Resistance to Pyrethroid Insecticides in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Indiana and Illinois

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**ABSTRACT** The corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae), can cause serious losses in many field and vegetable crops throughout the United States. Since their introduction, pyrethroid insecticides have become the primary insecticide class for managing *H. zea*. However, resistance has been reported in the southern United States and has recently become a concern in the Midwest after the observation of sporadic control failures and a decreased efficacy of pyrethroids in small-plot field trials. Larvae collected from Lafayette, IN, Vincennes, IN, and Collinsville, IL, were used to establish laboratory colonies in 2006 and 2007. Larvae from these colonies were tested for resistance to the pyrethroid insecticide bifenthrin by using topical assays. Adult males collected from pheromone traps in Lafayette were tested for resistance to cypermethrin by using the adult vial test (AVT) method. Resistance ratios of ≥8 were observed for the larval bifenthrin assays in 2006 and 2007 in all colonies except for the 2007 Illinois colony. AVT assays conducted with cypermethrin showed ∼15% survival in both 2006 and 2007 at the 5 μg per vial discriminating dose. These results suggest that low to moderate levels of pyrethroid resistance are present in these populations.

**KEY WORDS** corn earworm, insecticide resistance, pyrethroid

*Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) is a highly polyphagous and mobile insect best known for damaging cotton (*Gossypium* spp.); corn, *Zea mays* L.; tomato, *Lycopersicon esculentum* L.; soybean, *Glycine max* (L.) Merr.; and bean (*Phaseolus* spp.) crops in the United States and Canada (Capinera 2004). The larvae cause damage to crops by feeding on ears of corn and fruiting portions of other hosts, reducing yield and marketability. The pest’s overwintering range is limited because overwintering pupae cannot survive the cold temperatures normally encountered above the 40th parallel (Blanchard et al. 1942), which runs through southern Indiana and Illinois. However, adults from the southern United States migrate north on wind currents and expand this range up to 59°N by late summer (Farrow and Daly 1987).

Management programs for *H. zea* primarily consist of applying insecticides to kill newly hatched larvae, and pyrethroid insecticides have been the most effective materials for the past 15 yr (Hutchison et al. 2007). In the South, pyrethroids are frequently applied to high-value crops such as cotton and vegetable crops. Additional selection pressure occurs when pyrethroids are used in management programs for other hosts, such as soybean, corn, and sorghum, *Sorghum bicolor* (L.) Moench (Pietrantonio et al. 2007). The danger of resistance development is also compounded because migrating individuals can spread resistance genes over large geographic areas.

Resistance to pyrethroids in *H. zea* larvae was first reported in 1990 in Mississippi and since then has been reported in Arkansas, South Carolina, Florida, Louisiana, Texas, and Illinois (Stadelbacher et al. 1990, Hsu and Yu 1991, Abd-Elghafar et al. 1993, Kanga et al. 1996, Brown et al. 1998, Ottea and Holloway 1998). In addition, resistance levels to cypermethrin present in populations of field-collected adult *H. zea* in Louisiana, monitored since the late 1980s, has shown an increase in survivorship at each dose over time (Riley 1988; Graves et al. 1993, 1994; Bagwell et al. 1996, 1997). Also, adult males collected from pheromone traps in Texas have exhibited up to 10-fold resistance to cypermethrin (Pietrantonio et al. 2007). Field-level failures, however, have not been widespread and have not been experienced outside of the southern United States, including regions infested by southern migrants.

In Indiana and Illinois, *H. zea* is a severe pest of sweet corn, seed corn, and tomato. In Indiana, there are normally small numbers of overwintering individuals in the southern part of the state, but the majority...
of the problems caused by this pest are due to the migrants that arrive in late summer (Flood et al. 2007). In Illinois, H. zea moths have consistently been trapped earlier in the spring near Collinsville, and in some years in Mason County along the Illinois River. These moth flights are believed to represent an over-wintering population that is able to survive this far north, probably due to the combination of milder weather and lighter soils that allow H. zea to escape freezing at greater pupation depths. In addition, moth catches at these sites are not synchronized with catches that seem to be migration events, and flights of H. zea near Collinsville are earlier and heavier than elsewhere (R.W., unpublished data). To date, no widespread control failures have been reported for pyrethroid insecticides used to control H. zea in Indiana or Illinois, although sporadic control failures have occurred since 2004 in both states. In addition, small plot insecticide trials in Indiana and Illinois have shown a decrease in the efficacy of pyrethroid insecticides since 2003 (R.F., unpublished data; Weinzierl et al. 2007). Researchers in Minnesota and Wisconsin have documented similar declines in efficacy in small plot trials and have observed an increase in the sur-viviorship of adults tested at discriminating doses of cypermethrin by using the adult vial test method (AVT), described by Plapp et al. (1987), from 2003 to 2005 (Hutchison et al. 2007). Similarly, in Virginia and Pennsylvania low levels of survivivorship have occurred at these same discriminating doses of cypermethrin in 2003–2005 (Fleischer et al. 2007).

The decreased efficacy of pyrethroids in small plot trials, the sporadic control failures in Indiana and Illinois, and the survivivorship of adult males at the discriminating dose of cypermethrin in AVT trials conducted in neighboring states all suggest that pyrethroid resistance may be developing in Indiana and Illinois. A loss of effectiveness of this class of insecticides would be detrimental to sweet corn, seed corn, and tomato producers because no cost-effective insecticide alternatives are currently available. The objective of this research was to determine whether pyrethroid resistance is present in populations of H. zea infesting crops in Indiana and Illinois.

Materials and Methods

Insect Collection. In 2006 and 2007, larvae collected from untreated sweet corn fields at Throckmorton Purdue Agricultural Center near Lafayette, IN, the Southwest Purdue Agricultural Experimental Station near Vincennes, IN, and commercial farms near Collinsville, IL were used to establish laboratory colonies that were tested for pyrethroid resistance. Collections were made early in the growing season (late June–early July) in Vincennes and Collinsville where populations of H. zea were great enough to allow collection of sufficient (>300) larvae to start a laboratory colony. Most migrating insects arrive in late July, August, and September. Based on migration forecast models and field monitoring with pheromone traps, it is highly unlikely that Indiana or Illinois received migrat-ing insects before these insects were collected (AWARE 2007, Flood and Rabae 2007, Sandstrom et al. 2007). Based on climatological data it is possible that in 2006 and 2007 some overwintering pupae survived the winters in Lafayette, IN (NOAA 2006, 2007a,b, Sandstrom et al. 2007). However, in 2006 and 2007, only 34 and 50 individuals were collected from 0.1 and 0.2 ha, respectively, within sweet corn plots at this location. Therefore, collections in Lafayette were made in late August or early September when large populations were present after migrating adults had arrived. In addition, 300 pupae from a pyrethroid susceptible population of H. zea were obtained from the Monsanto Company (Union City, TN) to serve as a reference population in the LD50 trials. Larvae were reared on a synthetic corn earworm diet (Bio-Serv, Frenchtown, NJ) by using a standard rearing procedure (Waldbauer et al. 1984).

Insecticides. Technical grade bifenthrin (94.7%, FMC Corporation, Philadelphia, PA) was used in larval toxicity trials conducted with the laboratory colonies. Piperonyl butoxide (PBO) (Aldrich, Milwau-kee, WI) was evaluated as a synergist in the larval toxicity trials conducted with bifenthrin. Glass scin-tillation vials containing 0, 1.25, 2.5, 5, 7.5, 10, 20, and 30 µg of cypermethrin were obtained from Greg Payne (University of West Georgia, Newnan, GA) and used in the adult vial tests with males collected from pheromone traps. A 1-ml volume of insecticide solution dissolved in acetone was pipetted into 20-ml scin-tillation vials; the vials were rolled on their side on a hotdog roller with no heating element to allow acetone to evaporate.

Larval Toxicity Trials. Third-instar larvae were tested for resistance to bifenthrin using a topical bio-assay method. Bifenthrin was dissolved in acetone, and five concentrations of bifenthrin ranging from 0.02 to 6.0 µg were tested on at least 30 individuals per concentration with acetone used as a control. A 1-µl droplet of pesticide–acetone solution or acetone was administered to the thoracic tergum using a micropipette (Finpipette, Thermo Fisher Scientific, Waltham, MA). In addition, toxicity trials that included a pre-treatment with the insecticide synergist PBO were conducted to test for metabolic resistance. Thirty minutes before applying bifenthrin, a 1-µl droplet containing 1 µg of PBO was applied to the thoracic tergum of the larvae. Treated larvae were kept in individual cups with diet and held at 25°C in the environmental chamber used to rear the larvae. Mortality was assessed at 48 h; a larva was considered dead if it was unable to make a coordinated movement when gently prodded. Percentage of mortality was recorded for each treatment.

Adult Toxicity Trials. During summers 2006 and 2007, adult males collected from Hartstack pheromone traps baited with Pherocon cap lures (Trécé Inc., Salinas, CA) at Throckmorton Purdue Agricultural Center, Lafayette, IN, were tested for resistance to cypermethrin by using the AVT (Plapp et al. 1987). Moths caught in pheromone traps were kept overnight in a container with a 10% (wt:vol) sugar water solu-
moth was considered dead if it was unable to fly. Males were examined for mortality after 24 h; a vials treated with acetone were used as the control. Overnight at room temperature on an incline of 15º, placed individually into loosely capped vials and held for 24 h. Moths surviving the night with a healthy appearance, wings intact with scales still on them, were scored as alive.

2.3. Data Analysis

Control mortality was calculated using Abbott’s formula (Abbott 1925). For the larval toxicity trial conducted with bifenthrin, LD50 values were calculated for each colony with probit models by using SAS (SAS Institute, Cary, NC). Resistance ratios and their 95% confidence intervals (CIs) were calculated for all field-collected colonies to assess whether differences in toxicity observed between them and the susceptible colony were significant (Robertson and Preisler 1992). Mortality responses were considered significantly different if the 95% confidence intervals of the resistance ratios for the treatments that included PBO compared to bifenthrin, respectively. In 2007, there was a decrease in the LD50 values of the colonies established from Lafayette, IN, Vincennes, IN, and Collinsville, IL, larvae. In 2006, the Lafayette, IN, Vincennes, IN, and Collinsville, IL, larvae were 14, 22, and 17 times more tolerant to bifenthrin, respectively. In 2007, there was a decrease in the LD50 values in Vincennes, IN, and Collinsville, IL, and an increase in the LD50 values of the susceptible colony and in Lafayette, IN. In 2007, the resistance ratios for Lafayette, IN, Vincennes, IN, and Collinsville, IL, larvae were 20, 8, and 4, respectively. These increases in LD50 values were considered to show that resistance is occurring because the confidence intervals of the resistance ratios of field-collected colonies do not include 1.0 (Robertson and Preisler 1992).

A higher level of tolerance to bifenthrin, varying from 4- to 22-fold, was observed in larval toxicity trials conducted with colonies established from field-collected larvae in 2006 and 2007, but this varied between locations and years (Table 1). In 2006, the LD50 of the susceptible colony was significantly lower than the LD50 values of the colonies established from Lafayette, IN, Vincennes, IN, and Collinsville, IL, larvae. In 2006, the Lafayette, IN, Vincennes, IN, and Collinsville, IL, larvae were 14, 22, and 17 times more tolerant to bifenthrin, respectively. In 2007, there was a decrease in the LD50 values in Vincennes, IN, and Collinsville, IL, and an increase in the LD50 values of the susceptible colony and in Lafayette, IN. In 2007, the resistance ratios for Lafayette, IN, Vincennes, IN, and Collinsville, IL, larvae were 20, 8, and 4, respectively. These increases in LD50 values were considered to show that resistance is occurring because the confidence intervals of the resistance ratios of field-collected colonies do not include 1.0 (Robertson and Preisler 1992).

Larval toxicity trials also were conducted with the insecticide synergist PBO in both 2006 and 2007 (Table 2). The LD50 values, their 95% CIs, resistance ratios between the treatment with and without PBO for each colony, and the 95% CIs of the resistance ratios were calculated and compared (Table 2). In 2006, the resistance ratio for the Lafayette, IN, colony had a 95% CI that was slightly >1.0. The 95% CIs of the resistance ratios for the treatments that included PBO compared with the treatments that did not include PBO for the other colonies for both years all included 1.0, and were, therefore, not significantly different. This suggests that no synergism is occurring.

Results

Table 1. Responses of third-instar H. zea pyrethroid susceptible and field-collected colonies to topical applications of bifenthrin in 2006 and 2007.

<table>
<thead>
<tr>
<th>Colony</th>
<th>N</th>
<th>Slope (± SE)</th>
<th>t ratio</th>
<th>LD50 (µg/g)</th>
<th>95% CI</th>
<th>χ² (df)</th>
<th>P</th>
<th>RR</th>
<th>95% CI</th>
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<tbody>
<tr>
<td>2006</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lafayette, IN</td>
<td>350</td>
<td>1.55 ± 0.22</td>
<td>6.99</td>
<td>2.32</td>
<td>1.42–3.74</td>
<td>10.6</td>
<td>0.0607</td>
<td>14.2</td>
<td>9.51–21.2</td>
</tr>
<tr>
<td>Vincennes, IN</td>
<td>450</td>
<td>0.99 ± 0.28</td>
<td>3.54</td>
<td>3.67</td>
<td>0.55–8.67</td>
<td>22.1</td>
<td>0.0008</td>
<td>22.2</td>
<td>10.9–45.3</td>
</tr>
<tr>
<td>Collinsville, IL</td>
<td>350</td>
<td>1.51 ± 0.28</td>
<td>5.42</td>
<td>2.82</td>
<td>1.22–5.15</td>
<td>12.4</td>
<td>0.0248</td>
<td>16.6</td>
<td>6.99–39.2</td>
</tr>
<tr>
<td>Susceptible</td>
<td>350</td>
<td>2.39 ± 0.31</td>
<td>7.74</td>
<td>0.17</td>
<td>0.13–0.20</td>
<td>2.4</td>
<td>0.0877</td>
<td>1.0</td>
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</table>

<table>
<thead>
<tr>
<th>Colony</th>
<th>N</th>
<th>Slope (± SE)</th>
<th>t ratio</th>
<th>LD50 (µg/g)</th>
<th>95% CI</th>
<th>χ² (df)</th>
<th>P</th>
<th>RR</th>
<th>95% CI</th>
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<tr>
<td>2007</td>
<td></td>
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<tr>
<td>Lafayette, IN</td>
<td>210</td>
<td>1.81 ± 0.25</td>
<td>7.35</td>
<td>5.22</td>
<td>3.96–6.99</td>
<td>5.4</td>
<td>0.2441</td>
<td>20.2</td>
<td>14.5–28.2</td>
</tr>
<tr>
<td>Vincennes, IN</td>
<td>363</td>
<td>0.93 ± 0.16</td>
<td>5.78</td>
<td>2.10</td>
<td>1.35–3.00</td>
<td>3.0</td>
<td>0.0009</td>
<td>22.8</td>
<td>5.26–12.2</td>
</tr>
<tr>
<td>Collinsville, IL</td>
<td>320</td>
<td>0.99 ± 0.16</td>
<td>6.13</td>
<td>1.03</td>
<td>0.67–1.50</td>
<td>8.5</td>
<td>0.0117</td>
<td>22.2</td>
<td>10.9–45.3</td>
</tr>
<tr>
<td>Susceptible</td>
<td>180</td>
<td>2.90 ± 0.61</td>
<td>5.69</td>
<td>0.28</td>
<td>0.24–0.31</td>
<td>0.4</td>
<td>0.9325</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

a Number of larvae tested.

b The t ratio of the slope of the regression line calculated by the formula of Robertson and Preisler (1992). If the ratio does not exceed 1.96 the regression is not significant (P = 0.05).

c The 95% confidence intervals of the LD50.
d RR and 95% CI calculated by the formula of Robertson and Preisler (1992). Differences in the RR are considered significant if the CI does not include 1.0.
traps in Lafayette, IN, were tested for resistance to cypermethrin (Table 3). The discriminating dose of 5 μg used in the AVT tests should kill 99–100% of a susceptible population and field efficacy may be compromised if survival exceeds 25% at the 10-μg dose (Hutchison et al. 2007). The higher (10, 20, and 30 μg) doses were included in the tests due to observed resistance such as decreased cuticular penetration and metabolic detoxification enzymes inhibited by PBO, such as mixed function oxidases, glutathione transferases, and esterases also could be involved in detoxification of pyrethroids and should be investigated. Other pyrethroid resistance mechanisms such as decreased cuticular penetration and target site insensitivity also have been identified in *H. zea* (Abd-Elghafar and Knowles 1996, Ottea and Holloway 1998, Usmani and Knowles 2001). The results of these studies confirm that low-to-moderate levels of resistance to pyrethroid insecticides are present in *H. zea* collected from two locations in Indiana and one location in Illinois. The results from the adult vial assays conducted with bifenthrin and PBO did not show any differences between the larvae treated with bifenthrin versus the larvae treated with PBO and bifenthrin. These results suggest that the metabolic detoxification enzymes inhibited by PBO, primarily cytochrome P450 detoxification enzymes, do not have a large role in the detoxification of pyrethroids in the individuals tested. Other studies, however, have identified cytochromes P450 involved with the detoxification of the pyrethroid cypermethrin (Li et al. 2002, 2004). Metabolic detoxification enzymes not inhibited by PBO, such as mixed function oxidases, glutathione transferases, and esterases also could be involved in detoxification of pyrethroids and should be investigated. Other pyrethroid resistance mechanisms such as decreased cuticular penetration and target site insensitivity also have been identified in *H. zea* (Abd-Elghafar and Knowles 1996, Ottea and Holloway 1998, Usmani and Knowles 2001).

Toxicity trials were conducted using bifenthrin and cypermethrin. Bifenthrin is an effective pyrethroid
insecticide for many insect pests and is commonly used by producers in the Midwest (Hutchison et al. 2007). Cypermethrin is no longer widely used but serves as an important compound from a historical perspective because it has been used in resistance monitoring programs since the late 1980s. This method is still used in the southern United States and is being adopted to monitor for resistance in areas where the development of pyrethroid resistance is a concern. Testing adults for resistance to cypermethrin allows for the comparison of results among multiple states by using this method in their monitoring programs.

The findings of the larval and adult assays in this study are in general agreement with larval and AVT assays conducted in other midwestern states during the past few years. Larval toxicity trials indicated that increased levels of resistance to cypermethrin and lambda-cyhalothrin were present in Minnesota, Wisconsin, Louisiana, South Carolina, and Ontario, Canada, populations (Hutchison et al. 2007). Resistance also was detected in adults reared from field-collected larvae in Minnesota and Wisconsin in 2005 (Hutchison et al. 2007). In Minnesota, 48–66% of the adults survived a 5-μg dose and 43–45% of adults from Wisconsin survived, but this may be due to the larvae being collected from fields that had been treated with pyrethroids (Hutchison et al. 2007). Survivorship of up to 33% at the 5-μg dose of cypermethrin also was observed in field-collected adults from northeastern states (Fleischer et al. 2007). The H. zea infestations in northern states such as Wisconsin and Minnesota are composed entirely of migrants. In any given year, the response to pyrethroids probably reflects selection pressure in southern states (Pietrantonio et al. 2007) as well as additional selection pressure in the Midwest after moth migration and oviposition in northern crops (Hutchison et al. 2007).

The impact that migrating populations are having on the development of resistance in areas where overwintering populations exist is more challenging to address. If the local overwintering population is large enough, it is possible that this population may help to dilute resistance alleles that migrating populations introduce to the area. However, if the local population is also exposed to significant pyrethroid pressure each year, resistance would be enhanced. The role of the overwintering population in the development of resistance, however, cannot be conclusively determined because the size of the overwintering population relative to the size of the migrating population is unknown. If the impact of pyrethroid resistant migrants over an area is large, then the management practices of the producers in the area receiving migrants is less important than the management practices of producers in source areas.

In our study, the number of overwintering insects in Lafayette, IN, was shown to be small, whereas Vincennes, IN, had a substantial number overwintering H. zea. The location at Collinsville, IL, because of local moderating climatic conditions, is thought to have an even larger number of overwintering H. zea (Jacobson 2008). If these assumptions are true, it would explain why Collinsville had the lowest levels of resistance, possibly because of the dilution effect of overwintering individuals. This hypothesis is not supported as well by the comparison of the resistance levels of the two Indiana sites.

The number of overwintering and migrating H. zea inhabiting an area, however, is only one aspect of a dynamic life cycle in which many characteristics may contribute to the development of resistance. The variety of host plants used by H. zea throughout the year, how much movement occurs within the population, where the movements occur, the level of selection pressure in source and sink areas for migrants, the dominant or recessive nature of resistance, and fitness costs associated with resistance mechanisms also need to be considered when analyzing resistance (Kennedy and Storer 2000).

Currently, pyrethroids are still the most effective insecticides available for use in H. zea management programs and thus are still recommended to producers. Resistance problems, however, are possible and fields should continue to be monitored for control failures. Registration of several new insecticides with novel modes of action targeting ryanodine receptors is underway, and their products should be available to producers by the 2009–2010 growing season. Chemical rotation between products with different modes of action is a strategy that can delay the development of resistance. The effectiveness of this strategy with highly mobile pests, however, is dependent upon resistance management in locations with the greatest potential for serving as source areas for migrants. Management practices in areas that primarily receive migrants would only have an effect if there were reverse migration late in the season. There is indirect evidence of reverse migration in H. zea, but the extent to which this occurs is not known (Pair et al. 1987, Beerwinkle et al. 1994).

In conclusion, resistance to pyrethroids in H. zea was found in the Indiana and Illinois populations tested during this study. These results support other research findings in the upper Midwest, north central, and northeastern states where migrating insects make up most or all of the H. zea populations. The continuation of resistance monitoring programs is critical for detecting management problems for vegetable producers in this region, and future research should be conducted to better understand the relative impacts of local movement and migration on resistance development in H. zea.

Acknowledgments

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