Inhibiting Cytochrome P450 Detoxification Enzymes in Navel Orangeworm by Naturally Occurring Plant-Derived Synergists

Project No.: **08-ENTO1-Berenbaum**

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Objectives:

A. Ascertain the ability of essential oil constituents to synergize toxicity of insecticides, mycotoxins, and almond phytochemicals and reduce survival of Navel Orangeworm (NOW).
B. Identify the cytochrome P450 monooxygenases in NOW responsible for metabolism of insecticides, mycotoxins, and almond phytochemicals and test synergists in a heterologous expression system.
C. Use molecular modeling and substrate-docking to "virtually screen" three-dimensional chemical databases to identify other potential synergists.

Interpretive Summary:

The Navel Orangeworm (NOW), *Amyelois transitella*, is a destructive pest of almonds in California, tunneling into the nut and consuming most of the nutmeat. NOW feeding also leaves almonds vulnerable to infection by *Aspergillus* molds that produce toxic aflatoxins. NOW management depends on a combination of control tactics, including chemical control; chemical sprays in current use include organophosphates and pyrethroids. NOW can tolerate high levels of aflatoxins due to the fact they possess very active gut enzymes that metabolize these toxins. These same enzymes likely allow NOW to metabolize pesticides and naturally occurring toxins in their many

hostplants. Our study was aimed at identifying the genes that code for these enzymes and to identify naturally occurring substances that can inhibit the enzymes, reducing the ability of this insect to detoxify insecticides, aflatoxins, and hostplant toxins. We identified one compound, myristicin, which occurs naturally in nutmeg, that inhibits these enzymes and increases the toxicity of xanthotoxin, a constituent of figs (another hostplant), up to sevenfold. Moreover, we identified three candidate genes that code for detoxification enzymes. Characterizing these genes will help in designing other synergists that can interfere with the ability of NOW to metabolize pesticides and toxins in their diet. A new potentially safe and sustainable approach for managing NOW in almond orchards may be to use natural plant-derived synergists to suppress caterpillar growth while reducing applications of synthetic chemicals.

Materials and Methods:

A. Conduct bioassays with insecticides, mycotoxin, phytochemical, and essential oil synergists using artificial diets to determine LD50 with and without synergists. Synergists tested include apiole and myristicin; both inhibit aflatoxin production in *Aspergillus*. The phytochemical selected for bioassay is xanthotoxin, a constituent of figs, another NOW hostplant.
B. Characterize detoxification enzymes. Total RNAs isolated from midguts of final instars are RT-PCR amplified with an oligo dT primer and a degenerate primer designed based on a conserved sequence in P450s corresponding to an amino acid sequence of FDPER ca. 80 nucleotides upstream of the heme-binding region. The cDNA was then cloned to a pGEM-Teasy vector and 46 clones containing cDNA inserts were sequenced. Candidate P450s are expressed in the heterologous baculovirus Sf9 cell expression system. The *in vitro* metabolism reactions were conducted with pyrethroid insecticides, aflatoxin, and phytochemicals.

Results and Discussion:

A. Although the essential oil synergists selected for this study do not increase the toxicity of aflatoxin to NOW, myristicin is an effective synergist of xanthotoxin, increasing toxicity up to sevenfold.
B. Three unique P450s (CYP321C1, CYP6B44 and CYP6AB11) were confirmed by BLAST analysis; all have a high level of identity with the CYP6B subfamily of genes, which encodes insect detoxification enzymes. CYP321C1 was expressed in baculovirus; although aflatoxin B1, ochratoxin A and xanthotoxin were tested, no substrates have yet been identified. Screening with insecticides in current use for NOW management is in progress and molecular modeling is underway as well.

Myristicin should be explored further as a field treatment to reduce survival of NOW in orchard situations; determination of detoxification P450s with efficient metabolism of aflatoxins or insecticides may contribute to sustainable management of NOW in almond orchards by reducing inputs of synthetic organic insecticides.

Recent Publications:

Niu, G., J. Siegel, M.A. Schuler and M.R. Berenbaum. Comparison of toxicity and metabolism of mycotoxins (aflatoxin B1 and ochratoxin A) between two lepidopterans: *Helicoverpa zea* and A*myelois transitella.* J. Chemical Ecology in press.