Effect of enzyme inhibitors on insecticide susceptibility in caterpillars

Sage Wood, Lexi Baiter, and Dr. Niranjana Krishnan

Funding from Missouri S&T FYRE Program and Missouri S&T Biological Sciences Department. Thank you to Cassandra Gorman.

Introduction

The U.S. Environmental Protection Agency only requires pesticide toxicity data for one insect species: honeybees (Apis mellifera). However, there are an estimated 30 million insect species, and pesticide susceptibility varies within and across insect orders [1]. The overall goal of this project is to identify highly susceptible and non-susceptible pest and non-target species in major insect orders and determine reasons for susceptibility differences. Specifically, this poster aims to elucidate significant differences in susceptibility to imidacloprid (IMI), a commonly employed neonicotinoid insecticide, in corn earworms (Helicoverpa zea) and fall armyworms (Spodoptera frugiperda). Both species belong to the Lepidoptera order and Noctuidae family, but corn earworms are significantly more susceptible to imidacloprid [1]. We hypothesize that differences in susceptibility across the two species are due to differing activity of their metabolizing enzymes.

Objectives

1. Determine doses of IMI that result in intermediate levels of larval mortality
2. Determine if in vivo inhibition of metabolizing enzymes result in increased larval mortality
3. Determine the concentration of metabolizing enzymes through in vitro assays

Methods

Objective 1. Imidacloprid was topicaly applied on different larval instars of both species to determine doses that caused intermediate mortality (observations taken until pupation). Objective 2. Metabolizing enzyme inhibitors PBO (piperonyl butoxide; inhibits P450s/MFOs), TPP (triphenyl phosphate; inhibits esterases), and DEM (diethyl maleate; inhibits transferases) were topicaly applied 1 hour prior to selected imidacloprid doses. Mortality and adult emergence and health were noted (20 larvae were used per treatment). Objective 3. Larvae were crushed into a chilled phosphate buffered saline solution and centrifuged under ice-cold condition to extract microsomal enzymes. BCA assay [2] was conducted to determine protein concentrations in the lysate and a mixed function oxidase (MFO) assay [3] was done to determine concentrations of P450s in lysate with and without PBO.

Average size of corn earworms and fall armyworms

<table>
<thead>
<tr>
<th></th>
<th>CEW</th>
<th>FAW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight</td>
<td>224 (± 36) mg</td>
<td>179 (± 58) mg</td>
</tr>
<tr>
<td>Surface Area</td>
<td>3.4 (± 0.4) cm²</td>
<td>2.7 (±0.6) cm²</td>
</tr>
</tbody>
</table>

Life Cycle of a Moth

Eggs → 1st Instar → 2nd Instar → 3rd Instar → 4th Instar → 5th Instar → Cocoon

Results

Objective 1. Imidacloprid doses causing intermediate levels of mortality were identified for sixth instar corn earworms (0.1 and 1 µg/larva) and fourth instar fall armyworms (20 and 100 µg/larva).

Objective 2. Corn earworms exposed to PBO + 1 µg IMI had significantly greater mortality than those exposed to 1 µg IMI alone (see Figure 1; p = 0.0065 at 95% CI using an Exact Binomial Test). None of the other inhibitor treatments showed significant differences in mortality w.r.t. IMI (p ≥ 0.076). See Figure 2 for characteristics of pupated larvae.

Objective 3. BCA assay indicated necessary protein concentrations were present in lysate; however, 2 repetitions of MFO assay showed inconsistent results.

Discussion

- Sixth-instar corn earworms were more susceptible to imidacloprid than fourth-instar fall armyworms.
- In corn earworms, P450s play a critical role in imidacloprid metabolism and detoxification.
- Following optimization of MFO assay, P450 concentrations in both species will be determined.
- Results from this study will be incorporated into a larger project to predict insect responses to pesticides with implications in risk assessment and pest management.

References