Tree Fruit Research Commission, the Washington State Commission on Pesticide Registration, and Western Sustainable Agriculture and Research (Project No.: GW10-003).

References Cited

Ahmad, M., R. M. Hollingworth, and J. C. Wise. 2002. Broad-spectrum insecticide resistance in obliquebanded leafroller *Choristoneura rosaceana* (Lepidoptera: Tortricidae) from Michigan. Pest Manag. Sci. 58: 834–838.

Beers, E. H., J. F. Brunner, M. J. Willett, and G. M. Warner. 1993. Orchard pest management: a resource book for the Pacific Northwest. Good Fruit Grower, Yakima WA.

Brunner, J. F. 1996. Discovery of *Colpoclypeus florus* (Walker) in apple orchards of Washington. Pan-Pacific Entomol. 72: 5–12.

Brunner, J. F. 1999. New pests a challenge for area wide programs. Proc. Wash. St. Hortic. Assoc. 95: 154–158.

Cordova, D., E. A. Benner, M. D. Sacher, J. J. Rauh, J. S. Sopa, G. P. Lahm, T. P. Selby, T. M. Stevenson, L. Flexner, S. Gutteridge, D. F. Rhoades, L. Wu, R. M. Smith, and Y. Tao. 2006. Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. Pestic. Biochem. Physiol. 84: 196–214.

Dunley, J. E., J. F. Brunner, M. D. Doerr, and E. H. Beers. 2006. Resistance and cross-resistance in populations of

leafrollers, *Choristoneura rosaceana* and *Pandemis pyrusana,* in Washington apples. 7 pp. J. Insect Sci. 6: 14. (www.insectscience.org).

Dunley, J. E., and S. C. Welter. 2000. Correlated insecticide cross-resistance in azinphosmethyl resistant codling moth. J. Econ. Entomol. 93: 955–962.

Ffrench-Constant, R. H., and R. T. Roush. 1990. Resistance detection and documentation: the relative roles of pesticidal and biochemical assays, pp. 4–38. *In* R. T. Roush, and B. E. Tabashnik [eds.], Pesticide resistance in arthropods. Chapman & Hall, New York.

Georghiou, G. P. 1983. Management of resistance in arthropods, pp. 769–792. *In* G. P. Georghiou, and T. S. Saito [eds.], Pest resistance to pesticides. Plenum, New York.

Howitt, A. H. 1993. Common tree fruit pests. Michigan State University Extension, NCR 63.

Hull, L. A., N. K. Joshi, and F. U. Zaman. 2009. Large plot reduced risk insecticide study for lepidopteran pests infesting apples, 2008. Arthropod Management Tests 34. (doi: 10.4182/amt.2009.A11).

[IRAC] Insecticide Resistance Action Committee. 2009. IRAC mode of action classification, v. 6.3. IRAC Mode of Action Working Group, St. Louis, MO. (http://www.irac-online.org/documents/MoA%20classification_v6.3.3_28july09.pdf).

Lahm, G. P., T. P. Selby, J. H. Freudenberger, T. M. Stevenson, B. J. Myers, G. Seburyamo, B. K. Smith, L. Flexner, C. E. Clark, and D. Cordova. 2005. Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators. Med. Chem. Lett. 15: 4898–4906.

Lawson, D. S., W. H. Reissig, and C. M. Smith. 1997. Response of larval and adult obliquebanded leafroller (Lepidoptera: Tortricidae) to selected insecticides. J. Econ. Entomol. 90: 1450–1457.

LeOra Software. 1987. POLO-PC: A user's guide to probit and logit analysis. LeOra, Berkeley, CA.

Metcalf, R. L. 1980. Changing role of insecticide resistance in crop protection. Annu. Rev. Entomol. 25: 219–256.

[NRC] National Research Council. 1986. Pesticide resistance: strategies and tactics for management. Natl. Acad. Press, Washington, DC.

Ohlendorf, B.L.P. 1999. Integrated pest management for apples and pears, 2nd ed. Statewide Integrated Pest Management Project, University of California Division of Agriculture and Natural Resources Publication 3340.

Pree, D. J., K. J. Whitty, M. K. Pogoda, and L. A. Bittner. 2001. Occurrence of resistance to insecticides in populations of the obliquebanded leafroller from orchards. Can. Entomol. 133: 93–103.

Reissig, W. H., B. H. Stanely, and H. E. Hebding. 1986. Azinphosmethyl resistance and weight related response of obliquebanded leafroller (Lepidoptera: Tortricidae) larvae to insecticides. J. Econ. Entomol. 79: 329–333.

Robertson, J. L., R. M. Russel, H. K. Preisler, and N. E. Savin. 2007. Binary quantal response with one explanatory variable, pp. 21–34. Bioassays with Arthropods, 2nd ed. CRC, Boca Raton, FL.

Salgado, V. L. 1998. Studies on the mode of action of spinosad: insect symptoms and physiological correlates. Pestic. Biochem. Physiol. 60: 91–102.

Salgado, V. L., J. J. Sheets, G. B. Watson, and A. L. Schmidt. 1998. Studies on the mode of action of spinosad: the internal effective concentration, and the concentration dependence of neural excitation. Pestic. Biochem. Physiol. 60: 103–110.

Sanderson, E. D., and A. D. Jackson. 1909. The obliquebanded leafroller. J. Econ. Entomol. 2: 391–403.

Sauphanor, B., V. Brosse, C. Monier, and J. C. Bauvier. 1998. Differental ovicidal and larvicidal resistance to benzoylureas in the codling moth, *Cydia pomonella.* Entomol. Exp. Appl. 88: 247–253.

Shorey, H. H., and R. L. Hale. 1965. Mass-rearing of the larvae of nine noctuid species on a simple artificial medium. J. Econ. Entomol. 58: 522–524.

Smirle, M. J., D. T. Lowery, and C. L. Zurowski. 2002. Resistance and cross-resistance of four insecticides in populations of obliquebanded leafroller (Lepidoptera: Tortricidae). J. Econ. Entomol. 95: 820–825.

Smirle, M. J., D. T. Lowery, and C. L. Zurowski. 2003. Susceptibility of leafrollers (Lepidoptera: Tortricidae) from organic and conventional orchards to azinphosmethyl, spinosad, and *Bacillus thuringiensis.* J. Econ. Entomol. 96: 879–884.

[USEPA] U.S. Environmental Protection Agency. 1996. Food Quality Protection Act of 1996. U.S. Public Law 104–170. USEPA, Washington, DC. (http://www.epa.gov/pesticides/regulating/laws/fqpa/gpogate.pdf).

[USEPA] U.S. Environmental Protection Agency. 1997. Guidelines for expedited review of conventional pesticides under the reduced-risk initiative and for biological pesticides. Pesticide Registration (PR) Notice 97-3(1997). USEPA, Washington, DC. (http://www.epa.gov/opppmsd1/PR_Notices/pr97-3.html).

Waldstein, D. E., and W. H. Reissig. 2000. Synergism of tebufenozide in resistant and susceptible strains of obliquebanded leafroller (Lepidoptera: Tortricidae) and resistance to new insecticides. J. Econ. Entomol. 93: 1768–1772.

Waldstein, D. E., W. H. Reissig, J. G. Scott, and R. W. Straub. 1999. Susceptibility of obliquebanded leafroller (Lepidoptera: Tortricidae) populations from commercial orchards and unsprayed habitat in New York to tebufenozide. J. Econ. Entomol. 92: 1251–1255.

Weires, R., and H. Riedl. 1991. Other tortricids on pome and stone fruits, pp. 412–434. *In* L.P.S. van der Geest, and H. H. Evenhuis [eds.], Tortricid pests: their biology, natural enemies and control. Elsevier, New York.

Received 21 July 2009; accepted 29 September 2009.