Mechanism of resistance acquisition in navel orangeworms (*Amyelois transitella*) resistant to pyrethroid insecticides

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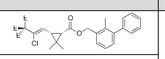
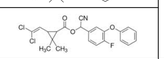
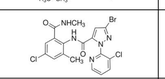
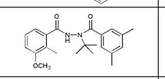
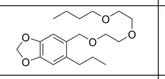
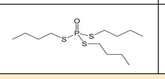
Introduction

- The navel orangeworm (*Amyelois transitella*) has a diverse range of hostplants and is considered the most destructive pest of introduced nut crops, including almonds, pistachios, and walnuts in California orchards.[1]
- Neonates tunnel into nuts, where they consume the nutmeat and increase the chance of infection by *Aspergillus* fungi that produce aflatoxins.[2,3]
- Management of this insect pest has typically been a combination of cultural control (removal of mummies) combined with insecticides, but the use of insecticides has increased along with the value of these commodities.
- **Genes encoding mechanisms of resistance are passed on to succeeding generations, resulting in populations that are not effectively controlled through insecticide use.[4]**
- Selective inhibitors that target specific detoxification enzyme systems can be used to identify detoxification pathways in resistant populations.
- Removal of selection pressures exerted through insecticide use may result in pest populations regaining sensitivity, particularly if there is a fitness cost associated with resistance.[4]

Chemical Family	Chemical Name	Trade Names	Structure	Mode of Action
Pyrethroid	Bifenthrin	Brigade®; Bifenture®		Binds sodium channels
	β-cyfluthrin	Baythroid®		
Anthranilic diamide	Chlorantraniliprole	Altacor®		Ryanodine receptor agonist
Diacylhydrazine	Methoxyfenozide	Intrepid®		Ecdysone agonist
Synergist	Piperonyl butoxide	Butacide®		Cytochrome P450 inhibitor
Organophosphate/ Synergist	S,S,S-tributyl phosphorotrithioate	Tribufos®		Esterase inhibitor

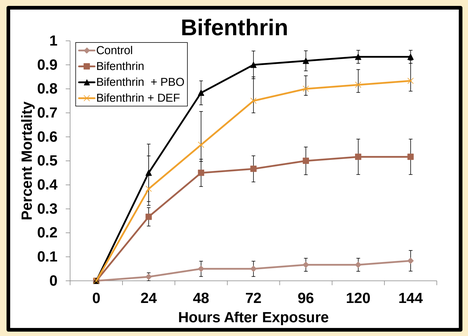
Methods

- Insecticides were tested against a resistant colony of *A. transitella* (B. Higbee:Paramount Farming) which was maintained at conditions of 28 ± 4ºC in the absence of a light cycle.
- Insecticides and synergists were incorporated into standard insect diet at specific concentrations and fed to neonate larvae.
- PROBIT analyses were conducted to generate the median lethal concentrations that would kill 50% of the sample population at 48 hours (LC_{50}) across successive generations.
- Median lethal concentrations determined for bifenthrin and β-cyfluthrin were used to assay for synergism with piperonyl butoxide and S,S,S-tributyl phosphorotrithioate
- Median lethal concentrations of bifenthrin and β-cyfluthrin were assayed for synergism with chlorantraniliprole and methoxyfenozide.
- Data were analyzed with ANOVA and Tukey's HSD test, using Statistical Analysis Software Version 9.3.

Results

Generation	*n*	Slope (SE)	LC_{50} ± 95% CL (µg/g)	x^2	*P*	LC_{50} chosen (µg/g)
F2	200	1.93 (0.227)	1.88 (1.36-2.46)	3.58	0.17	2.00
F3	238	1.32 (0.267)	1.47 (1.05-1.98)	0.35	0.84	1.60
F4	240	1.87 (0.291)	1.89 (1.53-2.38)	0.48	0.79	2.00
F5	300	1.69 (0.200)	1.75 (1.40-2.16)	1.82	0.61	1.90

Table 1. PROBIT analysis data for bifenthrin in resistant neonate *A. transitella* across multiple generations



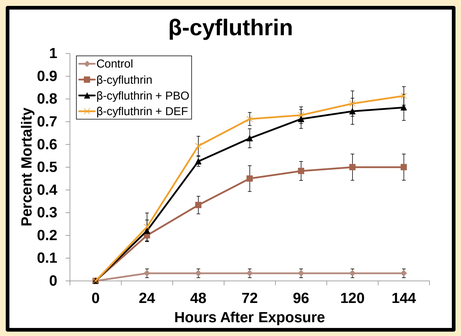
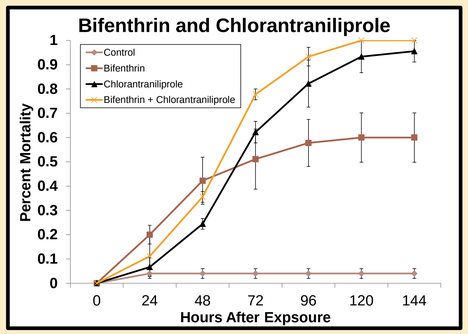


Figure 1,2. Effect of piperonyl butoxide (PBO) and s,s,s-tributyl phosphorotrithioate (DEF) on neonate mortality in the presence of bifenthrin and β-cyfluthrin. Percent mortality was significantly different between bifenthrin and both PBO and DEF treatments (F=14.46, P=0.015). Percent mortality was significantly different between β-cyfluthrin and both PBO and DEF treatments (F=9.33, P=0.0064)



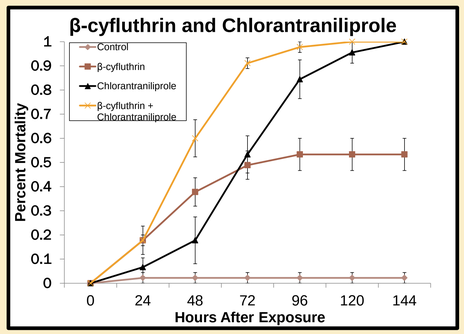
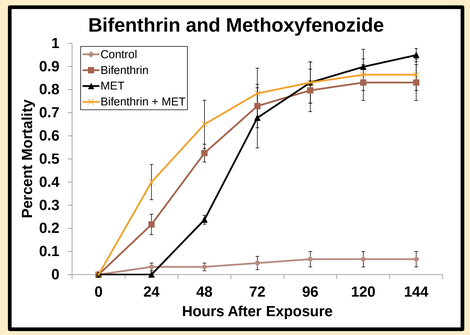


Figure 3,4. Effect of chlorantraniliprole on neonate mortality in the presence of bifenthrin and β-cyfluthrin. Percent mortality was not significantly different between bifenthrin with chlorantraniliprole and both bifenthrin and chlorantraniliprole after 72 hours (F=3.27, P=10.98). Percent mortality was significantly different between β-cyfluthrin with chlorantraniliprole and both β-cyfluthrin and chloranatraniliprole after 72 hours but not 48 hours (F=20.64, P=0.002) , (F=4.75, P=0.0579).



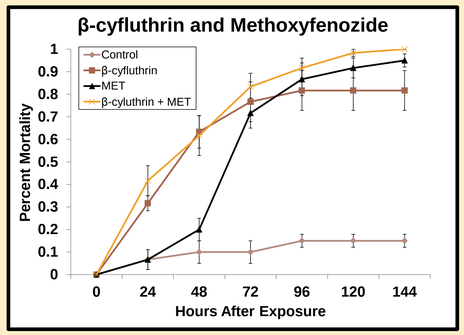


Figure 5,6. Effect of methoxyfenozide (MET) on neonate mortality in the presence of bifenthrin and β-cyfluthrin. Percent mortality was not significantly different between bifenthrin with methoxyfenozide and both bifenthrin and methoxyfenozide after 72 hours (F=0.34, P=0.7219). Percent mortality was not signifcantly different between β-cyfluthrin with methoxyfenozide and both β-cyfluthrin and methoxyfenozide after 72 hours (F=0.67, P=0.5477).

Discussion

- The overlap in median lethal concentration values of bifenthrin confirms that resistance is maintained in the absence of selection pressure
- Resistance across multiple generations suggests there is no fitness cost associated with resistance to bifenthrin
- The synergistic effects of both PBO and DEF toward bifenthrin and β-cyfluthrin indicate that elevated levels of cytochrome P450 and esterase activity are likely responsible for resistance in navel orangeworms
- Resistance is metabolic and not the result of target-site insensitivity
- The inclusion of chlorantraniliprole with pyrethroids may produce synergistic effects that can overcome resistance in navel orangeworms
- Methoxyfenozide did not enhance the toxicity of either bifenthrin or β-cyfluthrin in bioassays

Significance

Our project accentuates how selection pressures exerted by insecticide use may facilitate the emergence of resistance. Although insecticides used in mixes may have different modes of action, these combinations may not enhance toxicity toward the navel orangeworm. If resistance toward one insecticide class arises as the result of enhanced P450 and esterase activity, then cross-resistance may also arise rapidly toward other insecticides that share similar modes of detoxification. Results from this research may generate insights and lead to the establishment of novel management practices that reduce reliance upon insecticides.

Future Research

Our current resistant colony of navel orangeworms originated from a research ranch of Paramount Farming on the west side of Central Valley, California. We hope to assay additional populations from the west side of Central Valley as well on the eastern side. This project will allow us to determine the geographic distribution of resistance, in addition to sampling for resistance in populations with different histories of pyrethroid use.

References

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