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17th Annual Undergraduate Research
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Apr 14th, 2022 - 3:00 PM

Enzyme Inhibitors and Insecticide Effect on Caterpillars

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Baiter, Alexis, "Enzyme Inhibitors and Insecticide Effect on Caterpillars" (2022). *Undergraduate Research Conference at Missouri S&T*. 19.

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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

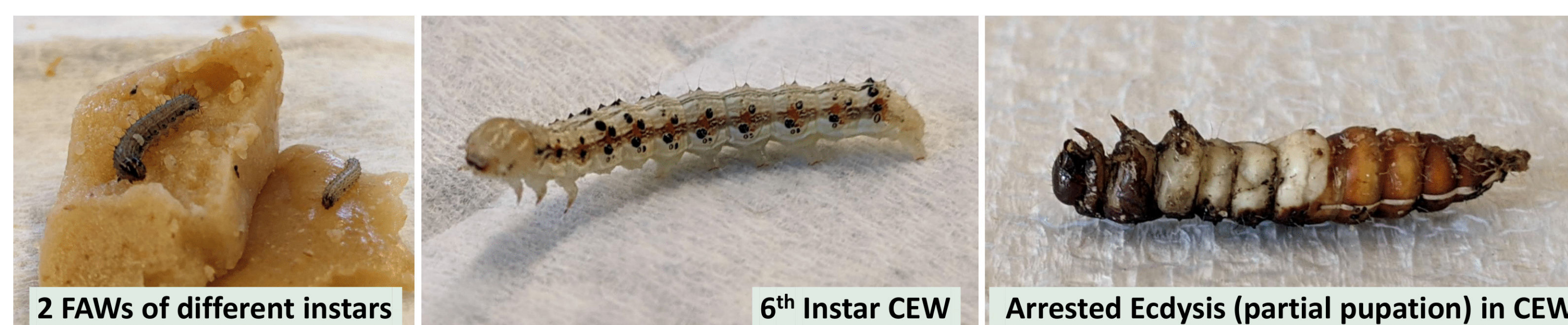
Objective 1. Imidacloprid was topically applied on different larval instars of both species to determine doses that caused intermediate mortality (observations taken until pupation).

Objective 2. Metabolizing enzyme inhibitors PBO (piperonyl butoxide; inhibits P450s/MFOs), TPP (triphenyl phosphate; inhibits esterases), and DEM (diethyl maleate; inhibits transferases) were topically applied 1 hour prior to selected imidacloprid doses. Mortality and adult emergence and health were noted (20 larvae were used per treatment).

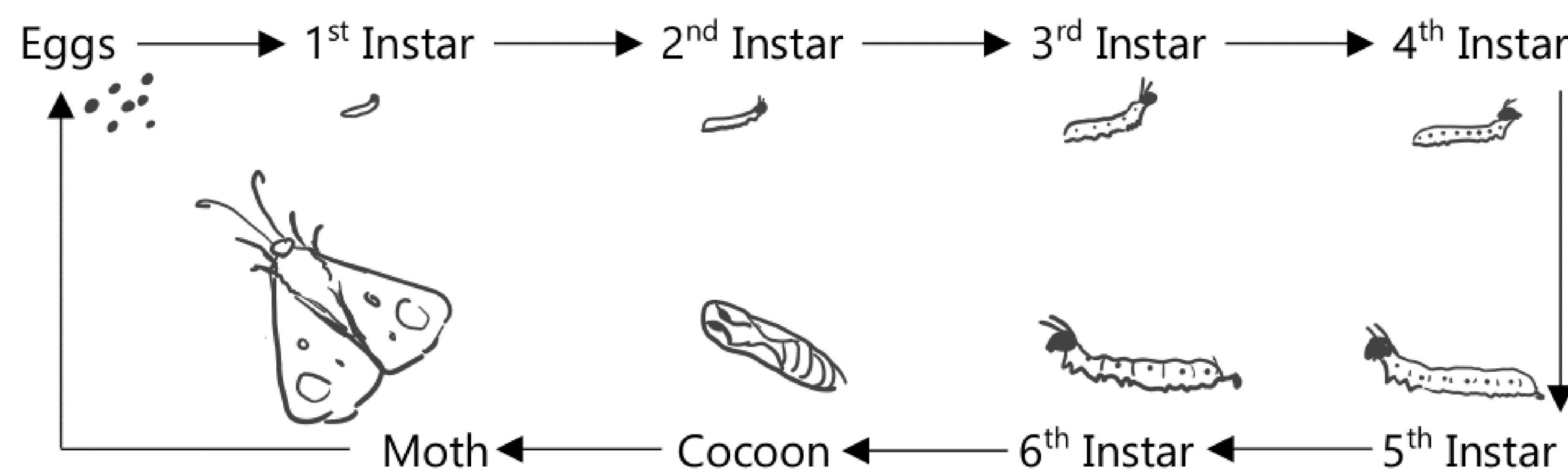
Objective 3. Larvae were crushed into a chilled phosphate buffered saline solution and centrifuged under ice-cold condition to extract microsomal enzymes. BCA assay [2] was conducted to determine protein concentrations in the lysate and a mixed function oxidase (MFO) assay [3] was done to determine concentrations of P450s in lysate with and without PBO.

Average size of corn earworms and fall armyworms

	CEW	FAW
Weight	224 (\pm 36) mg	179 (\pm 58) mg
Surface Area	3.4 (\pm 0.4) cm ²	2.7 (\pm 0.6) cm ²



Life Cycle of a Moth



Results

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

Objective 2. Corn earworms exposed to PBO + 1 μ g IMI had significantly greater mortality than those exposed to 1 μ g IMI alone (see Figure 1; p = 0.0065 at 95% CL using an Exact Binomial Test). None of the other inhibitor treatments showed significant differences in mortality w.r.t. IMI (p \geq 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.

References

- [1] Krishnan N. 2021. Assessing risk of insecticide exposures on monarch butterflies. [Doctoral dissertation, Iowa State University].
- [2] Pierce BCA Protein Assay. Thermo Fisher Scientific.
- [3] Narayanan, *et al.* 2020. Toxicity of cypermethrin and enzyme inhibitor synergists in red hairy caterpillar *Amsacta albistriga* (Lepidoptera: Arctiidae). *JoBAZ* 81, 45.

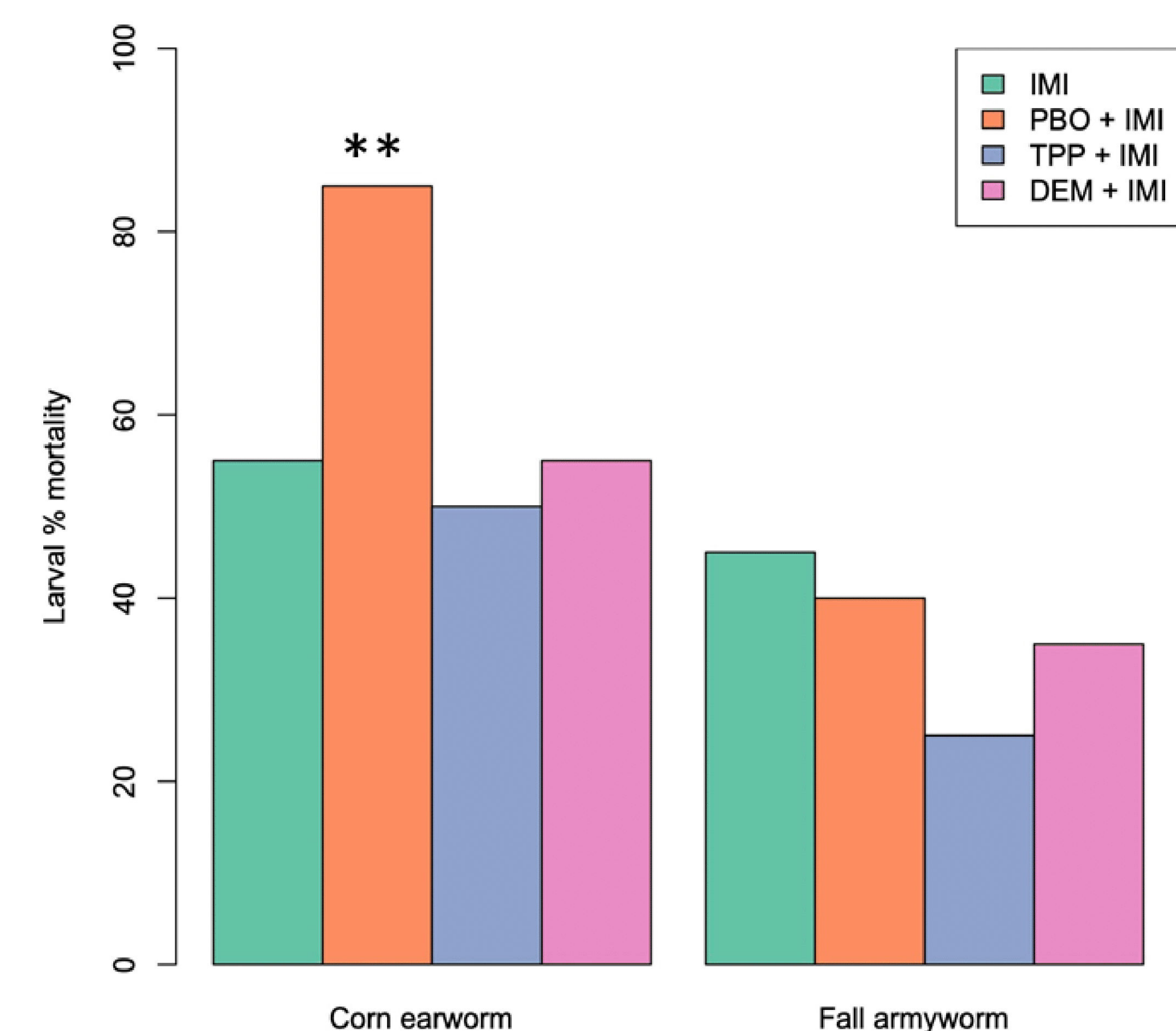


Figure 1. Response to inhibitors of metabolic enzymes

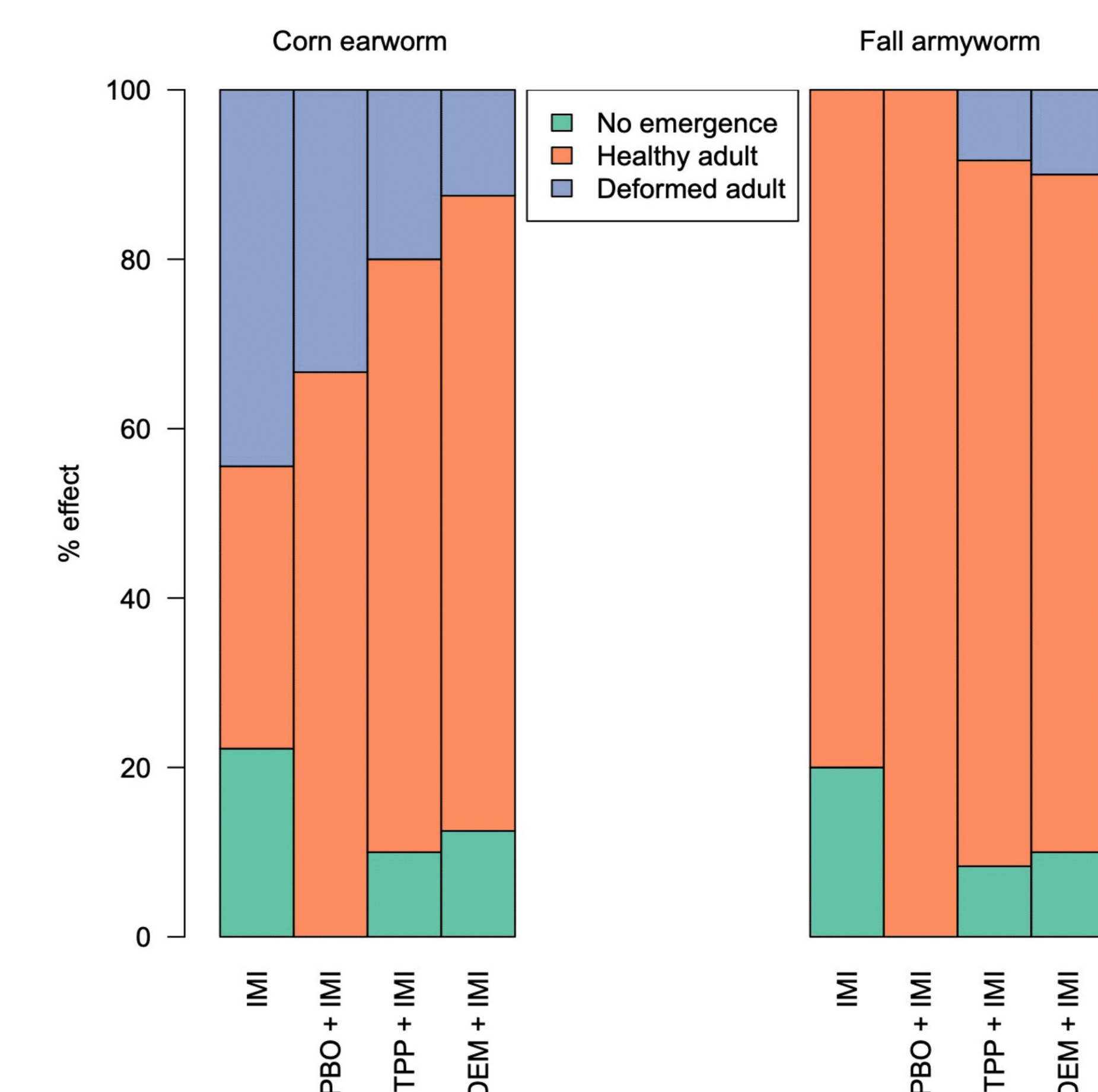


Figure 2. Treatment effects on adult emergence

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