Investigating Navel Orangeworm (NOW) Resistance to Pyrethroid Insecticides and Detoxification of Phytochemicals in Hostplants

Project No.: **14-ENT01-Berenbaum**

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Objectives:

1) Determine whether navel orangeworm resistance to bifenthrin is maintained in the absence of selection pressure.
2) Assess whether or not navel orangeworm resistant to bifenthrin has an enhanced ability to detoxify phytochemicals in hostplants relative to a susceptible navel orangeworm population.
3) Examine neonate survivorship in contact toxicity assays involving filter papers sprayed with bifenthrin at a range of concentrations in a resistant and susceptible colony.
4) Identify survivorship differences in resistant and susceptible adults sprayed with bifenthrin .
5) Exploratory research: identify survivorship differences in resistant and susceptible adults in response to consumption of hostplant phytochemicals and compare with survivorship in a hostplant-adapted population.

Interpretive Summary:

The polyphagous navel orangeworm (*Amyelois transitella*; Walker; Lepidoptera: Pyralidae), is considered the most destructive pest of introduced nut crops, including almonds and pistachios, in California orchards (Zalom et al. 2012). Neonates tunnel into nuts where they consume the nutmeat and generate large quantities of frass and webbing (Bentley et al. 2008). Management of this insect pest has typically been a combination of cultural control (removal of unharvested fruits) combined with insecticides, but the use of insecticides has substantially increased along with the value of these commodities.

Infestation by navel orangeworm (NOW) also results in an increased susceptibility to infection by *Aspergillus* species, many of which produce aflatoxins (Campbell et al. 2003). The highly toxigenic fungal species *Aspergillus flavus*, which infects damaged or overripe tree nuts and fruits, is closely associated with the navel orangeworm. Recent work suggests that this association is a facultative mutualism; *A. flavus* opportunistically infects nuts damaged by navel orangeworm and may be phoretic on mobile life stages of the insect (Palumbo et al. 2014). Together, these two organisms comprise the most destructive pest complex associated with California nut crops (Zalom et al. 2012).

Navel orangeworm resistance to the pyrethroid insecticide bifenthrin (Brigade, Bifenture, and Fanfare) was first reported in 2012 in almond orchards (B. Higbee: Paramount Farming Company). In 2013, we received eggs from wild-caught moths resistant to bifenthrin and established a colony (R347) of resistant NOW at the University of Illinois at Urbana-Champaign. With the support of both the Almond Board of California and Pistachio Research Board, resistance levels were quantified in first instars of this population relative to a susceptible colony (CPQ). Median-lethal concentrations (LC_{50}) were determined through neonate feeding assays, which revealed an approximate 10-fold difference in toxicity of the pyrethroids bifenthrin and beta-cyfluthrin (Baythroid) between the resistant and susceptible colonies. Feeding assays using inhibitors of major detoxification pathways in NOW demonstrated that resistance is metabolic and likely the result of elevated cytochrome P450 and esterase activity.

In these experiments we assessed the stability of resistance over time in the absence of selection pressure through continuation of feeding assays with first instar NOW by testing a cohort from every generation of R347. Additionally, we examined the capacity of R347 to detoxify xanthotoxin and bergapten, natural furanocoumarins present in many hostplants of NOW, relative to the susceptible (CPQ) colony and a colony reared from NOW collected in fig orchards (FIG). Further differences in bifenthrin toxicity between the R347 and CPQ colonies were also investigated through contact toxicity bioassays involving neonates and adults sampled from each colony.

The results generate insights that could improve chemical management strategies for navel orangeworm. The research shows there is a decline in resistance over time (generations 10 through 13 – although a still five-fold resistance level for these generations) in the absence of selection pressure and suggests that a reduction in the use of pyrethroids could restore efficacy of the chemical class.

Materials and Methods:

Colonies of *A. transitella* were raised under conditions of 28 ± 4ºC with a 16:8 (L:D) hour photoperiod. Insecticides were incorporated into the artificial diet at specific concentrations and fed to neonates. Insecticides were mixed in with the diet in its liquid phase at a range of concentrations and then poured into separate 28-ml (1-oz) cups to harden. Mortality levels were assessed and recorded at each concentration after 48 hours. SPSS version 22 software (SPSS Inc., Chicago, IL) was used to run the Probit analysis and generate an estimated calculation of the median lethal concentration that would kill 50% of the sample population at 48 hours (LC_{50}) for bifenthrin, xanthotoxin, and bergapten. Significant results in these experiments were identified when there was no overlap between treatments in the 95% confidence intervals generated through Probit analysis. These methods were also used for bioassays of R347, CPQ, and larvae from a strain derived from individuals collected infesting figs, to determine toxicity of xanthotoxin in the presence and absence of piperonyl butoxide, a well-known inhibitor of P450-mediated detoxification. Results were analyzed by two-way analysis of variance with strain and PBO as main effects.

Filter papers were sprayed with bifenthrin at 0.3 ppm, 3 ppm, 30 ppm, and 300 ppm. Eggs from R347 and CPQ colonies were placed on sprayed filter papers in Petri dishes surrounded by a wheat bran-based diet. Hatching neonates received bifenthrin exposure through contact with the filter papers, and survivorship was recorded two weeks after egg hatch. Survivors after two weeks were monitored for time to adult emergence on the wheat bran diet. In a separate series of assays, adults from R347 and CPQ colonies were separated by sex, sealed into mesh bags, and sprayed with bifenthrin at 3 ppm. Survivorship was recorded after 48 hours. Data were analyzed with dummy-variable regression, using JMP Pro Version 10 (SAS Institute, Cary, NC).

Results and Discussion:

Median-lethal concentration values for bifenthrin were consistent across 9 generations (**Figure 1**). The resistance factor between colonies declined to an approximate 5-fold difference in generations 10 through 13. In feeding assays with furanocoumarins, the LC_{50}s for both xanthotoxin and bergapten were significantly greater in the R347 and FIG strains compared to the LC_{50}s in CPQ, but not significantly different between R347 and FIG for each furanocoumarin (**Figure 2**).

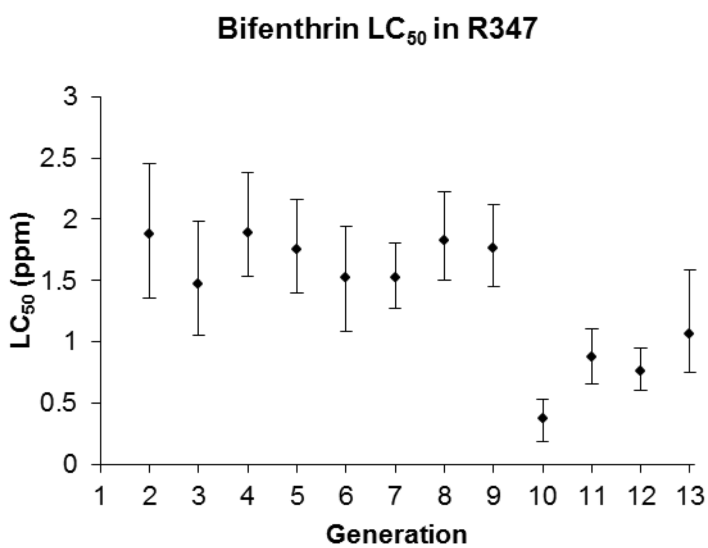

Figure 1. Median-lethal concentration values in a pyrethroid-resistant colony of NOW (R347) through 13 generations.

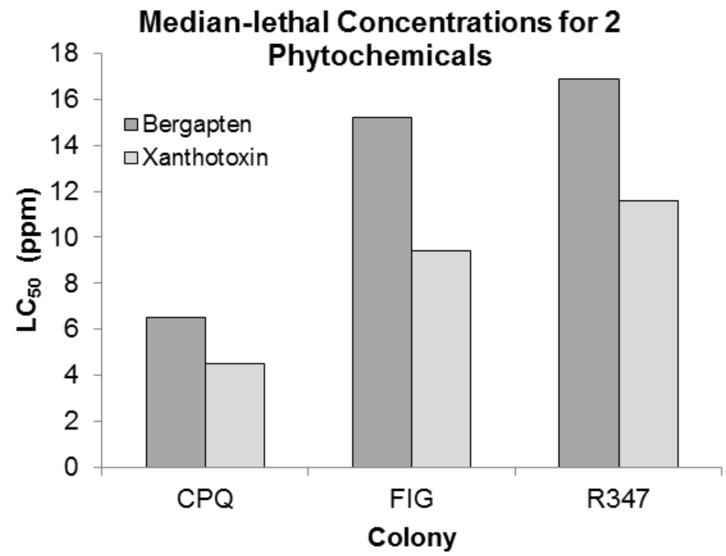

Figure 2. Median-lethal concentration values for bergapten and xanthotoxin from a susceptible laboratory colony (CPQ), a colony derived from fig populations (FIG), and a pyrethroid-resistant colony of NOW (R347).

Bioassays were subsequently conducted with CPQ, FIG and R347 strains to determine the extent to which piperonyl butoxide, an inhibitor of P450-mediated detoxification, enhances toxicity of xanthotoxin to neonates. In all strains, PBO enhanced mortality caused by xanthotoxin ingestion, with strains responding significantly differently both to xanthotoxin alone and xanthotoxin in the presence of piperonyl butoxide (**Table 1**).

Table 1. Percent mortality of three strains of navel orangeworm after 84 hours on artificial diets containing xanthotoxin (4.52 mg/g) in the presence or absence of piperonyl butoxide.

	Strain		
	R347	Fig	CPQ
Xanthotoxin	55	50	60
Xanthotoxin + PBO	85	78	98

Two-way ANOVA shows a significant main effect of strain ($p<0.0001$) and PBO ($p<0.0001$), and a significant strain X PBO interaction ($p<0.0004$)

In neonate filter paper assays, R347 larvae displayed significantly greater survivorship than CPQ larvae ($P<0.001$) after two weeks in the 30 ppm and 300 ppm doses (**Figure 3**). R347 adults that survived bifenthrin exposure in these assays emerged approximately three days earlier ($P<0.001$) than CPQ adults across all doses of bifenthrin (**Figure 4**). In adult assays, both males and females of the R347 strain exhibited significantly greater survivorship ($P<0.001$) when sprayed with bifenthrin at 3 ppm (**Figure 5**).

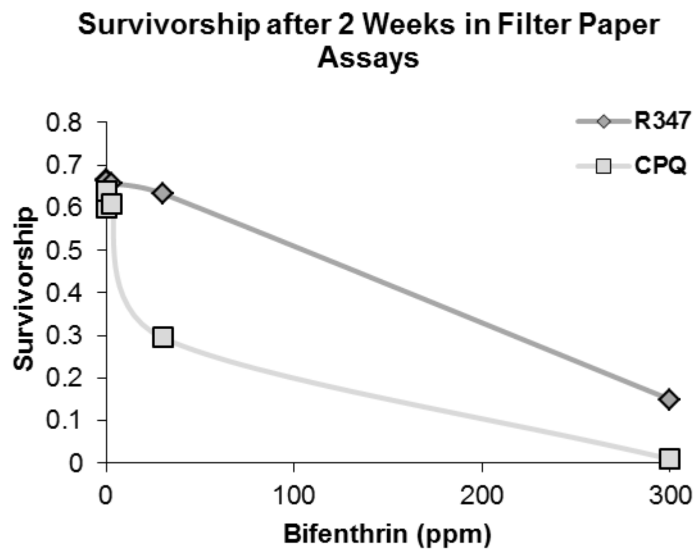

Figure 3. Navel orangeworm survival 14 days after eggs were placed on filter papers sprayed with bifenthrin at 0 ppm, 3 ppm, 30 ppm, and 300 ppm in a resistant colony (R347) and a susceptible colony (CPQ).

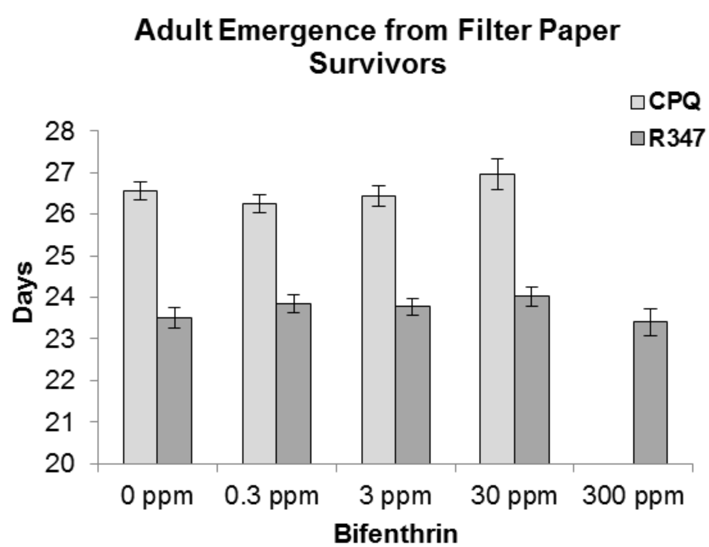

Figure 4. Navel orangeworm days to adult emergence from survivors of bifenthrin filter paper assays in a resistant colony (R347) and susceptible colony (CPQ).

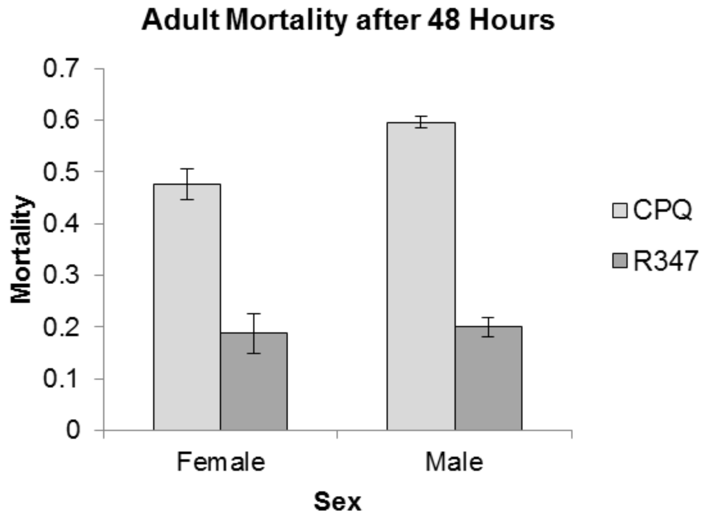

Figure 5. Mortality of adult navel orangeworm sprayed with bifenthrin at 0 ppm and 3 ppm in a resistant (R347) colony and susceptible colony (CPQ). Water was used as the insecticide carrier.

This research may generate insights that improve chemical management strategies for navel orangeworm. Although filter paper assays and adult spray assays were conducted with larvae from recent generations (F_{11}/F_{12}) in the R347 with only 5-fold resistance levels (vs 10-fold in early generations), their survivorship was still significantly greater than that of a susceptible strain at both the neonate and adult stages after bifenthrin exposure. If navel orangeworm populations resistant to pyrethroids can complete development faster than susceptible populations, then an additional generation could potentially emerge during the growing season. A decline in resistance over time in the absence of selection pressure suggests that a reduction in the use of pyrethroids could restore efficacy of the chemical class.

Research Effort Recent Publications:

Demkovich, M., J.P. Siegel, B.S. Higbee, and M.R. Berenbaum. 2015. Mechanism of resistance acquisition and potential associated fitness costs in navel orangeworm (*Amyelois transitella*) exposed to pyrethroid insecticides. Env. Entomol. 44: 855-863.

Demkovich, M., C.E. Dana, J.P. Siegel, and M.R. Berenbaum. 2015. Effect of piperonyl butoxide on the toxicity of four classes of insecticides to navel orangeworm (*Amyelois transitella*) (Lepidoptera: Pyralidae). J. Econ. Entomol. (in press).

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Brancato, F.P. and Golding, N.S. 1953. The diameter of the mold colony as a reliable measure of growth. *Mycological Society of America* 45(6): 848-864.

Bentley, W., Siegel, J. P., Holtz, B.A., and Daane, K.M. 2008. Navel orangeworm (*Amyelois transitella*) (Walker) and obliquebanded leafroller as pests of pistachio. *Pistachio Production Manual*: 179-191.

Palumbo, J.D., Mahoney, N.E., Light, D.M., Siegel, J., Puckett, R.D., and Michailides T.J. 2014. Spread of *Aspergillus flavus* by navel orangeworm (*Amyelois transitella*) on almond. *Plant Disease* 98(9): 1194-1199. doi: 10.1094/PDIS-09-13-1015-RE

Zalom, F.G., Pickle, C., Bentley, W.J., Haviland, D.R., Van Steenwyk, R.A., Rice, R.E., Hendricks, L.C., Coviello, R.L., and M.W. Freeman. 2012. UC IPM Pest Management Guidelines: Almond. University of California, ANR Publication 3431.