FINAL REPORT

WSARE Project Number: GW10-003 (Graduate Student Grant)

Project Title: A Proactive Approach to Understanding Resistance to Novel OP Alternatives as a Strategy for Sustainable Management of Obliquebanded Leafroller

Graduate Student: Ashfaq A. Sial (ashfaqsial@yahoo.com)

Major Advisor: Dr. Jay F. Brunner
Professor and Director
Washington State University
Tree Fruit Research and Extension Center
Wenatchee, WA 98801
jfb@wsu.edu (email)
509-663-8181 (phone)
509-662-8714 (fax)

Organization: Washington State University

1. SUMMARY:

Studies were conducted to assess the current levels of resistance and cross-resistance of obliquebanded leafroller (OBLR) populations, genetic potential of OBLR to develop resistance, and characterize mechanisms responsible for conferring resistance to the recently registered reduced-risk OP alternatives, rynaxypyr and spinetoram. Bioassays of field-collected populations showed that low levels of resistance to the new chemicals, rynaxypyr and spinetoram already exist in the field populations, and that resistance of field populations to spinetoram was correlated with their resistance to spinosad. Significant levels of resistance to rynaxypyr and spinetoram were recorded in a laboratory population as a result of selection for resistance in the laboratory. The evidence of resistance and cross-resistance in the field as well as development of resistance in response to laboratory selection indicates that the risk of resistance evolution against these chemicals exists. However, in the absence of selection pressure, the selected populations reverted to being susceptible indicating that resistance to both rynaxypyr and spinetoram was unstable. Moreover, biochemical enzyme assays of the selected populations indicated that esterase activity was significantly increased in the rynaxypyr-selected population whereas mixed-function oxidase levels were elevated in the spinetoram-selected population. The fact that resistance to both rynaxypyr and spinetoram was unstable and that these chemicals do not share detoxification mechanisms indicates that rynaxypyr and spinetoram could be effectively incorporated into resistance management programs through strategies of rotation. Implementation of such strategies at this point would be a proactive approach and would lead to management of OBLR and other major pests of tree fruits on a sustainable basis.

2. INTRODUCTION:

The tree fruits are one of the biggest industries in Washington State producing crops valued at nearly $1.5 billion, with an economic impact of over $6 billion on the state's economy by providing for more than 140,000 jobs and service related activities (1). Washington's tree fruit growers produce high quality fruit for the state, the nation, and the world with over 241,986 acres dedicated to tree fruit crops (NASS-USDA). Insect pests are one of the major concerns of tree fruit growers, and have the potential to reduce crop production substantially. Obliquebanded leafroller (OBLR), *Choristoneura rosaceana* (Harris) (Lepidoptera: Tortricidae), is one of the most destructive lepidopteran pests of tree fruits, causing severe damage by feeding on leaves and developing fruits (2). Historically, broad-spectrum insecticides have been the primary tools to manage insect pests in tree fruits. Use of broad-spectrum insecticides such as organophosphates (OPs) for decades has resulted in the development of OP resistance and cross-resistance to other classes of insecticides in major pests including OBLR leading to control failure in some cases (3, 4). Additionally, regulatory actions such as Food Quality Protection Act of 1996 (FQPA) have put restrictions on the use of broad-spectrum insecticides leading to an OP phase-out (5). The development of insecticide resistance and implementation of FQPA along with restrictions in international markets have led to the development of new chemicals such as rynaxypyr and spinetoram which are safer to humans.

Rynaxypyr is an anthranilic diamide which belongs to insecticide resistance action committee (IRAC) mode of action class 28 (6). Anthranilic diamides selectively bind to ryanodine receptors (RyR) in insect muscles resulting in an uncontrolled release of calcium from internal stores in the sarcoplasmic reticulum (7, 8), causing impaired regulation of muscle contraction leading to feeding cessation, lethargy, paralysis, and death of target organisms. Anthranilic diamides have very low vertebrate toxicity due to a >500-fold differential selectivity toward insect over mammalian RyR (8). Spinetoram is a recently developed spinosyn belonging to IRAC mode of action class 5 (6). Spinosyns primarily activate the nicotinic acetylcholine receptors by acting on a unique and yet unknown binding site (9-11). Both rynaxypyr and spinetoram were highly effective against OBLR in both laboratory and field trials (12, Brunner unpublished data).

With the availability of these products, it was critical for tree fruit growers to incorporate the novel reduced-risk insecticides into IPM programs but resistance remains a threat. Resistance management strategies are usually developed after it has occurred in the field, which is too late. In this situation, characterizing resistance in various field populations could be extremely valuable for OBLR management programs by detecting potential problem of resistance at an earlier stage, thereby allowing growers to change their OBLR control strategies and slow the spread of resistance. The successful management of insecticide resistance depends ultimately on a thorough understanding of the resistance mechanisms. The understanding of genetic basis of resistance and mechanisms conferring resistance, especially, before its occurrence in the field would be a proactive approach, and could be extremely valuable in developing strategies to manage susceptibility leading to delay the development of resistance. Therefore, this project was proposed to assess current levels of resistance and cross-resistance of OBLR populations, genetic potential of OBLR to develop resistance, and characterize mechanisms involved in resistance of OBLR to rynaxypyr and spinetoram.

3) OBJECTIVES/PERFORMANCE TARGETS:

The primary goal of this project was to provide tree fruit growers with information that will enable them to incorporate the recently developed reduced-risk OP alternatives, rynaxypyr and spinetoram, into IPM programs to control OBLR and possibly other pests on sustainable basis in an economical and environmentally friendly manner. This goal was achieved under the following objectives:

1) To determine the current levels of resistance and cross-resistance in field-collected populations of OBLR to the novel reduced-risk chemicals,
2) To assess the genetic potential of OBLR to develop resistance against the novel reduced-risk chemicals, and
3) To characterize mechanisms conferring resistance against these chemicals in OBLR.

4) MATERIALS AND METHODS: [Please refer to the attached publications for details]

Objective 1: Current Levels of Resistance and Cross-resistance in Field Insects

Susceptibility of Field Populations: In order to determine susceptibility of field populations to two new reduced-risk insecticides, rynaxypyr and spinetoram, field populations were collected from orchards in Chelan, Douglas, Grant, and Okanogan counties of Washington. The field populations were collected as third to fifth instars, returned to the laboratory and reared to the adult stage. The neonate larvae of the first laboratory generation, and in some cases the second laboratory generation, were tested. A diet incorporation bioassay, as described in Sial et al. (13), was used to test the field-collected populations in the laboratory. Field populations were also tested for their susceptibility to azinphosmethyl and spinosad in order determine the possibility of cross-resistance between the new insecticides and the ones that have been used in the field for several years.

Lethal concentration values (LC_{50} and LC_{90}) and their corresponding 95% fiducial limits (FL) were estimated using POLO (14) and lethal concentration ratios (LCR) at LC_{50} and LC_{90} values and their corresponding 95% confidence limits (CL) were calculated using lethal concentration ratio significance test (15). 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.

Objective 2: **Genetic Potential of OBLR to Develop Resistance**

Selection for Resistance: In order to determine the genetic potential of OBLR to develop resistance to rynaxypyr and spinetoram, cohorts of larvae from the laboratory colony were selected with rynaxypyr (RYN) or spinetoram (SPIN) while another cohort treated in the same way but without exposure to insecticides served as a control (LAB) (16). 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 rynaxypyr and spinetoram used to select each subsequent generation was the $\approx LC_{70}$ based on the results of bioassays from the previous generation. The number of neonate larvae used for each generation varied (1000-

2000) depending on availability. Based on availability, a subset of the progeny of the OBLR 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.

Lethal concentration values (LC_{50} and LC_{90}) and their corresponding 95% fiducial limits (FL) were estimated using POLO (14) and lethal concentration ratios (LCR) at LC_{50} values and their corresponding 95% confidence limits (CL) were calculated using lethal concentration ratio significance test (15). A laboratory population 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.

Resistance Risk Assessment: In order to assess the risk of resistance development in OBLR against rynaxypyr and spinetoram, data from the selection experiments were used to estimate realized heritability (h^2) of resistance to these insecticides using a quantitative genetic approach (17) as described in Sial and Brunner (16).

Objective 3: Characterization of Resistance Mechanisms

Biochemical Enzyme Assays: In order to determine mechanisms responsible for resistance of OBLR to rynaxypyr and spinetoraam, biochemical assays were performed to determine the activities of three major classes of detoxification enzymes including general esterases, mixed-function oxidases, and glutathione-S-transferases. For enzyme assays, 30 third-instar *C. rosaceana* larvae (10-15 mg each) from the susceptible colony (LAB) and each of the selected resistant colonies (RYN and SPIN) were used. Individual insects were homogenized in 200 µl ice-cold potassium phosphate buffer (0.1M, pH 7.2), and then spun in a microfuge at ~21,000*g* for 2 min. All reactions were carried out in disposable 96-well microplates (Greiner Bio-One, VWR International, West Chester, PA). Total protein contents were determined by the method of Bradford (18) using Bio-Rad dye reagent (Bio-Rad Laboratories, Hercules, CA) with bovine serum albumin (BSA) as a standard. Esterase, oxidase, and glutathione-*S*-tranferase activities were measured using α-naphthyl acetate (αNA), 3,3',5,5'-tetramethyl benzidine dihydrochloride (TMBZ), and 1-chloro-2,4-dinitrobenzene (CDNB) as substrates, respectively. Mean enzyme activities recorded in larvae from the rynaxypyr-selected (RYN) and spinetoram-selected (SPIN) colonies were compared with those from larvae in the unselected LAB colony using *t*-test. Significance was accepted at $\alpha = 0.05$ in all statistical tests used in this study.

Bioassays with Metabolic Synergists: A diet overlay bioassay as described by Ahmad and Hollingworth (19) was used to test the effect of metabolic synergists DEF, DEM and PBO on toxicity of rynaxypyr and spinetoram to RYN and SPIN (resistant), and the LAB (susceptible) populations of *C. rosaceana*.

Reversion toward Susceptibility: After six generations of selection and after resistance had been documented, a subset of *C. rosaceana* larvae from each of the selected RYN and SPIN populations was removed from selection to establish two new populations, RYN-Rev and SPIN-Rev, respectively. The main objective of establishing these colonies was to determine whether or not the resistance in the selected populations was stable. The *C. rosaceana* larvae in RYN-Rev and SPIN-Rev populations were reared in the laboratory without any further exposure to insecticides. Susceptibility of neonate larvae from the RYN-Rev and SPIN-Rev populations was assessed and compared with that of the neonate larvae from the unselected LAB populations at each generation using a diet incorporation bioassay.

5) <u>RESULTS AND DISCUSSIONS</u>: [Please refer to the attached publications for details]

Objective 1: Current Levels of Resistance and Cross-resistance in Field Insects

Significant variation was detected in response to all four insecticides including rynaxypyr and spinetoram, which had never been used in the field. LCRs were 1.2-5.3 for rynaxypyr (Tables 1.1 and 1.2), 0.5-4.1 for spinetoram (Table 1.3), 0.5-3.6 for spinosad (Table 1.4), and 3.9-39.7 for azinphosmethyl (Table 1.5). Correlation analysis indicated possibility of cross-resistance between spinosad and spinetoram ($R^2 = 0.85$) (Fig. 1.1), which are both members of the spinosyn class suggesting the possibility of cross-resistance. This was the first study to document the evidence of correlated cross-resistance between spinosad and spinetoram. The prevalence of azinphosmethyl resistance can be attributed to the decades of its use in tree fruit orchards. However, the occurrence of low but significant levels of resistance against rynaxypyr 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. 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. Our findings establish baseline susceptibility of the field-collected *C. rosaceana* populations to rynaxypyr and spinetoram and serve 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.

Table 1.1: Results of probit analyses for rynaxypyr using diet incorporation bioassays on *C. rosaceana* neonate larvae from a laboratory as well as field-collected populations

Population	*n*	Slope (± SE)	χ^2	*P*	LC_{50} (ppm) (95% FL)[1]	LC_{90} (ppm) (95% FL)[1]	LCR-LC_{50}[2] (95% CL)[4]	LCR-LC_{90}[3] (95% CL)[4]
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 = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[3] LCR-LC_{90}, lethal concentration ratio at LC_{90} = LC_{90} (field population)/LC_{90} (LAB population).
[4] 95% confidence limits estimated using lethal concentration 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 population (Robertson et al. 2007).

Table 1.2: Results of probit analyses for rynaxypyr using diet incorporation bioassays on *C. rosaceana* neonate larvae from a laboratory as well as field-collected populations

Population	*n*	Slope (± SE)	χ^2	*P*	LC_{50} (ppm) (95% FL)	LC_{90} (ppm) (95% FL)[1]	LCR-LC_{50}[2] (95% CL)[4]	LCR-LC_{90}[3] (95% CL)[4]
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 = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[3] LCR-LC_{90}, lethal concentration ratio at LC_{90} = LC_{90} (field population)/LC_{90} (LAB population).
[4] 95% confidence limits estimated using lethal concentration 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 population (Robertson et al. 2007).

Table 1.3: Results of probit analyses for spinetoram using diet incorporation bioassays on *C. rosaceana* neonate larvae from a laboratory as well as field-collected populations

Population	*n*	Slope (± SE)	χ^2	*P*	LC_{50} (ppm) (95% FL)[1]	LC_{90} (ppm) (95% FL)[1]	LCR-LC_{50}[2] (95% CL)[4]	LCR-LC_{90}[3] (95% CL)[4]
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	2.28	0.68	0.06	0.16	3.00	3.08

		(0.59)			(0.04-0.09)	(0.11-0.33)	(1.86-4.84)*	(1.66-5.70)*
ROB	180	3.27	0.97	0.91	0.08	0.20	4.05	3.80
		(0.39)			(0.06-0.11)	(0.15-0.37)	(2.57-6.39)*	(2.10-6.89)*

n = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[3] LCR-LC_{90}, lethal concentration ratio at LC_{90} = LC_{90} (field population)/LC_{90} (LAB population).
[4] 95% confidence limits estimated using lethal concentration 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 population (Robertson et al. 2007).

Table 1.4: Results of probit analyses for spinosad using diet incorporation bioassays on *C. rosaceana* neonate larvae from a laboratory as well as field-collected populations

Population	*n*	Slope (± SE)	χ^2	*P*	LC_{50} (ppm) (95% FL)[1]	LC_{90} (ppm) (95% FL)[1]	LCR-LC_{50}[2] (95% CL)[4]	LCR-LC_{90}[3] (95% CL)[4]
Spring Brood:								
LAB	180	2.43	13.45	0.01	0.26	0.87		
		(0.33)			(0.16-0.43)	(0.50-2.63)		
STM	180	1.78	5.59	0.23	0.13	0.68	0.50	0.78
		(0.25)			(0.08-0.19)	(0.41-1.66)	(0.32-0.78)*	(0.38-1.60)
KMP	180	1.86	5.90	0.21	0.61	2.96	2.36	3.41
		(0.22)			(0.28-1.45)	(1.28-21.60)	(1.52-3.66)*	(1.71-6.83)*
WEB	180	2.09	14.07	0.01	0.25	1.01	0.95	1.16
		(0.27)			(0.17-0.36)	(0.63-2.17)	(0.62-1.45)	(0.59-2.28)
First Summer Brood:								
LAB	180	1.78	15.02	0.01	0.16	0.84		
		(0.24)			(0.11-0.24)	(0.50-1.96)		
GRF	180	3.75	5.08	0.28	0.58	1.27	3.61	1.52
		(1.02)			(0.33-0.78)	(0.93-2.49)	(2.22-5.89)*	(1.08-2.77)*
ROB	180	3.46	1.45	0.84	0.49	1.16	3.09	1.39
		(0.89)			(0.30-0.66)	(0.84-2.31)	(1.91-5.00)*	(0.70-2.75)

n = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[3] LCR-LC_{90}, lethal concentration ratio at LC_{90} = LC_{90} (field population)/LC_{90} (LAB population).
[4] 95% confidence limits estimated using lethal concentration 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 population (Robertson et al. 2007).

Table 1.5: Results of probit analyses for azinphosmethyl using diet incorporation bioassays on *C. rosaceana* neonate larvae from a laboratory as well as field-collected populations

Population	*n*	Slope (± SE)	χ^2	*P*	LC_{50} (ppm) (95% FL)[1]	LC_{90} (ppm) (95% FL)[1]	LCR-LC_{50}[2] (95% CL)[4]	LCR-LC_{90}[3] (95% CL)[4]
Spring Brood:								
LAB	210	4.25 (0.88)	8.20	0.15	1.33	2.66		
					(1.04-1.66)	(2.05-4.42)		
KMP	210	2.11 (0.27)	1.17	0.95	9.92	40.04	7.46	15.04
					(7.29-13.52)	(26.99-72.11)	(5.13-10.58)*	(8.40-26.94)*
WEB	180	1.52 (0.18)	17.32	0.004	52.69	368.33	39.65	138.29
					(33.85-83.81)	(202.03-963.10)	(25.88-60.75)*	(68.54-279.0)*
CLK	210	1.64 (0.19)	10.81	0.06	15.56	94.32	11.71	35.42

					(10.52-23.24)	(56.26-206.07)	(7.77-17.65)*	(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 = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[3] LCR-LC_{90}, lethal concentration ratio at LC_{90} = LC_{90} (field population)/LC_{90} (LAB population).
[4] 95% confidence limits estimated using lethal concentration 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 population (Robertson et al. 2007).

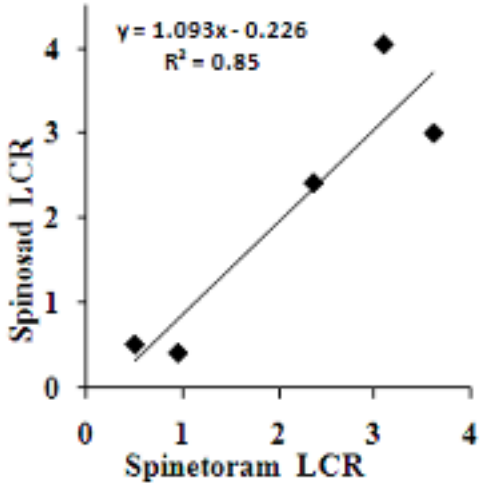

Fig. 1.1: Regression between spinosad and spinetoram lethal concentration ratios at LC_{50} in populations of *C. rosaceana* collected from pome fruit orchards in Washington.

Objective 2: Genetic Potential of OBLR to Develop Resistance

After six generations of selection, 6.58- and 3.64-fold increases in LC_{50} were recorded for rynaxypyr (Table 2.1) and spinetoram (Table 2.2), respectively. The realized heritability (h^2) of resistance was estimated as 0.17 for rynaxypyr and 0.18 for spinetoram using threshold trait analysis. These results indicate that ~17% of the total variation in rynaxypyr susceptibility and ~18% of that in spinetoram susceptibility of the LAB population was caused by additive genetic variation. The rates of resistance development were compared using the response quotient (Q), which was estimated as 0.11 and 0.07 for rynaxypyr and spinetoram, respectively, suggesting that resistance to rynaxypyr could evolve faster than to spinetoram in *C. rosaceana*. 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 rynaxypyr and ten generations for spinetoram.

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 (20-22). It took only six generations of selection of a susceptible laboratory strain of *C. rosaceana* with rynaxypyr 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.

Relatively quick response of a laboratory population selected with rynaxypyr and spinetoram suggests that a risk for resistance development in *C. rosaceana* to both insecticides exists, but resistance development in *C. rosaceana* would be slower against spinetoram than rynaxypyr. 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. These insecticides must be used wisely in the framework of a well thoughtout 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. Therefore, further studies are required to explore the biochemical and molecular basis of mechanisms conferring resistance to rynaxypyr 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.

Table 2.1: The effect of rynaxypyr contaminated diet on *Choristoneura rosaceana* neonate larvae from rynaxypyr selected population (RYN) and unselected susceptible laboratory population (LAB)

Selected Generation	Population	n	Slope (± SE)	χ^2	LC_{50} (ppm) (95% FL)[1]	LC_{90} (ppm) (95% FL)[1]	LCR-LC_{50}[2] (95% CL)[3]
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)	

n = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[3] 95% confidence limits estimated using lethal concentration ratio significance test (Robertson et al. 2007).
* Indicates that Lethal Concentration Ratio was significant ($\alpha = 0.05$) (Robertson et al. 2007).

Table 2.2: The effect of spinetoram contaminated diet on *Choristoneura rosaceana* neonate larvae from rynaxypyr selected population (RYN) and unselected susceptible laboratory population (LAB)

Selected Generation	Population	n	Slope (± SE)	χ^2	LC_{50} (ppm) (95% FL)[1]	LC_{90} (ppm) (95% FL)[1]	LCR-LC_{50}[2] (95% CL)[3]
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)	

n = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] LCR-LC_{50}, lethal concentration ratio at LC_{50} = LC_{50} (field population)/LC_{50} (LAB population).
[3] 95% confidence limits estimated using lethal concentration ratio significance test (Robertson et al. 2007).
* Indicates that Lethal Concentration Ratio was significant ($\alpha = 0.05$) (Robertson et al. 2007).

Objective 3: Characterization of Resistance Mechanisms

Enzyme assays performed after nine generation of selection indicated that esterase activity was significantly increased in rynaxypyr-selected (RYN) colony (Fig. 3.1), whereas mixed-function oxidase levels were elevated in spinetoram-selected (SPIN) colony (Fig. 3.2). No difference in glutathione-*S*-transferase activity was seen in either of the insecticide-selected colonies (Fig. 3.3). These results indicate the potential involvement of esterases and mixed-function oxidases as detoxification mechanisms responsible for resistance to rynaxypyr and spinetoram, respectively.

In synergist bioassays performed after twelve generations of selection, S,S,S-tributylphosphoro trithioate (DEF) and piperonyl butoxide (PBO) synergized the toxicity of rynaxypyr (Tables 3.1) and spinetoram (Tables 3.2), respectively, suggesting the involvement of esterases in rynaxypyr resistance and that of mixed-function oxidases in spinetoram resistance. The results also confirm the results of the enzyme assays. These findings suggest that rynaxypyr and spinetoram could be incorporated into *C. rosaceana* resistance management programs by using rotational strategies.

In the absence of selection pressure, susceptibility of a subset of larvae from both rynaxypyr- and spinetoram-selected populations reverted to pre-selection levels after five and six generations, respectively (Figs. 3.4 and 3.5), indicating that resistance to both rynaxypyr and spinetoram was unstable in *C. rosaceana*. These results of this study suggest that rynaxypyr and spinetoram do not share detoxification mechanisms and that the resistance to both of these chemicals in OBLR was unstable, and could therefore be incorporated into resistance management programs in tree fruits by using rotational strategies leading to sustainable management of *C. rosaceana*.

Insecticide resistance presents a major risk to the sustainability of integrated pest management (IPM) programs for *C. rosaceana*. Resistance management strategies could slow the development of resistance only if implemented in a timely manner. However, the effectiveness of resistance management strategies may be reduced without the knowledge of biochemical mechanisms conferring resistance to insecticides used in IPM programs.

This study represents the first report on the mechanisms involved in resistance to rynaxypyr and spinetoram in *C. rosaceana*. The higher activities of esterases in *C. rosaceana* larvae from the RYN-selected colony are indicative of the possible involvement of esterases in conferring resistance to rynaxypyr. The esterase enzymes have previously been reported to be involved in resistance to azinphosmethyl in *C. rosaceana* (23, 24) and other tortricid moths, such as light brown apple moth, *Epiphyas postvittana* (25). The azinphosmethyl-resistance in *C. rosaceana* mediated by general esterases usually extends to several types of organophosphates, carbamates, and other classes of insecticides (24), and has been associated with cross-resistance to pyrethroids (26-28).

Spinetoram is a second-generation spinosyn which was recently registered for *C. rosaceana* control in tree fruit. Resistance to spinosad has been characterized in several species of insects; however, mechanisms responsible for spinetoram-resistance have not yet been reported. Significant elevation in the level of oxidases in *C. rosaceana* larvae from the SPIN-selected colony suggests that resistance to spinetoram in this laboratory-selected colony was mediated by oxidases. Our findings are in agreement with the previous studies reporting the involvement of oxidases as a mechanism for resistance to spinosad in *Musca domestica* (29), *Spodoptera exigua* (30), and *Helicoverpa armigera* (31), an anticipated result since spinosad and spinetoram are both spinosyns.

For the first time, our studies also demonstrate other new characteristics of rynaxypyr and spinetoram resistance in *C. rosaceana*: the instability of resistance in the absence of selection pressure, and the synergism of toxicity of rynaxypyr and spinetoram by DEF and PBO, respectively. It is evident from the results of reversion experiments where, in the absence of selection pressure, both of the selected populations reverted to being susceptible that the rynaxypyr and spinetoram resistance in *C. rosaceana* was unstable. These findings are encouraging for resistance management programs aimed at slowing the process of resistance evolution against rynaxypyr and spinetoram, and prolonging the useful life of these new insecticides against *C. rosaceana* in the field. Our results suggest that rynaxypyr and spinetoram resistance could revert in *C. rosaceana* in the field when selection pressure was relaxed, however, it could take several generations for this to occur. One of the operational strategies that can be used to reduce selection pressure is rotation of rynaxypyr and spinetoram treatments with other chemicals that do not have cross-resistance to these insecticides. Reversion of resistance to pre-selection levels has been demonstrated in *C. rosaceana* (23) and other species (32, 33) and is sometimes cited as a pre-requisite for the success of rotational strategies for resistance management in the field (34).

The synergism of the toxicity of rynaxypyr and spinetoram primarily by DEF and PBO, respectively, suggests that rynaxypyr resistance was mediated by esterases whereas oxidases were the primary mechanism responsible for spinetoram resistance in *C. rosaceana*. In other species, PBO has been previously reported to synergize the toxicity of spinosad (29-31) indicating possible involvement of oxidases in resistance to spinosad, which is a spinosyn just like spinetoram.

Our findings that the resistance to rynaxypyr and spinetoram in *C. rosaceana* is unstable and that these two new insecticides appear to be detoxified by different enzyme systems suggest that rynaxypyr and spinetoram could be incorporated into *C. rosaceana* management programs, and management of resistance may be possible with rotational strategies. The information that resistance to rynaxypyr and spinetoram was esterase- and oxidase-based, respectively, will also be helpful in making sound choices regarding the best alternation of materials to be used in such

management programs. Furthermore, synergism of rynaxypyr and spinetoram by DEF and PBO, respectively, also indicates that DEF and PBO could be useful in improving the efficacy of these compounds. However, prior to such use, further studies should be conducted to determine the effects of metabolic synergists on toxicity of these insecticides to field populations of *C. rosaceana*.

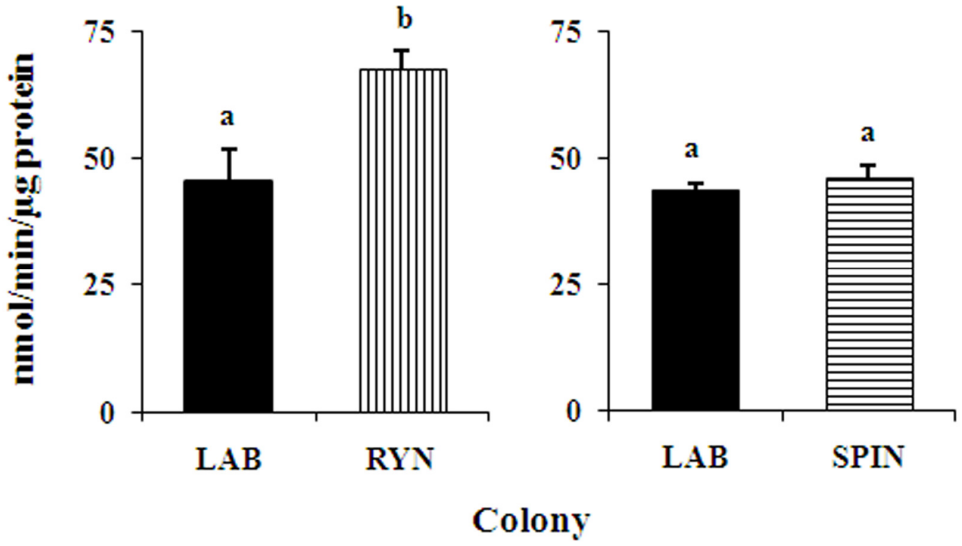

Fig. 3.1: Activity of esterases (Mean + SEM) in rynaxypyr-selected (RYN) and spinetoram-selected (SPIN) colonies after nine generations of selection for resistance in laboratory, and the unselected laboratory (LAB) colony of *C. rosaceana*. Graph bars containing similar letters on the top are not significantly different ($\alpha = 0.05$, *t*-test).

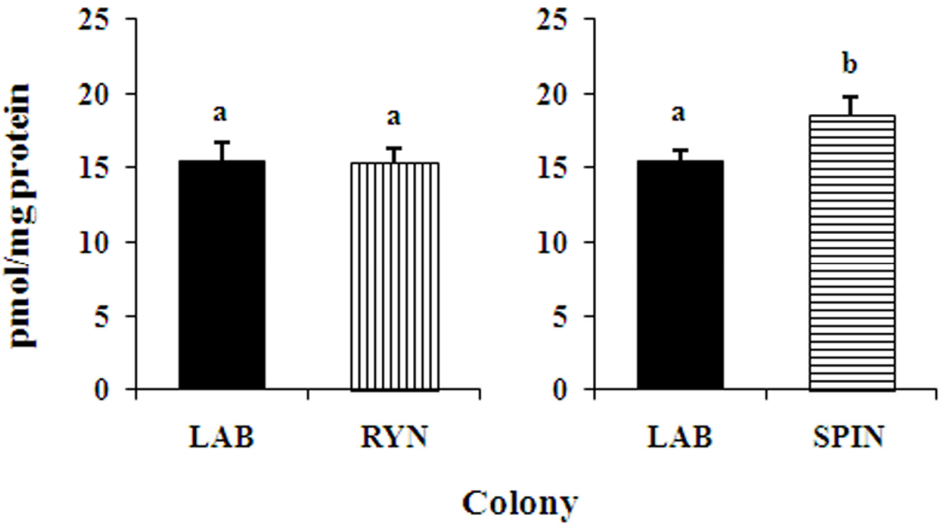

Fig. 3.2: Level of oxidases (pmol equivalent cytochrome-P450 U) (Mean + SEM) in rynaxypyr-selected (RYN) and spinetoram-selected (SPIN) colonies after nine generations of selection for resistance in laboratory, and the unselected laboratory (LAB) colony of *C. rosaceana*. Graph bars containing similar letters on the top are not significantly different ($\alpha = 0.05$, *t*-test).

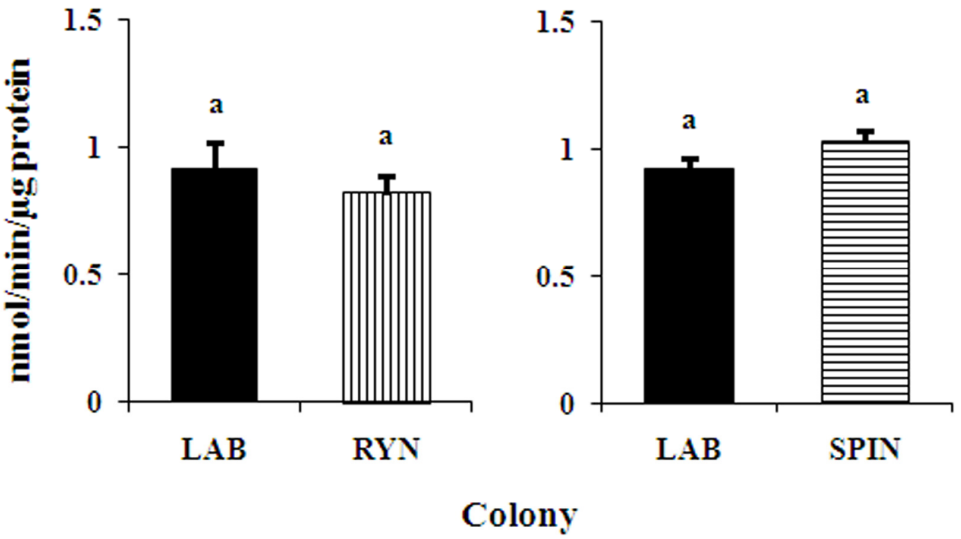

Fig. 3.3: Activity of glutathione-*S*-transferases (Mean + SEM) in rynaxypyr-selected (RYN) and spinetoram-selected (SPIN) colonies after nine generations of selection for resistance in laboratory, and the unselected laboratory (LAB) colony of *C. rosaceana*. Graph bars containing similar letters on the top are not significantly different (α = 0.05, *t*-test).

Table 3.1: Toxicity of rynaxypyr to *C. rosaceana* neonate larvae from a colony (RYN) subjected to selection for resistance to rynaxypyr for 12 generations and the unselected laboratory (LAB) colony after synergism.

Colony	Compound	N	Slope (± SE)	LC_{50} (ppm) (95% FL)[1]	SR^2 (95% CL)[3]
LAB	Rynaxypyr	180	1.51 (0.18)	0.12 (0.08-0.18)	
	Rynaxypyr + DEF	180	1.59 (0.20)	0.09 (0.06-0.12)	1.44 (0.86-2.39)
	Rynaxypyr + DEM	180	1.44 (0.18)	0.13 (0.09-0.19)	0.95 (0.56-1.61)
	Rynaxypyr + PBO	180	1.71 (0.26)	0.13 (0.08-0.20)	0.94 (0.54-1.64)
RYN	Rynaxypyr	180	2.60 (0.24)	1.05 (0.31-1.91)	
	Rynaxypyr + DEF	180	1.65 (0.25)	0.41 (0.26-0.62)	2.54 (1.41-4.6)*
	Rynaxypyr + DEM	180	1.63 (0.29)	1.08 (0.51-1.90)	0.97 (0.53-1.78)
	Rynaxypyr + PBO	180	1.63 (0.25)	0.99 (0.41-2.02)	1.07 (0.59-1.92)

n = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] SR, synergistic ratio = LC_{50} (without synergist)/LC_{50} (with synergist).
[3] 95% confidence limits estimated using lethal concentration ratio significance test (Robertson et al. 2007).
* Indicates that synergistic ration was significant (α = 0.05) (Robertson et al. 2007).

Table 3.2: Toxicity of spinetoram to *C. rosaceana* neonate larvae from a colony (SPIN) subjected to selection for resistance to spinetoram for 12 generations and the unselected laboratory (LAB) colony after synergism.

Colony	Compound	*n*	Slope (± SE)	LC_{50} (ppm) (95% FL)[1]	SR[2] (95% CL)[3]
LAB	Spinetoram	300	1.86 (0.18)	0.06 (0.03-0.14)	
	Spinetoram + DEF	300	2.56 (0.44)	0.07 (0.05-0.09)	0.95 (0.65-1.40)
	Spinetoram + DEM	300	2.11 (0.29)	0.06 (0.04-0.08)	1.11 (0.75-1.64)
	Spinetoram + PBO	300	1.71 (0.16)	0.04 (0.02-0.06)	1.83 (1.26-2.64)*
SPIN	Spinetoram	300	2.71 (0.34)	0.34 (0.27-0.42)	
	Spinetoram + DEF	300	2.58 (0.32)	0.33 (0.26-0.41)	1.05 (0.76-1.44)
	Spinetoram + DEM	300	1.99 (0.19)	0.21 (0.12-0.38)	1.66 (0.68-2.31)
	Spinetoram + PBO	300	1.76 (0.27)	0.10 (0.04-0.16)	3.58 (2.28-5.61)*

n = number of larvae assayed.
[1] 95% fiducial limits estimated using POLO (LeOra Software 1987).
[2] SR, synergistic ratio = LC_{50} (without synergist)/LC_{50} (with synergist).
[3] 95% confidence limits estimated using lethal concentration ratio significance test (Robertson et al. 2007).
* Indicates that synergistic ration was significant (α = 0.05) (Robertson et al. 2007)

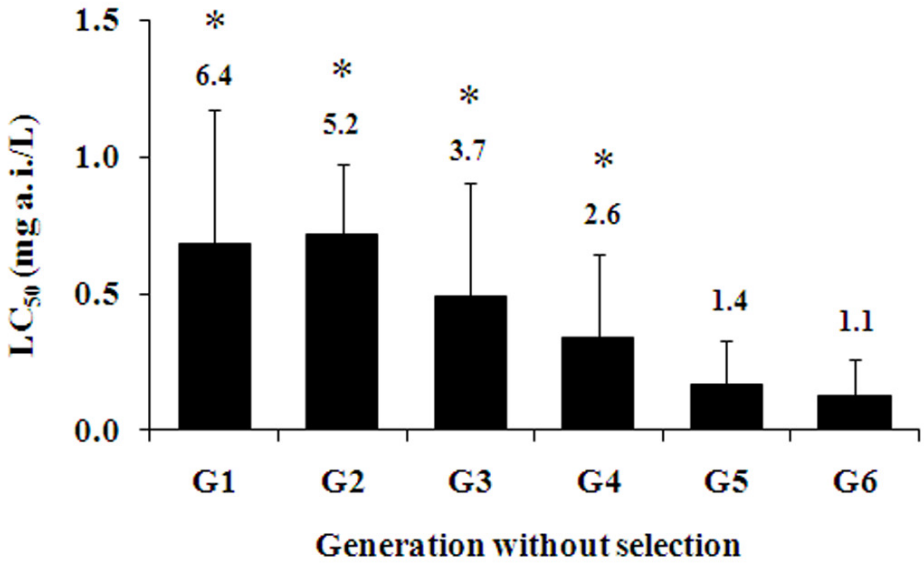

Fig. 3.4: Toxicity of rynaxypyr (LC_{50} + 95% CL) to *C. rosaceana* neonate larvae from rynaxypyr-selected colony when reared in the absence of selection pressure (RYN-Rev). Numbers on top of the graph bars represent resistance ratios (RR); and *Indicates that the RR is significant (α = 0.05).

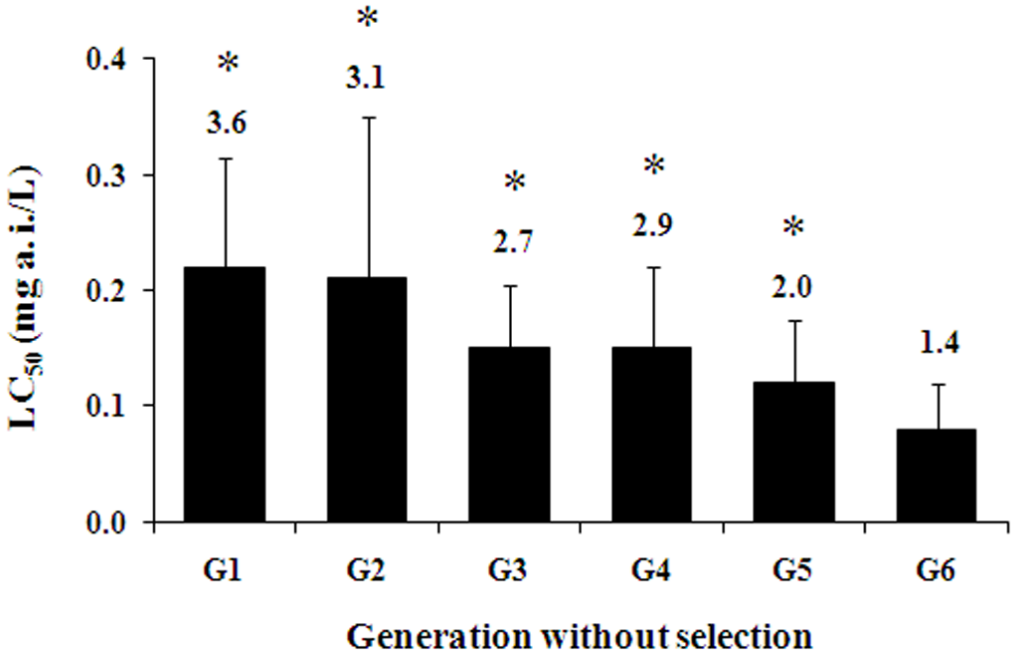

Fig. 3.5: Toxicity of spinetoram (LC_{50} + 95% CL) to *C. rosaceana* neonate larvae from spinetoram-selected colony when reared in the absence of selection pressure (SPIN-Rev). Numbers on top of the graph bars represent resistance ratios (RR); and *Indicates that the RR is significant ($\alpha = 0.05$).

6) <u>IMPACTS OF RESULTS/OUTCOMES</u>:

The results of this project show that the risk of resistance development against these new chemicals in OBLR exists, the resistance was unstable, and the mechanisms conferring resistance to rynaxypyr and spinetoram were different. 1) This information would allow tree fruit growers not only from Washington State but also those from all tree fruit growing regions in the US to incorporate these reduced risk chemicals into IPM programs judiciously, develop rational resistance management strategies based on scientific principles, and implement those strategies in a timely manner. The use of these selective chemicals will also allow for the conservation of natural bio-control agents, and integration of other IPM tactics into OBLR management programs. Consequently, the growers will be able to manage OBLR and potentially other pest populations on a sustainable basis leading to better yield and higher profits. 2) Sustainable management of OBLR and other pests of tree fruits using these reduced-risk chemicals will enhance the quality of farmers' life by increasing economic return for their investment in orchard business, which will ensure the viability of rural communities by increasing orchard success leading to more stable jobs in tree fruit industry. 3) The replacement of broad-spectrum pesticides with these selective ones will ensure the protection of health and safety of orchard workers as well as fruit consumers, especially children. It will also save growers a significant portion of their investment on expensive personal protective equipment (PPE) for workers, getting workers tested for acetylcholine-esterase levels, paying workers for not working due to health issues associated with pesticide exposures, and litigation issues due to pesticide poisoning. Moreover, growers will also get benefit from extremely short re-entry intervals (REI) and pre-

harvest intervals (PHI) for these chemicals by more efficient use of labor. 4) The ability of growers to use these chemicals on different tree fruit crops and against other major pests such as codling moth will allow growers to diversify their cropping systems by growing different crops leading to increased and more sustainable income for farmers as well as expanded duration and number of job opportunities for the rural communities. 5) Replacing the broad-spectrum insecticides with these environmentally benign chemicals will help growers maintain soil quality as well as the quality of surface and ground water. It will also promote good environmental stewardship by as well as prolonging the use of environmentally friendly chemicals.

7) ECONOMIC ANALYSIS:

Although a formal economic impact analysis of this project was not conducted, the replacement of broad-spectrum insecticides with these reduced-risk chemicals in tree fruit production will enhance global acceptability of the US fruit and competitiveness of the US tree fruit growers in export markets. Additionally, the judicious use of the novel chemicals could save growers at least one pesticide application, which costs ~$45 per acre, and this alone would save tree fruit growers millions of dollars each year.

8) PUBLICATIONS/OUTREACH:

Peer-Reviewed Publications:

The work directly related to this project resulted in the following four peer-reviewed publications (Two of which have been published in Journal of Economic Entomology, the third one has been published in Pesticide Biochemistry and Physiology, and the fourth manuscript is ready and will be submitted to Pesticide Biochemistry and Physiology soon):

1. **A. A. Sial,** J. F. Brunner, M. D. Doerr, Susceptibility of obliquebanded leafroller (Lepidoptera: Tortricidae) to two new reduced-risk insecticides, J. Econ. Entomol. 103 (2010) 140-146.
2. **A. A. Sial,** J. F. Brunner, Assessment of resistance risk in obliquebanded leafroller (Lepidoptera: Tortricidae) to the reduced-risk insecticides chlorantraniliprole and spinetoram, J. Econ. Entomol. 103 (2010) 1378-1385.
3. **A. A. Sial,** J. F. Brunner, S. F. Garczynski, Biochemical characterization of chlorantraniliprole and spinetoram resistance in obliquebanded leafroller (Lepidoptera: Tortricidae), Pestic. Biochem. Physiol. 99(3): 274-279.
4. **A. A. Sial,** J. F. Brunner. Selection for resistance, reversion toward susceptibility, and synergisms of chlorantraniliprole and spinetoram in obliquebanded leafroller (Lepidoptera: Tortricidae). Ready to be submitted in **Pesticide Biochemistry and Physiology.**

Research & Extension Presentations:

In addition to the peer-reviewed publications, findings of this project were also disseminated in the form of oral and display presentations to a variety of audiences including grower and professional scientists by the graduate student:

Oral Presentations:

1. **Sial, A. A.** 2011. Developing sustainable IPM programs using reduced-risk insecticides. The Annual Meeting of Fresno/Madera CAPCA organization, 31 March 2011, The Ramada Inn, Fresno, CA.

2. **Sial, A. A.**, and J. F. Brunner. 2010. Are we ready to replace broad-spectrum insecticide with reduced-risk chemicals in tree fruit systems? The 58th Annual Meeting of the Entomological Society of America, 12-15 December 2010, Town and Country Resort & Convention Center, San Diego, CA.
3. **Sial, A. A.** 2010. Developing sustainable IPM programs for tree fruits using reduced-risk insecticides. The 94th Annual Meeting of ESA- Pacific Branch, 11-14 April 2010, The Grove Hotel, Boise, ID.
4. **Sial, A. A.**, J. F. Brunner, Stephen F. Garczynski, and J. E. Dunley. 2010. Obliquebanded Leafroller (Lepidoptera: Tortricidae) Resistance to Novel Chemistries: Is it Possible, Stable, and Manageable? The 84th Annual Western Orchard Pest and Disease Management Conference, 13-15 January 2010, Hilton Hotel, Portland, OR.
5. **Sial, A. A.**, J. F. Brunner, S. F. Garczynski and J. E. Dunley. 2009. Evolution of resistance against novel chemistries. *In* IPMIS section symposium "Evolutionary arms race of resistance against novel chemistries: Lessons from the native and agricultural systems". The 57th Annual Meeting of the Entomological Society of America, 13-16 December 2009, Indiana Convention Center, Indianapolis, IN.
6. **Sial, A. A.**, J. F. Brunner, and S. F. Garczynski. 2009. Resistance risk assessment and strategies for managing resistance against novel reduced-risk insecticides in obliquebanded leafroller, *Choristoneura rosaceana* (Lepidoptera: Tortricidae). The 57th Annual Meeting of the Entomological Society of America, 13-16 December 2009, Indiana Convention Center, Indianapolis, IN.
7. **Sial, A. A.** and J. F. Brunner. 2009. Risk assessment and strategies for managing resistance in obliquebanded leafroller (Lepidoptera: Tortricidae) to the reduced-risk insecticides chlorantraniliprole and spinetoram. Dr. William R. Wiley Exposition of Research and Scholarship, 10 November 2009, WSU Pullman, WA.
8. **Sial, A. A.** and J. F. Brunner. 2009. Resistance risk assessment for novel reduced-risk insecticides in obliquebanded leafroller (Lepidoptera: Tortricidae). The 90th Annual Meeting of American Association for the Advancement of Science – Pacific Division (AAAS), 14-19 August 2009, San Francisco State University and the California Academy of Sciences, San Francisco, CA.
9. **Sial, A. A.** and J. F. Brunner. 2009. Toxicodynamics of novel reduced-risk insecticides in obliquebanded leafroller (Lepidoptera: Tortricidae). The 80th Rocky Mountains Conference of Entomologists, 3-4 August 2009, Silverton, CO.
10. **Sial, A. A.**, J. F. Brunner, and J. E. Dunley. 2009. Resistance risk assessment for novel reduced-risk insecticides in obliquebanded leafroller (Lepidoptera: Tortricidae). The 93rd Annual Meeting of Pacific Branch - Entomological Society of America, 29 March-1 April 2009, Bahia Resort and Convention Center, San Diego CA.
11. **Sial, A. A.** 2009. Insecticide Resistance: Evolution in the visible spectrum. *In* a symposium, "Entomology and evolutionary theory: Celebrating 150 years of "On the Origin of Species". The 93rd Annual Meeting of Pacific Branch - Entomological Society of America, 29 March-1 April 2009, Bahia Resort and Convention Center, San Diego CA.
12. **Sial, A. A.** 2009. Risk and evidence of resistance in major pests against novel reduced risk insecticides in perennial crops. *In* a symposium, **"**IPM in Perennial Cropping Systems: A Preferred Future Based on a Sound Past". The 93rd Annual Meeting of Pacific Branch - Entomological Society of America, 29 March-1 April 2009, Bahia Resort and Convention Center, San Diego CA.

13. **Sial, A. A.**, J. F. Brunner, and J. E. Dunley. 2009. Resistance risk assessment for novel reduced-risk insecticides in obliquebanded leafroller (Lepidoptera: Tortricidae). 83rd Annual Western Orchard Pest and Disease Management Conference, 14-16 January 2009, Hilton Hotel, Portland, OR.

Display:

14. **Sial, A. A.**, J. F. Brunner, and S. F. Garczynski. 2010. A Proactive Approach to Understanding Resistance to Novel OP Alternatives as a Strategy for Sustainable Management of Obliquebanded Leafroller (Lepidoptera: Tortricidae). Washington State University Showcase for Innovation in Science and Technology, 26 March 2010, WSU Pullman, WA.

15. **Sial, A. A.,** J. F. Brunner and S. F. Garczynski. 2009. Strategies to manage resistance in obliquebanded Leafroller to the novel reduced-risk insecticides: chlorantraniliprole and spinetoram. The 105th Annual Meeting of Washington State Horticultural Association, 7-9 December 2009, Wenatchee Convention Center, Wenatchee, WA.

16. **Sial, A. A.** and J. F. Brunner. 2009. Risk Assessment for resistance against the novel insecticides, chlorantraniliprole and spinetoram, in obliquebanded leafroller (Lepidoptera: Tortricidae). Washington State University Showcase for Innovation in Science and Technology, 27 March 2009, WSU Pullman, WA.

In addition to these presentations, findings of this project were also disseminated to the growers and pest management professionals at various formal and non-formal meetings by the Major Professor. In all of the publications and presentations, Western SARE was properly acknowledged as sponsor of this research funded under WSARE Project No. GW10-003.

9) FARMER ADOPTION:

The information generated in this project was incorporated into the WSU Decision Aid System (DAS) through which it will be utilized by more than 3000 growers currently managing over a quarter-million acres of tree fruit orchards in Washington and Oregon. The dissemination of the findings of this project through publications and presentations will most likely result in judicious use of these chemicals in tree fruit orchards throughout the United States and overseas.

10) AREAS NEEDING ADDITIONAL STUDY:

Based on the amount of funding and duration of the project, we were able to characterize mechanisms of resistance against rynaxypyr and spinetoram in OBLR only at biochemical level. Further studies should be conducted to determine molecular basis of resistance to these reduced-risk chemicals in OBLR and then to develop molecular markers which could be used to detect resistance even in an individual insect collected from a field rather than performing traditional bioassays which require larger number of insects from each population, which in most cases is not even possible.

REFERENCES (cited in INTRODUCTION & MATERIALS AND METHODS):

(1) Jensen, W. S. 2004. Washington State Horticultural Association and Washington Tree Fruit Research Commission, 52pp.

(2) J. F. Brunner, Discovery of *Colpoclypeus florus* (Walker) in apple orchards of Washington, Pan-Pacific Entomol. 72 (2): 5-12.

(3) M. J. Smirle, D. T. Lowery, and C. L. Zurowski, Resistance and cross-resistance of four insecticides in populations of obliquebanded leafroller (Lepidoptera: Tortricidae), J. Econ. Entomol. 95 (2002) 820-825.
(4) J. E. Dunley, J. F. Brunner, M. D. Doerr, E. H. Beers, Resistance and cross-resistance in populations of leafrollers, *Choristoneura rosaceana* and *Pandemis pyrusana*, in Washington apples, 7pp, Journal of Insect Science, 6 (2006) 14, available online: insectscience.org/6.14.
(5) (USEPA) U.S. Environmental Protection Agency. 1996. Food Quality Protection Act of 1996. U.S. Public Law 104-170. http://www.epa.gov/pesticides/regulating/laws/fqpa/gpogate.pdf . USEPA, Washington, DC.
(6) (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). http://www.epa.gov/opppmsd1/PR_Notices/pr97-3.html. USEPA, Washington, DC.
(7) G. P. Lahm, T. P. Selby, J. H. Freudenberger, T. N. Stevenson, B. J. Myers, G. Seburyamo, B. K. Smith, L. Flexner, C. E. Clark, D. Cordova, Insecticidal anthranilic diamides: a new class of potent ryanodine receptor activators, Med. Chem. Lett. 15 (2005) 4898-4906.
(8) D. Cordova, 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, Y. Tao, Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation, Pestic. Biochem. Physiol. 84 (2006) 196-214.
(9) V. L. Salgado, Studies on the mode of action of spinosad: Insect symptoms and physiological correlates, Pestic. Biochem. Physiol. 60 (1998) 91-102.
(10) V. L. Salgado, J. J. Sheets, G. B. Watson, A. L. Schmidt, Studies on the mode of action of spinosad: The internal effective concentration, and the concentration dependence of neural excitation, Pestic. Biochem. Physiol. 60 (1998) 103-110.
(11) N. Orr, A. J. Shaffner, K. Richey, G. D. Crouse, Novel mode of action of spinosad: Receptor binding studies demonstrating lack of interaction with known insecticidal target sites, Pestic. Biochem. Physiol. 95 (2009) 1-5.
(12) L. A. Hull, N. K. Joshi, F. U. Zaman, Large plot reduced risk insecticide study for lepidopteran pests infesting apples, 2008, Arthropod Management Tests 34 (2009), doi: 10.4182/amt.2009.A11.
(13) A. A. Sial, J. F. Brunner, M. D. Doerr, Susceptibility of Obliquebanded Leafroller (Lepidoptera: Tortricidae) to Two New Reduced-Risk Insecticides, J. Econ. Entomol. 103 (2010) 140-146.
(14) LeOra Software, POLO-PC: A user's guide to probit and logit analysis, LeOra, Berkeley, CA, 1987.
(15) J. L. Robertson, R. M. Russel, H. K. Preisler, N. E. Savin, Binary quantal resposne with one explanatory variable, in: Bioassays with Arthropods, 2nd ed. CRC Press, Boca Raton, FL, 2007, pp. 21-34.
(16) A. A. Sial, J. F. Brunner, Assessment of Resistance Risk in Obliquebanded Leafroller (Lepidoptera: Tortricidae) to the Reduced-Risk Insecticides Chlorantraniliprole and Spinetoram, J. Econ. Entomol. 103 (2010) 1378-1385.
(17) B. E. Tabashnik, Resistance risk assessment: realized heritability of resistance to *Bacillus thuringiensis* in diamondback moth (Lepidoptera: Plutellidae), tobacco budworm

(Lepidoptera: Noctuidae), and colorado potato beetle (Coleoptera: Chrysomelidae), J. Econ. Entomol. 85 (1992) 1551-1559.
(18) M. M. Bradford, A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem. 72 (1976) 248-254.
(19) M. Ahmad, R. M. Hollingworth, Synergism of insecticides provides evidence of metabolic mechanisms of resistance in the obliquebanded leafroller *Choristoneura rosaceana* (Lepidoptera: Tortricidae), Pest Manag. Sci. 60 (2004) 465-473.
(20) Keiding, J. 1986. Prediction of resistance risk assessment, pp. 279-297. *In* Pesticide resistance: strategies and tactics for management. National Academy of Sciences, Washington, DC.
(21) Tanaka, Y. and V. Noppun. 1989. Heritability estimates of phenthoate resistance in diamondback moth. Entomol. Exp. Appl. 52: 39-47.
(22) Firko, M. J. and J. L. Hayes. 1990. Quantitative genetic tools for insecticide resistance risk assessment: Estimating the heritability of resistance. J. Econ. Entomol. 83: 647-654.
(23) M. J. Smirle, C. Vincent, C. L. Zurowski, B. Rancourt, Azinphosmethyl resistance in the obliquebanded leafroller, *Choristoneura rosaceana*: Reversion in the absence of selection and relationship to detoxification enzyme activity, Pestic. Biochem. Physiol. 61 (1998) 183-189.
(24) D. J. Pree, K. J. Whitty, L. A. Bittner, M. K. Pogoda, Mechanisms of resistance to organophosphorus insecticides in populations of the obliquebanded leafroller *Choristoneura rosaceana* (Harris)(Lepidoptera: Tortricidae) from southern Ontario, Pest Manag. Sci. 59: (2002) 79-84.
(25) K. F. Armstrong, D. M. Suckling, Correlation of azinphosmethyl resistance with detoxification enzyme activity in the light brown apple moth *Epiphyas postvittana* (Lepidoptera: Tortricidae), Pestic. Biochem. Physiol. 36 (1990) 281-289.
(26) Y. Carriere, J. P. Deland, D. A. Roff, Obliquebanded leafroller (Lepidoptera: Tortricidae) resistance to insecticides: Among orchard variation and cross-resistance, J. Econ. Entomol. 89 (1996) 577-582.
(27) A. L. Devonshire, G. D. Moores, A carboxylesterase with broad specificity causes organophosphorus, carbamate and pyrethroid resistance in peach-potato aphids (*Myzus persicae*), Pestic. Biochem. Physiol. 18 (1982) 235-246
(28) M. E. Scharf, J. J. Neal, G. W. Bennet, Changes of insecticide resistance levels and detoxification enzymes following insecticide selection in the German cockroach *Blatella germanica* L. Pestic. Biochem. Physiol. 59 (1998) 67-70.
(29) J. G. Scott, Toxicity of spinosad to susceptible and resistant strains of house flies, *Musca domestica*, Pestic. Sci. 54 (1998) 131-133.
(30) W. Wang, J. Mo, J. Cheng, P. Zhuang, Z. Tang, Selection and characterization of spinosad resistance in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), Pestic. Biochem. Physiol. 84 (2006) 180-187.
(31) D. Wang, X. Qui, X. Ren, F. Niu, K. Wang, Resistance selection and biochemical characterization of spinosad resistance in *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), Pestic. Biochem. Physiol. 95 (2009) 90-94.
(32) J. L. Flexner, P. H. Westigard, B. A. Croft, Field reversion of organotin resistance in the twospotted spider mite (Acari: Tetranychidae) following relaxation of selection pressure, J. Econ. Entomol. 81 (1988) 1516-1520.

(33) M. R. Bush, Y. A. I. Abdel-Aal, K. Saito, G. C. Rock, Azinphosmethyl resistance in the tufted apple bud moth (Lepidoptera: Tortricidae): Reversion, diagnostic concentrations, associated esterases, and glutathione transferases, J. Econ. Entomol. 86 (1993) 213-225.
(34) B. E. Tabashnik, Modeling and evaluation of resistance management tactics, in: R. T. Roush, B. E. Tabashnik (Eds.), Pesticide Resistance in Arthropods, Chapman and Hall, New York, 1990, pp. 153-182.