Determining the Route of Detoxification of Insecticides Used to Control Navel Orangeworm (NOW) in Tree Nuts

Project No.: **13-ENTO1B-Berenbaum**

Project Leader: May Berenbaum
Department of Entomology
320 Morrill Hall
University of Illinois at Urbana-Champaign
505 S. Goodwin
Urbana, IL 61801-3795
217.333.7784
maybe@illinois.edu

Project Cooperators and Personnel:
Mark Demkovich, Univesity of Illinois Department of Entomology
Joel Siegel, USDA-ARS, Parlier

Objectives:

1. Determination of LD50 (Lethal Dose, 50%) of pesticides to which navel orangeworm is exposed, either as a target pest in almonds or as a nontarget species exposed via proximity to nearby crops. *Measuring LD50 on a standard diet allows for comparisons of efficacy/toxicity across multiple classes of pesticides*.

2. Identification of principal enzyme system(s) responsible for metabolism of pesticides used for insect control in or near almond orchards by use of specific inhibitors. *Identifying the enzyme systems principally responsible for detoxification of different classes of pesticides can provide insight into the likelihood of acquisition of cross- or multiple- resistance.*

3. Identification of the specific genes encoding the enzymes conferring resistance to pesticides used for navel orangeworm pest management. *Identifying the specific genes encoding enzymes contributing to detoxification and resistance opens up possibilities for novel management strategies, such as use of RNAi, that are both sustainable and environmentally compatible*.

Interpretive Summary:

Representatives of six pesticide classes (organophosphates, pyrethroids, diamides, diacyl hydrazines, avermectins and spinosyns) are registered for use in management of navel orangeworm (*Amyelois transitella*), yet little is known of the mechanisms by which this insect detoxifies or otherwise processes them. Although the assumption is often made that rotating insecticides with different modes of action delays resistance acquisition, applied individually in rotation or simultaneously in combination, insecticides can select for cross- or multiple-resistance, which presents a challenge to sustainable management. To delay resistance evolution and preserve efficacy of available control chemicals, it is important to understand the relative sensitivity of navel orangeworm to the insecticides used in almond orchards and neighboring row crops and to characterize the mechanisms of detoxification for the different pesticide classes. In this project, we have used inhibitors of 3 major detoxification enzyme

systems—cytochrome P450 monooxygenases (P450), glutathione-S-transferases and esterases--to identify the detoxification systems principally responsible for metabolism and potential resistance to the six classes of control chemicals in use today. Results suggest that organophosphates are bioactivated, or made more toxic, by P450s, whereas pyrethroids are detoxified by these enzymes. Thus, if P450-mediated resistance develops in navel orangeworms, control may be rescued by using organophosphates instead.

Pesticide failures in some orchards in Kern County have suggested that, indeed, bifenthrin resistance may have already evolved in the field. We have used synergist assays to demonstrate that both P450s and esterases contribute to this resistance. Up to tenfold resistance is maintained in the laboratory across 8 generations; as well, resistant strains have lower pupal weights. Together, these findings indicate that resistance is genetically based and may be associated with a fitness cost in the absence of the pesticide. In terms of recommendations, switching away from pyrethroids to a pesticide that is not metabolized by P450s may restore control.

Materials and Methods:

In a series of bioassays in which pesticides and specific enzyme inhibitors were incorporated into an artificial diet, the effects of the cytochrome P450 monooxygenase (P450) inhibitor piperonyl butoxide (PBO) and the glutathione-S-transferase (GST) inhibitor diethyl maleate (DEM) were assessed on the toxicity of the insecticides azinphos-methyl, chlorpyrifos, chlorantraniliprole, β-cyfluthrin, and bifenthrin to first instar *A. transitella* larvae from a laboratory strain

Results and Discussion:

Piperonyl butoxide interacted antagonistically with the organophosphate insecticides azinphos-methyl and chlorpyrifos, indicating that they likely are bioactivated by P450s. Piperonyl butoxide synergized the toxicity of the pyrethroids β-cyfluthrin and bifenthrin, which suggests that P450s are involved in detoxification of pyrethroid insecticides. Only azinphos-methyl was a substrate for GSTs in *A. transitella*, as evidenced by diethyl maleate synergism assays. Neither piperonyl butoxide (PBO) nor diethyl maleate (DEM) influenced the toxicity of the anthranilic diamide chlorantraniliprole. Results suggest that if *A. transitella* detoxify other classes of insecticides used in management through enhanced P450 activity, and resistance begins to develop by this route, then incorporating organophosphate insecticides into rotations may provide an effective means of prolonging efficacy of chemical control because their speed of activation will increase.

In a related series of studies to characterize a putatively pyrethroid-resistant strain of navel orangeworm, eggs from adults originating from almond orchards in which pesticide failures were reported in Kern County, California were shipped to the University of Illinois at Urbana-Champaign. Their susceptibility to bifenthrin and β-cyfluthrin was compared to that of an established colony of navel orangeworms. Administration of piperonyl butoxide and S,S,S-tributyl phosphorotrithioate (DEF) in bioassays with the pyrethroids bifenthrin and β-cyfluthrin produced synergistic effects and demonstrated that P450s and carboxylesterases (COEs) contribute to resistance in this navel orangeworm population. Resistance is therefore primarily

metabolic and likely the result of overexpression of specific P450 and COE genes. Bioassays involving pesticides routinely used in tank mixtures indicate that chlorantraniliprole, which is not detoxified by P450s, may be more effective than methoxyfenozide in overcoming existing pyrethroid resistance because chlorantraniliprole enhanced pyrethroid toxicity and methoxyfenozide had no effect.

Results from median-lethal concentration (LC_{50}) assays revealed that resistance was maintained across eight generations in the laboratory. Life history trait comparisons between the resistant strain and susceptible strain revealed significantly lower pupal weights in resistant males and females reared on the same wheat bran-based artificial diet across three generations. The number of days until the first molt was significantly greater in the resistant strain than the susceptible strain, although overall development time was not significantly different between strains. These experiments indicate that resistance is heritable and may have an associated fitness cost, which could influence the dispersal and expansion of resistant populations

Research Effort Recent Publications:

Demkovich M., 2014. Insecticide detoxification in the navel orangeworm *Amyelois transitella* (Lepidoptera: Pyralidae. Master's thesis, University of Illinois at Urbana-Champaign.

Noble K, G Niu, JP Siegel, MR Berenbaum. Pyrethroid tolerance of navel orangeworm (*Amyelois transitella*) after dietary exposure to almond phytochemicals. J. Pest Science (in review).

Table 1.1. Insecticides and synergists tested against first instar larvae from a laboratory strain (SPIRL-1966) of *Amyelois transitella*.

Active Ingredient	Commercial Name(s)	Chemical Family	Mode of Action	Dosage used (µg/g)
Azinphos-methyl	Guthion	Organophosphate	Acetylcholinesterase inhibitor	1.60
β-cyfluthrin	Baythroid	Pyrethroid	Sodium channel modulators	0.04
Bifenthrin	Brigade Bifenture	Pyrethroid	Sodium channel modulators	0.20
Chlorantraniliprole	Altacor Coragen	Anthranilic diamide	Ryanodine receptor modulators	4.00
Chlorpyrifos	Lorsban	Organophosphate	Acetylcholinesterase inhibitor	0.40
Diethyl Maleate		Synergist	Glutathione-S-transferase inhibitor	200
Piperonyl Butoxide	Butacide	Synergist	Cytochrome P450 monooxygenase inhibitor	200

Table 1.2. Probit analysis data for azinphos-methyl, chlorpyrifos, chlorantraniliprole, β-cyfluthrin, and bifenthrin in neonate *A. transitella* from a laboratory strain (SPIRL-1966).

Insecticide	n	Slope (SE)	LC_{50} ± 95% CL (µg/g)	χ^2	P
Azinphos-methyl	636	2.73 (0.19)	1.50 (1.35-1.65)	6.22	0.40
Chlorpyrifos	555	4.20 (0.33)	0.41 (0.38-0.45)	7.49	0.19
Chlorantraniliprole	320	1.10 (0.33)	3.20 (0.92-4.83)	0.76	0.68
β-cyfluthrin	355	1.33 (0.22)	0.03 (0.01-0.05)	5.65	0.23
Bifenthrin	240	2.82 (0.35)	0.21 (0.18-0.25)	0.31	0.86

References Cited:

None.