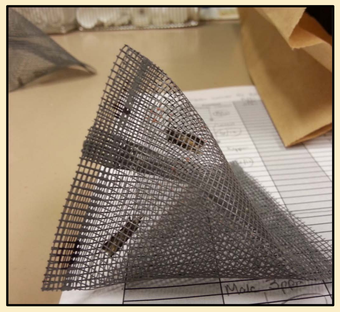


# Detoxification of Insecticides Individually and in Combination in Navel Orangeworm (NOW) Populations Resistant to Pyrethroid Insecticides

Mark Demkovich*, Vikram Bagchi*, Joel Siegel†, Spencer Walse†, and May Berenbaum*
* Department of Entomology, University of Illinois at Urbana-Champaign
† USDA, Agricultural Research Service, Parlier, CA

ILLINOIS
UNIVERSITY OF ILLINOIS AT URBANA-CHAMPAIGN

## Objective 1: Using our bifenthrin resistant (R347), susceptible (CPQ), and navel orangeworm colony collected from fig orchards (FIG), conduct neonate feeding assays to calculate median-lethal concentrations of pesticides (chlorantraniliprole, spinosad) and phytochemicals (chlorogenic acid, bergapten, xanthotoxin)

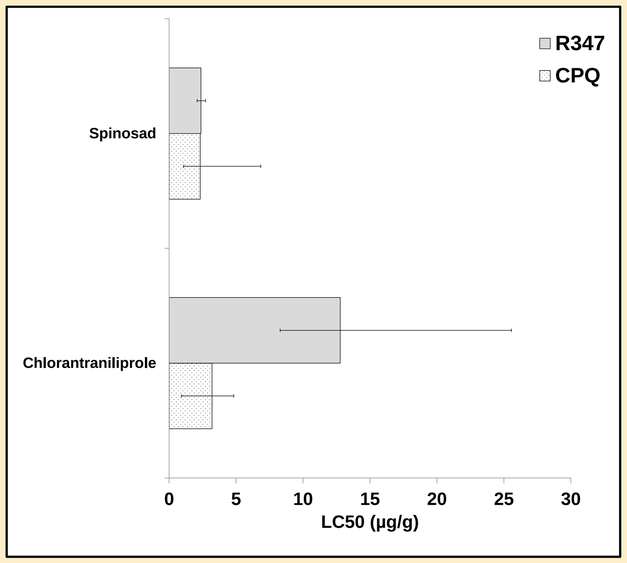



Figure 1. Median-lethal concentrations for chlorantraniliprole (diamide, IRAC Group 28) and spinosad (spinosyn, IRAC Group 5) in a bifenthrin-resistant strain (R347) and a susceptible strain (CPQ).

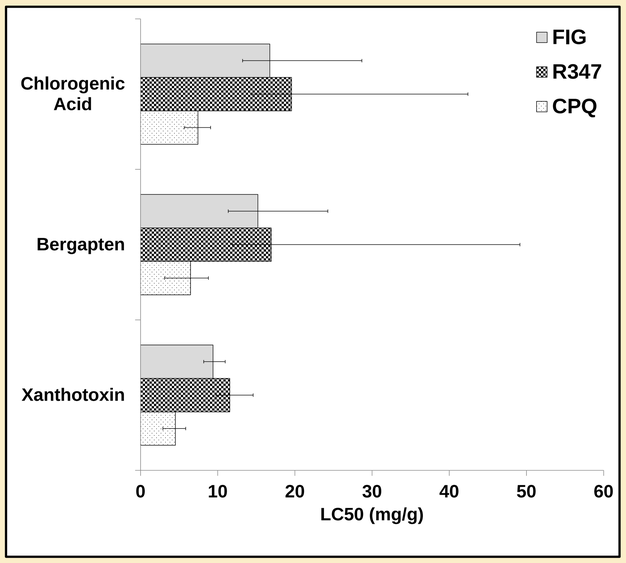



Figure 2. Median-lethal concentrations for phytochemicals present in NOW hostplants (chlorogenic acid-almond, bergapten-fig, xanthotoxin-almond) in a resistant strain (R347), fig population (FIG), and susceptible strain (CPQ).

Discussion: Insecticide feeding assays with the resistant and susceptible strain revealed an approximate 4-fold difference between the median-lethal concentration for chlorantraniliprole and no difference for spinosad (Figure 1). These results suggest an overlapping mechanism in the detoxification of pyrethroids in the resistant strain of navel orangeworm and the diamide insecticide chlorantraniliprole. When challenged in feeding assays with phytochemicals present in almonds and figs, the resistant line and population collected from fig orchards exhibited a greater tolerance than the susceptible line (Figure 2). These results suggest that the FIG colony may have a robust detoxification system in addition to the R347 strain as a result of exposure to furanocoumarins (Bergapten, Xanthotoxin) present in its hostplants. Sustained exposure to such furanocoumarins may facilitate hostplant expansion and acquisition of insecticide tolerance/resistance by fig populations.

## Objective 2:Apply synergists piperonyl butoxide (PBO), diethyl maleate (DEM), and S,S,S-tributyl phosphorotrithioate (DEF) with an LC50 dose of each insecticide to determine if cytochrome P450s, GSTs, and/or esterases, respectively, are involved in detoxification

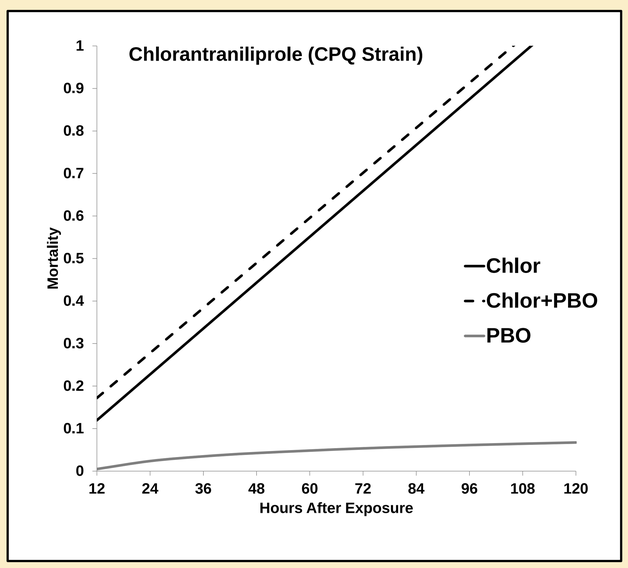



Figure 3. Predicted NOW mortality during the first 120 h in feeding assays with the CPQ strain and 4 µg/g chlorantraniliprole in the presence and absence of 200 µg/g PBO. Figure taken from Demkovich et al. (2015)[1].

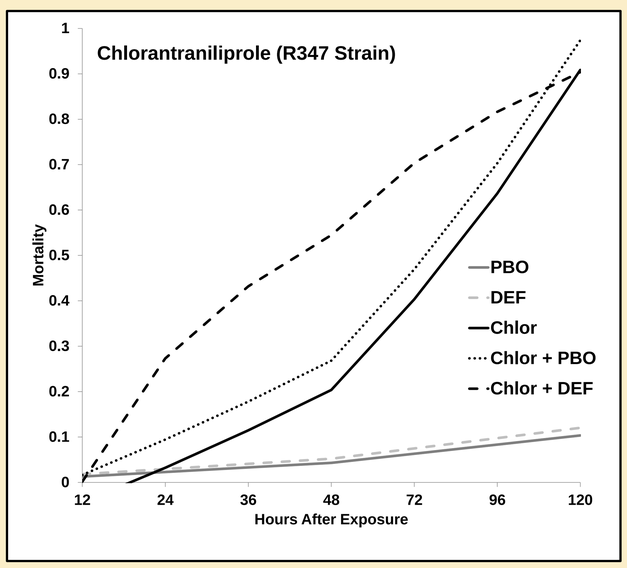
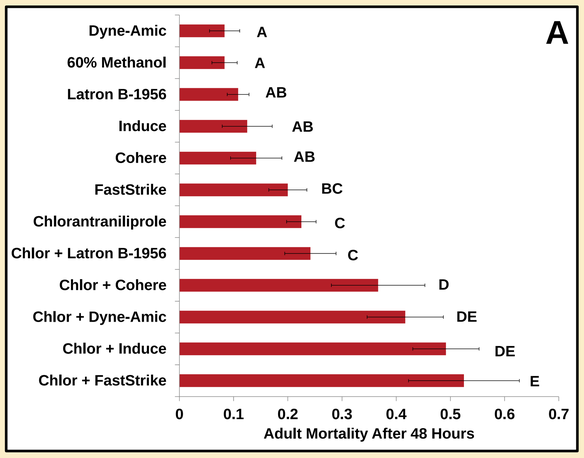
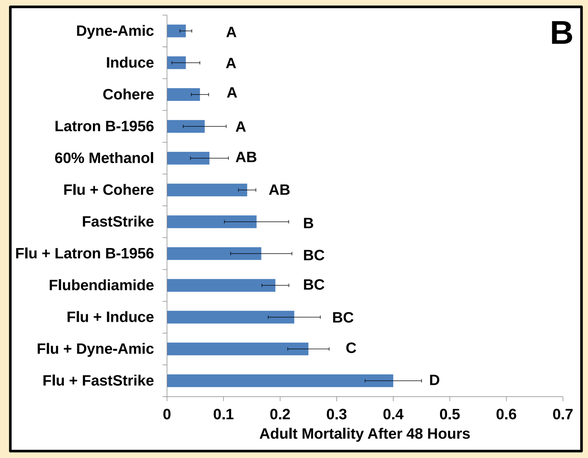


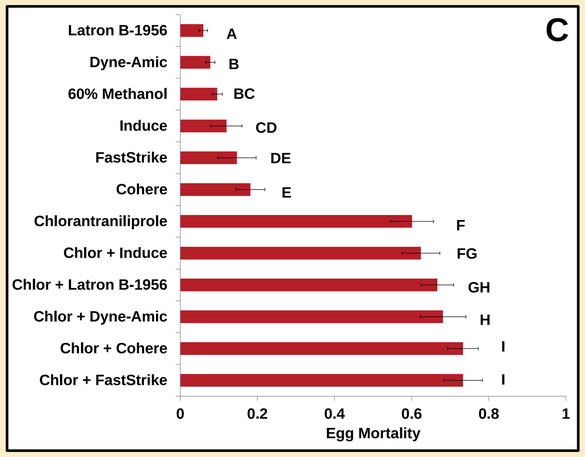

Figure 4. Predicted NOW mortality during the first 120 h in feeding assays with the R347 strain and 8 µg/g chlorantraniliprole in the presence and absence of 200 µg/g PBO and 200 µg/g DEF.

Discussion: Demkovich et al. (2015)[1] investigated the role of cytochrome P450 monooxygenases (P450s) in the detoxification of chlorantraniliprole in the susceptible CPQ strain in navel orangeworm and found no effect of the P450 inhibitor piperonyl butoxide (PBO), indicating P450s are not involved in the metabolism of the insecticide (Figure 3). When PBO was applied to the bifenthrin-resistant R347 strain, there was also no impact on chlorantraniliprole toxicity; however, **the esterase inhibitor S,S,S-tributyl phosphorotrithioate enhanced the toxicity of chlorantraniliprole in the resistant strain (Figure 4).** The resistance mechanism of R347 was identified in Demkovich et al. (2015)[2] as overexpression by P450 and esterase enzymes. If esterases are involved in chlorantraniliprole detoxification in a strain of navel orangeworm with enhanced metabolic capabilities, then cross-resistance may arise with diamide exposure.

## Objective 3: Use the Parlier USDA-ARS spray tower to test the effects of adjuvants on pesticide toxicity to eggs, larvae, and adults in the CPQ strain of NOW



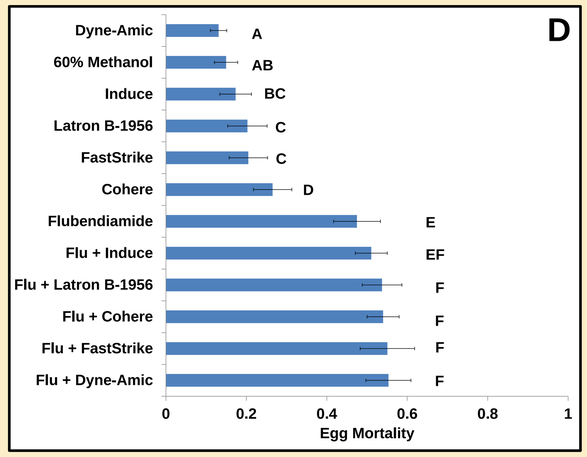




Figure 5-8. Toxicity of **125 ppm chlorantraniliprole and flubendiamide** with the adjuvants Dyne-Amic™, FastStrike™, Induce™, Cohere™, and Latron B-1956™ against NOW adults and eggs. **Adjuvant application rates scaled down to 10 ml sprays were Dyne-Amic 8 oz/100 gal; FastStrike 64 oz/100 gal; Induce 8 oz/100 gal; Cohere 8 oz/100 gal; Latron B-1956 3.5 oz/100 gal. (A)** Adult mortality (n=120 per control/treatment) after 48 hours when sprayed with 10 ml chlorantraniliprole at 125 ppm with and without adjuvants. **(B)** Adult mortality (n=120 per control/treatment) after 48 hours when sprayed with 10 ml flubendiamide at 125 ppm with and without adjuvants. **(C)** Egg mortality (n≥750 per control/treatment) when sprayed with 10 ml chlorantraniliprole at 125 ppm without and without adjuvants. **(D)** Egg mortality (n≥750 per control/treatment) when sprayed with 10 ml flubendiamide at 125 ppm without and without adjuvants.

Discussion: FastStrike was the only adjuvant that was more toxic than the 60 % methanol carrier solution among the controls in adult assays with chlorantraniliprole and flubendiamide. FastStrike enhanced mortality for both chlorantraniliprole and flubendiamide when applied to adults and eggs. Egg mortality was enhanced by each class of adjuvant except for the wetter-spreader Induce for both chlorantraniliprole and flubendiamide. Results from these sets of assays indicate that navel orangeworm may be more vulnerable to certain insecticide-adjuvant combinations at different stages in its life cycle. **If adjuvants have differential impact on the toxicity of current insecticides used to control navel orangeworm, then this may result in new chemical management strategies that incorporate effective insecticide-adjuvant combinations in field sprays.**

## Objective 4: With the newly available NOW genome, compare our susceptible CPQ strain with the R347 resistant strainby deep sequencing transcriptomes, mapping the cDNA reads to the reference genome , and identifying differences in detoxification loci that distinguish the strains

Discussion: The NOW genome is complete and being uploaded to the i5K workspace (https://i5k.nal.usda.gov/). Once the genome and NCBI annotation models are incorporated into the i5k WebApollo, manual annotation by the community can begin. A new cost-effective method, Pool-Seq (Schlötterer et al. 2014), will allow us to examine genome-wide polymorphisms between resistant R347 and susceptible CPQ strains. We are collecting adults from these two strains for the analysis

## References

1. Demkovich, M., Dana, C. E., Siegel, J. P., and Berenbaum, M. R. (2015). Effect of piperonyl butoxide on the toxicity of four classes of insecticides to navel orangeworm (*Amyelois transitella*) (Lepidoptera: Pyralidae). *Journal of Economic Entomology* 1–8 (2015); DOI: 10.1093/jee/tov237.
2. Demkovich, M., Siegel, J. P., Higbee, B. S., and Berenbaum, M. R. (2015). Mechanism of Resistance Acquisition and Potential Associated Fitness Costs in Amyelois transitella (Lepidoptera: Pyralidae) Exposed to Pyrethroid Insecticides. *Environmental Entomology* 44(3): 855-863.
3. Schlötterer, C., Tobler, R., Kofler, R., and Nolte, V. (2014). Sequencing pools of individuals—mining genome-wide polymorphism data without big funding. *Nature Reviews Genetics* 15: 749-763.

## Acknowledgments

We thank the Almond Board of California and California Pistachio Research Board for research funding.

## Contact Information

Mark Demkovich – mdemkov3@illinois.edu
Joel Siegel – joel.siegel@ars.usda.gov
May Berenbaum – maybe@illinois.edu