Identifying Detoxification Enzymes in Navel Orangeworm Responsible for Insecticide and Hostplant Chemicals

Project No.: **10-ENTO1-Berenbaum**

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Objectives:

A. Determine the effect of hostplant identity on insecticide LD50 and metabolism
B. Ascertain the degree to which cytochrome P450s, esterases, and/or glutathione-S-transferases are involved in NOW detoxification of pesticides, mycotoxins and phytochemicals.
C. Characterize at the molecular level the genes encoding the principal detoxification enzymes responsible for metabolizing a set of ecologically important synthetic and natural toxins.

Interpretive Summary:

The navel orangeworm (NOW), *Amyelois transitella*, is among the most destructive pests of almonds in California, as well as a serious problem in pistachios, figs and walnuts. Larvae tunnel into the nut and successive instars consume the nutmeat, generating large quantities of frass and webbing. In addition to causing such direct losses, NOW feeding leaves almonds vulnerable to infection by fungi that produce toxic aflatoxins (Campbell et al., 2003). NOW adults lay eggs in mummy fruits when new crop nuts are unavailable (Molyneux et al., 2007) and the caterpillars have been reported as scavengers on mummified fruits or nuts of at least 25 plant species. NOW management depends on a combination of control tactics, including cultural practices and insecticide sprays, particularly when infested adjacent crops, such as pistachios, provide immigrants into almond orchards (Higbee and Siegel, 2009). Biological control agents, although identified, cannot yet provide complete control. Chemical sprays for hull split include insecticides in several chemical classes; however, little is known as to how NOW metabolizes these compounds. Our overall goal in this project has been to determine the metabolic basis for insecticide and hostplant detoxification. Characterizing the precise enzyme system utilized by NOW in detoxifying pesticides will also facilitate identification of synergists that

can be used to enhance pesticide toxicity and circumvent pesticide resistance should it evolve.

The remarkably broad host range of NOW presents several unique challenges to chemical control. This year we were able to demonstrate that certain hostplant phytochemicals can, administered concurrently with pesticide, reduce the toxicity of that pesticide, presumably by inducing detoxification enzymes that metabolize that pesticide. Specifically, in bioassays, the toxicity of the model pyrethroid insecticide cypermethrin is decreased in the presence of the hostplant flavonoids chlorogenic acid and quercetin. To explore the effects of hostplant identity on detoxification capacity, we also undertook a series of studies aimed at identifying the possible native hostplants of NOW; most of its crop hosts are not native to North America and identifying its ancestral hosts may provide insights into how its detoxification abilities evolved. Because its close relatives in tropical Central America are associated with Fabaceae / Caesalpiniaceae we conducted a series of trials assaying the suitability of native species in the family for sustaining NOW growth and development. Individuals could complete development on two species tested--*Cassia fasciculata* (partridge pea) and *Gleditschia triacanthos* (honey locust). We also successfully characterized the gene encoding a detoxification enzyme, CYP6AB11, and demonstrated that the enzyme can metabolize imperatorin, a phytochemical either occurring structurally similar to compounds occurring in several crop hosts of NOW (e.g., citrus and fig).

To achieve our objective of ascertaining the degree to which different classes of detoxification enzymes are involved in NOW metabolism of natural and synthetic chemicals, we changed our experimental plan, which had been based on the candidate gene approach that allowed us to identify CYP6AB11 as a phytochemical-metabolizing P450. Instead, because genome sequencing costs have dropped significantly, for a comparable investment sequencing the entire genome will allow us to identify all detoxification genes (P450s, esterases, and glutathione-S-transferases) in approximately one year. This substantially more efficient approach will provide genomic information that will be invaluable for comprehensively identifying all genes contributing to hostplant adaptation and pesticide resistance. We have begun our sequencing effort, which we anticipate will be completed by the end of 2012.

Materials and Methods:

Objective A. Determine the effect of hostplant identity on insecticide LD50 and metabolism. We used standard bioassay methods, using artificial diets, to obtain pyrethroid (cypermethrin) toxicity and to measure the impact of simultaneous ingestion of two hostplant flavonoids, chlorogenic acid and quercetin, on toxicity.

Objective B. Ascertain the degree to which cytochrome P450s, esterases, and/or glutathione-S-transferases are involved in NOW detoxification of pesticides, mycotoxins and phytochemicals. We changed our experimental plan, which had been based on the candidate gene approach that allowed us to identify CYP6AB11 as a phytochemical-metabolizing P450. Instead, we initiated an effort to sequence the entire genome. ILLUMINA sequencing-by-synthesis is being used to sequence the genome, followed by intensive manual annotation of the three detoxification gene families. To date, we have sequencing data from a single female

moth done on Illumina with one lane of 180 base- pair insert shotgun library and one lane of 1.5 kB insert shotgun library, representing ca 14X coverage.

Objective C. Characterize at the molecular level the genes encoding the principal detoxification enzymes responsible for metabolizing a set of ecologically important synthetic and natural toxins To identify substrate specificity of cytochrome P450s, P450 cDNAs were co-expressed in Sf9 insect cells with house fly P450 reductase cDNA and fruit fly cytochrome *b*5 cDNA. Expression conditions were optimized by adjusting the ratio of P450: P450 reductase: cytochrome *b*5 to achieve maximum activity toward test substrates. Sixteen chemicals including four furanocoumarins (xanthotoxin, angelicin, bergapten, imperatorin), a coumarin (coumarin), a phenylpropanoid (myristicin), four flavonoids (quercetin, kaempferol, flavone, α-naphthoflavone), a phenolic acid (chlorogenic acid), a mycotoxin (AFB1, and two synthetic compounds (the insecticide α-cypermethrin, the synergist PBO) were tested to determine their suitability as substrates based on the predicted structures of two P450s, CYP321C1 and CYP6AB11.

Results and Discussion:

A. Three replicates of 48-hour bioassays with cypermethrin in the presence or absence of chlorogenic acid and quercetin demonstrated that the presence of 2 mg/g chlorogenic acid or 5 mg/g quercetin in diet containing 50 ng/g cypermethrin allowed for significantly more survival than 50 ng/g dietary cypermethrin alone
B. Analysis of substrate disappearance in these assays indicated that imperatorin is efficiently metabolized by CYP6AB11 (0.88nmol/min/nmol P450) and PBO is turned over by CYP6AB11 at a slower rate (0.11nmol/min/nmol P450). One metabolite was generated and analyzed by HPLC. LC-MS analysis was performed to further characterize the structure of the imperatorin metabolite by CYP6AB11. In positive mode electrospray, the metabolite yields four fragments. This metabolite is identical to that reported for CYP6AB3-mediated metabolism of imperatorin, which also appears to target the double bond in the isoprenyl side chain (Mao et al., 2006). No metabolite of PBO was observed in our GC-MS analysis. The structure of CYP6AB11, which was predicted using MOE programs, suggests that its catalytic site contains a doughnut-like constriction over the heme that excludes aromatic rings on substrates and allows only their extended side chains to access the catalytic site. Docking of imperatorin in the active site predicts that imperatorin binds with an interaction energy of 38.4 kcal/mol and at a distance from the heme estimated to be 7.2 Å. The Ile310-to-Val308 replacement in CYP6AB11 enlarges the opening to the heme and is predicted to allow imeratorin to acess the CYP6AB11 catalytic site. That CYP6AB11 can metabolize the principal insecticide synergist piperonyl butoxide (PBO, a methylenedioxyphenyl synergist), in wide use to circumvent pesticide resistance, raises the possibility that, once acquired, insecticide resistance in this species may be difficult to counter.
C. To date, we have sequencing data from a single female moth done on Illumina with one lane of 180 base- pair insert shotgun library and one lane of 1.5 kB insert shotgun library, representing ca 14X coverage. Illumina assembly of these data suggests that the genome is 300-350 Mb in size.

Research Effort Recent Publications:

Niu, G., S. G. Rupasinghe, A.R. Zangerl, J P. Siegel, M A. Schuler, M. R. Berenbaum, 2011. A substrate-specific cytochrome P450 monooxygenase, CYP6AB11, from the polyphagous navel orangeworm (*Amyelois transitella*). Insect Biochem. Mol. Biol. 41: 244-253

Niu, G., H. S. Pollock, A. Lawrance, J. Siegel, and M. R. Berenbaum. Effects of naturally occurring and synthetic synergists on the toxicity of insecticides, phytochemicals and mycotoxins to *Amyelois transitella* (Lepidoptera: Pyralidae). Journal of Economic Entomology, Accepted pending revision.

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