Apr 14th, 2022 - 3:00 PM

# Insecticide Metabolic Responses of Two Lepidoptera

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# Risk Assessment of insecticide exposure on two members of the Lepidoptera order: the soybean looper (*Chrysodeixis includens*) and the corn earworm (*Helicoverpa zea*)

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**INTRODUCTION:** Soybean loopers (*Chrysodeixis includens*) and corn earworms (*Helicoverpa zea*) are moths that belong to the Noctuidae family of Lepidoptera. They are commonly a pest of soybean and corn plants, respectively. Both species are found in Missouri. Their life cycles consist of egg, larval, pupal, and adult stages, as shown in Figure 1.

A variety of insecticides are employed to manage soybean loopers (SL) and corn earworms (CEW). The most used insecticide class is pyrethroid, which prevents closure of the voltage-gated sodium channels in the neurons. This causes depolarization of neurons which leads to paralysis and death. Cypermethrin is a commonly used pyrethroid insecticide that is registered as a foliar spray to manage both SL and CEW. Interestingly, literature studies indicate that both species show different susceptibilities to cypermethrin; however, these studies were performed on different instars, in different labs, and had different observation periods, all of which are known to influence susceptibility [1].

The overall goal of our project is to elucidate the toxicity of cypermethrin on SL and CEW. We hypothesize that since both species belong to the same family, they would show similar toxic and metabolic responses to cypermethrin if the studies were conducted similarly, i.e., in the same lab, using the same methods and same instars. We also hypothesize that cypermethrin applications in the field would be effective in controlling populations of both pest species. Specifically, our objectives are to:

1. Determine toxicity of cypermethrin on CEW and SL
2. Elucidate metabolic factors contributing to cypermethrin toxicity
3. Assess risk of cypermethrin applications on both species.

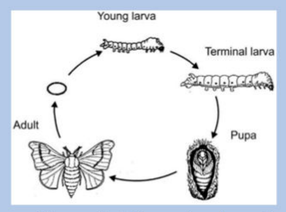



Figure 1. Life cycle of moths

Table 1. Characteristics of larvae at time of treatment

| Species | Instar treated | Mean weight (± SD) in mg | Mean surface area (± SD) in $cm^2$ |
|---|---|---|---|
| Corn earworm | $5^{th}/6^{th}$ instar | 224 (± 36) | 3.4 (± 0.4) |
| Soybean looper | $5^{th}/6^{th}$ instar | 216 (± 59) | 2.7 (± 0.9) |

**METHODS:**

Objective 1. Fifth/sixth instars of both species were topically exposed to five doses of cypermethrin:acetone solution and acetone alone. Fifteen larvae were treated per dose per insect and mortality was recorded until pupation. Prior to treatment, weights and surface areas of larvae were recorded (see Table 1) and, following pupation, pupal weights and adult emergence were noted. Dose-response curves and lethal dose (LD) values were generated using "drm" package in R while ANOVA and Dunnett's test was used to compare pupal weights across treatments.

Objective 2. Synergistic studies were undertaken with PBO, TPP, and DEM, inhibitors of three major metabolizing enzymes in insects: cytochrome P450s, esterases, and gluthathione-S-tranferases, respectively. Larvae were provided 50 mM of an inhibitor and a cypermethrin dose that caused low levels of mortality. The inhibitor was provided an hour prior to cypermethrin (or acetone) and mortality was recorded until pupation. Clopper-Pearson exact binomial test was used to assess differences between treatments.

**METHODS (continued):**

Objective 3. A representative cypermethrin label, Hero®, was selected and maximum application rates to manage SL and CEW were determined. This application rate was input into AgDRIFT, a spray drift model that estimates the concentration of insecticide landing per cm2 field [2]. The AgDRIFT-estimated concentration for cypermethrin was 600 ng/cm2. This was multiplied with the surface area of the larvae (see Table 1) to estimate larval exposure. Larval exposure was compared with the dose-response curves (see Figure 2) to estimate risk (see Table 3).

**RESULTS:**

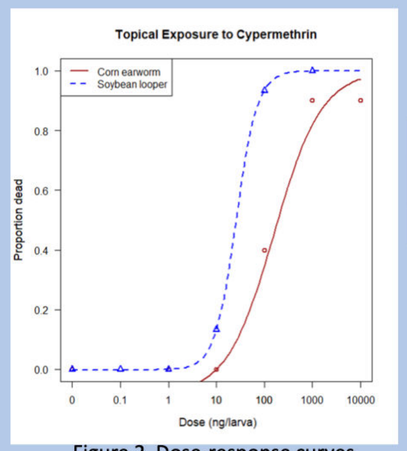



Figure 2. Dose-response curves

Table 2. Cypermethrin LD values and 95% CIs

| LD values (ng/larva) | Soybean looper | Corn earworms |
|---|---|---|
| LD10 | 8.6 (3.9 to 19) | 13 (6.8 to 26) |
| LD50 | 26 (14 to 49) | 160 (96 to 270) |
| LD90 | 80 (31 to 204) | 1900 (1400 to 2600) |

Comparison across species show different LD50s and LD90s (non-overlapping 95% CIs)

At least 90% of pupae in all treatments produced viable adults

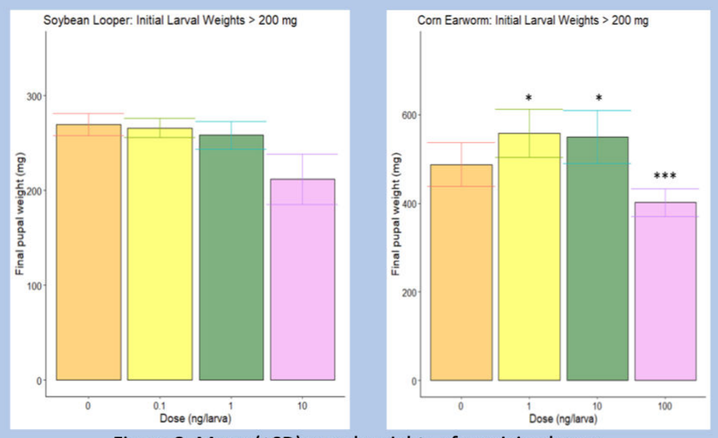
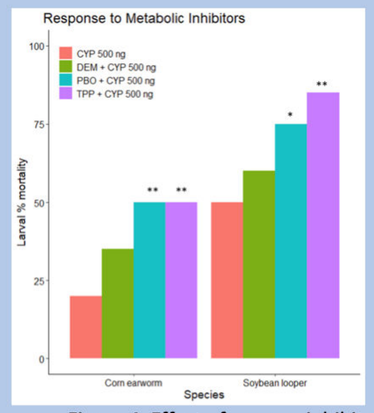



Figure 3. Mean (±SD) pupal weights of surviving larvae

Table 3. Risks to larvae from foliar cypermethrin applications (see Methods)

| Species | Larval exposure (ng) | Predicted mortality (%) |
|---|---|---|
| Soybean looper | 1620 | 100 |
| Corn earworm | 2000 | ~ 90 |

**RESULTS (continued)**




Note: None of the enzyme inhibitors caused mortality in CEW, and PBO and TPP were also non-toxic to SL. DEM was toxic however, and its mortality was corrected by applying Abbott's formula.

Figure 4. Effect of enzyme inhibitors on cypermethrin toxicity

**DISCUSSION:**

- Despite belonging to the same family, employment of the same testing methods show that SL and CEW show different susceptibilities to cypermethrin.
- High doses of cypermethrin significantly reduce pupal weights in CEW, likely due to reduced larval feeding from paralysis. Cypermethrin did not affect adult emergence in both species.
- Both species show increased toxicity in the presence of PBO and TPP, indicating cytochrome P450s and esterases are important for the breakdown of cypermethrin. This finding is supported by studies in literature [3].
- Ongoing studies are evaluating the activity of metabolizing enzymes through *in vitro* enzymatic assays [4]. Future studies will evaluate if differences in susceptibility in the two species are due to differences in larval absorption/excretion and/or binding of cypermethrin at target site.
- Comparison of toxicity (dose-response curve) data to exposure (AgDRIFT) estimates indicate foliar cypermethrin applications in soybean and corn fields will be effective in managing SL and CEW, despite differences in susceptibility between the two species.

**ACKNOWLEDGEMENT:** Biological Sciences department at Missouri University of Science and Technology.

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April 2022