Developmental and environmental impacts on pesticide detoxification in navel orangeworm (*Amyelois transitella*)

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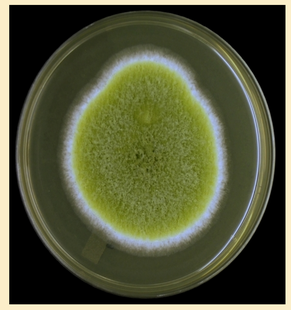
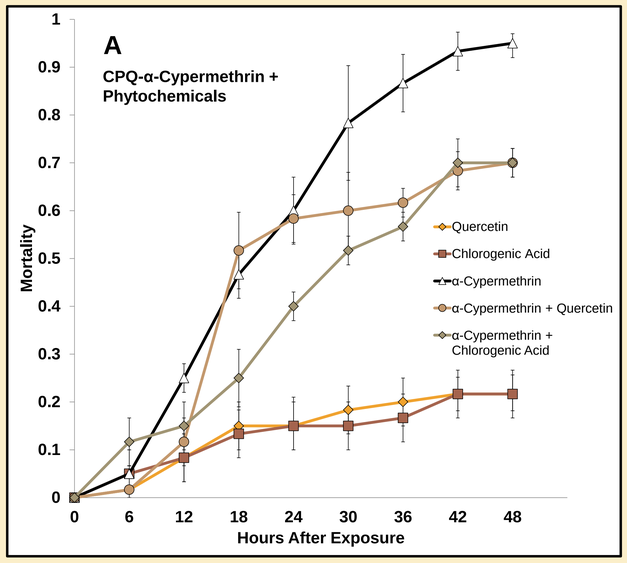
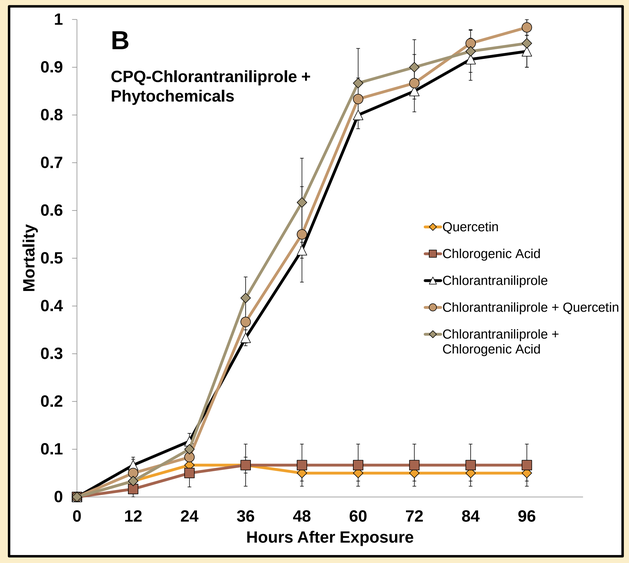


Objective 1: Determine the degree to which NOW larval detoxification capacity changes developmentally

Results: We have now established colonies of navel orangeworm (*Amyelois transitella*) (NOW) collected recently from almonds and figs, in addition to pyrethroid-resistant populations (R347) collected in both 2015 and 2016 (B. Higbee, Wonderful Orchards). Feeding assays with first instars to determine median-lethal concentrations (LC_{50}) in 2016 resistant populations have allowed us to conclude that the LC_{50} has increased to at least 25 ppm, greater than a two-fold increase over populations collected in 2015 where the reported LC_{50} was 11 ppm. In order to identify developmental changes in detoxification capacity in NOW, we continued feeding assays to identify LC_{50} values in second instar larvae. The LC_{50} for bifenthrin increased ~7-fold from first to second instar in the susceptible strain. The ability to metabolize insecticides in the R347, almond, and fig strains are currently under investigation. Initial efforts to estimate fifth instar LC_{50} revealed that low concentrations of bifenthrin may stop larvae from feeding on artificial diet without killing them. For susceptible populations of NOW, a 2 ppm concentration of bifenthrin caused half of the larvae to stop eating artificial diet; in the R347, this concentration was 8 ppm.

Objective 2: Measure the degree to which co-occurring phytochemicals (**quercetin**, **chlorogenic acid**) in different parts of almonds influence toxicity and detoxification capacity





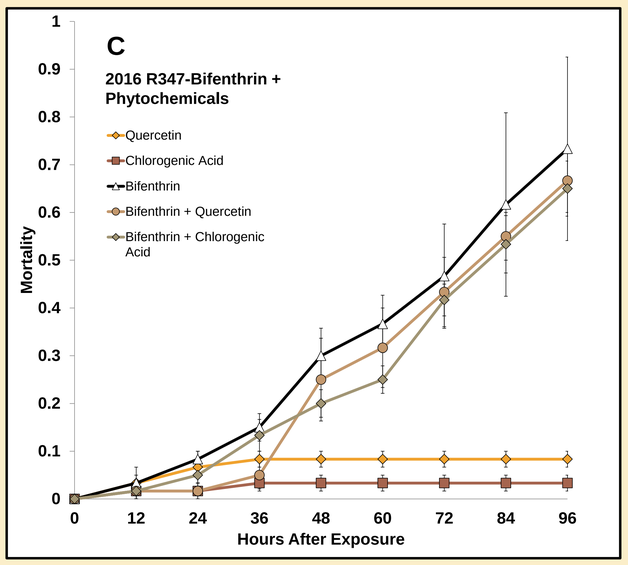
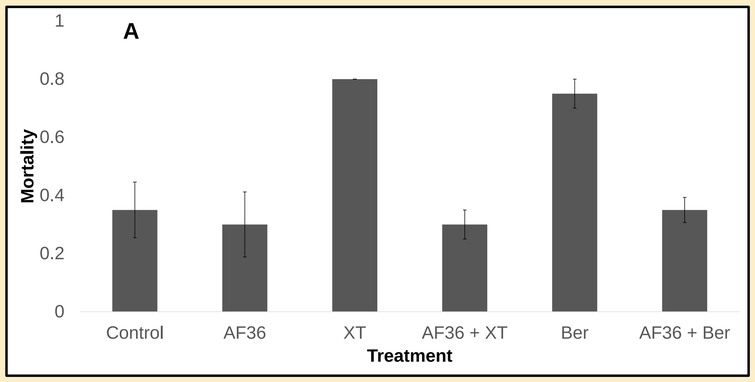


Figure 1A, 1B, 1C. NOW mortality during feeding assays with first instar larvae. **(A)** Feeding assays using the **susceptible CPQ strain** of NOW with 50 ng/g α-cypermethrin (IRAC Group 3A), 5 mg/g quercetin, and 2 mg/g chlorogenic acid. Figure taken from Noble 2012, MS Thesis. **(B)** Feeding assays using the **susceptible CPQ strain** of NOW with 4 µg/g chlorantraniliprole (Altacor) (IRAC Group 28), 5 mg/g quercetin, and 2 mg/g chlorogenic acid. **(C)** Feeding assays using the **pyrethroid-resistant R347 strain** of NOW with 16 µg/g bifenthrin (Bifenture) (IRAC Group 3A), 5 mg/g quercetin, and 2 mg/g chlorogenic acid. Concentrations are displayed as grams of chemical per gram of artificial diet. Controls consisted of the phytochemicals quercetin and chlorogenic acid without insecticide.

Discussion: **Phytochemicals are natural chemical compounds in plants. NOW larvae are challenged by the diversity of phytochemicals encountered in their various hosts in addition to insecticides**. Ingestion of **quercetin and chlorogenic acid**, found in both almonds and pistachios, reduced the toxicity of the pyrethroid α-cypermethrin in feeding assays with first instars in the susceptible strain (Figure 1A). The mechanism of increased tolerance to α-cypermethrin may be the induction of cytochrome P450 monooxygenase genes (P450s) by ingestion of quercetin or chlorogenic acid that facilitate detoxification of the pesticide. In contrast, ingestion of phytochemicals had no impact on the toxicity of bifenthrin in the resistant strain and in chlorantraniliprole assays with the susceptible strain. Demkovich et al. (2015)[1] showed that chlorantraniliprole is not detoxified by P450s and therefore its toxicity should not be altered by the addition of quercetin or chlorogenic acid (Figure 1B). Results with R347 were unexpected because P450s are a suspected mechanism of resistance[2] (Figure 1C). Phytochemical experiments with the resistant line will continue, to elucidate the mechanisms by which NOW metabolizes both synthetic and naturally occurring chemicals. Identifying these mechanisms can inform efforts to reduce damage in orchards.

Objective 3: Ascertain the extent to which *Aspergillus* fungi contribute to pesticide detoxification



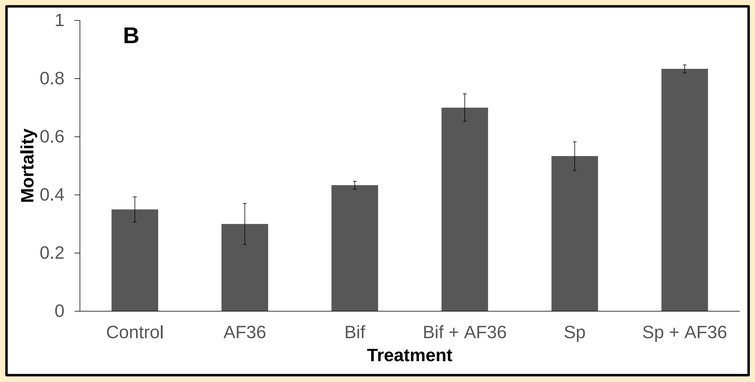


Figure 2A and 2B. NOW mortality in the presence of the fungus ***Aspergillus flavus* (AF36)** and phytochemicals or insecticides. **(A)** Larval mortality on diets containing *A. flavus* and **the phytochemicals xanthotoxin (XT) and bergapten (Ber)**. **(B)** Larval mortality on diets containing *A. flavus* and the **insecticides bifenthrin (Bif)** (IRAC Group 3A) and **spinetoram (Sp)** (IRAC Group 5).

Discussion: We investigated the toxicity of the naturally occurring furanocoumarins xanthotoxin (XT) and bergapten (Ber), in addition to the pyrethroid bifenthrin (Bif) and the spinosyn spinetoram (Sp), on laboratory strain NOW performance in the presence and absence of the fungus *Aspergillus flavus* (AF36). **Although ingestion of xanthotoxin and bergapten was detrimental to larval performance, larval survivorship increased and development time decreased in the presence of *A. flavus*, suggesting that the fungus may be detoxifying phytochemicals encountered in its hostplants (2A). Larval mortality rates on diets containing the insecticides rose significantly (2-fold) in the presence of AF36, indicating that fungal presence exacerbated the negative effects of insecticides (2B).** However, development time was shortened significantly (by 19%) in the spinetoram assays when *A. flavus* was present. We noticed that larvae refused to eat diets containing insecticide for the first few days of the experiment, and we suggest that this period allows the fungus to outcompete and infect the weakened caterpillars.

Genomics Projects with NOW:

With the newly available NOW genome, compare our susceptible CPQ strain, bifenthrin-resistant R347 strain, and a FIG strain through Pool-Seq analysis[3] to examine genome-wide polymorphisms. Using the annotated CYPome of NOW, identify cytochrome P450 monooxygenase genes involved in R347 resistance.

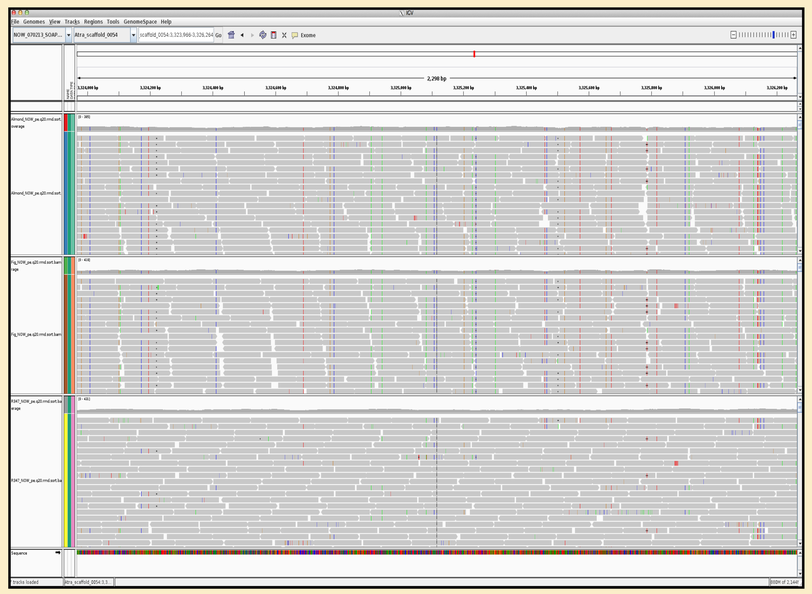


Figure 3. Screenshot taken in Integrated Genome Viewer of reads in the susceptible almond population (top), fig strain (middle), and the pyrethroid-resistant R347 strain aligned to the reference genome. There are 7301 scaffolds in total.

Results: The NOW genome has been uploaded to the i5K workspace (https://i5k.nal.usda.gov/). In the Pool-Seq analysis, reads are currently in the alignment stage for populations of NOW collected from almonds, figs, and areas where pyrethroid resistance has been reported (B. Higbee). Figure 3 displays a region of scaffold_0054 where the reads for Almond (top) and Fig (middle) have polymorphism compared to the resistant strain (bottom). Midguts were dissected from a susceptible 4th generation almond strain and from pyrethroid-resistant strains collected in Kern County in 2015 and 2016 (B.Higbee). We will begin RNA extractions soon with these midguts and qPCR amplifications to identify candidate P450 detoxification genes involved in pesticide resistance.

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

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