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## Evidence for Decreasing *Helicoverpa zea* Susceptibility to Pyrethroid Insecticides in the Midwestern United States

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### Abstract

The corn earworm (CEW), *Helicoverpa zea* (Boddie), is a highly mobile pest of numerous field and vegetable crops in much of North America. In the Midwestern United States, CEW is a voracious pest of several high-value vegetable crops, including sweet corn, tomatoes, and snap beans, and seed corn grown for the field corn industry. Historically, synthetic pyrethroid insecticides have been cost-effective and have provided excellent control of CEW, with control of larvae in sweet corn exceeding 90%. However, since 2000 pyrethroids have only provided 35 to 45% control in field tests conducted in the Midwest, as indicated by small-plot sweet corn efficacy trials. During 2005, the four most commonly used pyrethroids averaged only 19.3 to 37.3% control of CEW (mean of 5 trials in Minnesota, Wisconsin, Illinois, and Indiana). In addition, the survival of moths exposed to the standard pyrethroid, cypermethrin, using the Adult Vial Test (AVT), indicated high survival rates (44 to 66% at 5 µg cypermethrin and up to 45% survival at 10 µg). These levels are similar to, or higher than recent AVT results from Louisiana and Texas. These states reflect two possible "source" regions of late-season CEW that likely migrate north to the Midwestern states each summer. These results, including the stability of resistance each year in the Midwest, are discussed within the context of developing and expanding a North American resistance monitoring and management network.

### Introduction

In the Midwestern United States, the corn earworm (CEW), *Helicoverpa zea* (Boddie), continues to be one of the most damaging and economically important pests of several vegetable crops, including sweet corn, snap beans, tomatoes and peppers (10,11). The on-farm value of the horticultural crops in the Midwest region was estimated at \$400 million in 2004 (21). In addition, seed corn produced in the Midwest is also valuable and vulnerable to late-season CEW infestations.

Corn earworm infestations in the Midwest can develop quickly as moths are highly mobile (13,16,22), and females are capable of ovipositing 2,000 or more eggs over a 12-night period (11). After eggs hatch, larvae often feed directly on valuable fruit (10). In corn, most eggs are laid on fresh silk tissue of developing ears (9). After hatch, larvae move relatively quickly down the silk channel and begin feeding on kernels within the ear tip. After becoming established in the ear, larvae are protected by husk tissue, and it is virtually impossible to control

infestations with insecticides. Thus, CEW control must occur during the critical "treatment window" of the active silking period, when CEW moths are laying eggs and young larvae are hatching. Integrated pest management (IPM) programs have therefore focused on timely CEW monitoring systems, which primarily rely on the use of pheromone trap networks (8,12) and the judicious use of insecticides, primarily pyrethroids, applied two to five times from first to late silk (9). Compared to conventional "calendar-based sprays," much of the savings in insecticide use have occurred early- to mid-season, before CEW infestations occur or are at very low levels (e.g., Fig. 1).

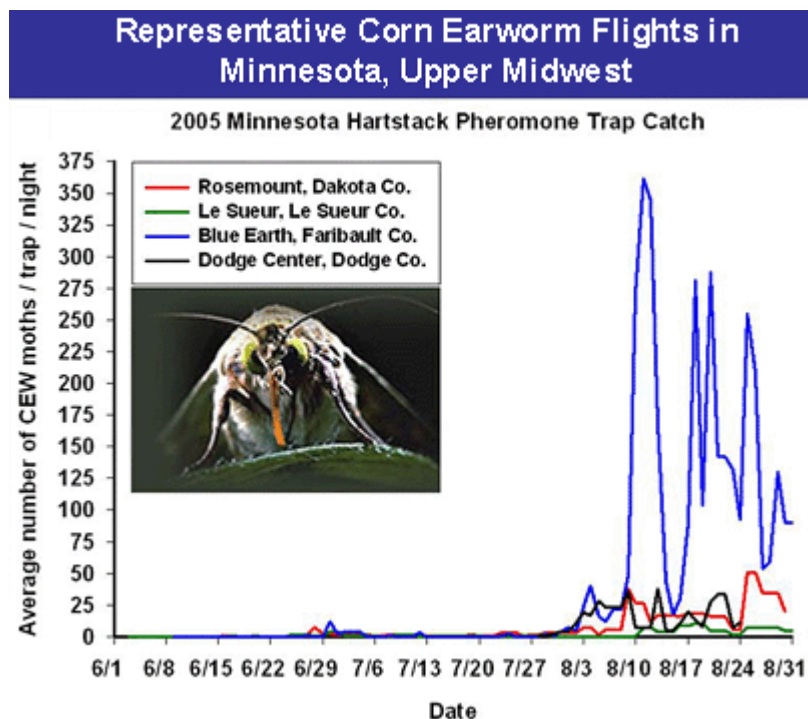


Fig. 1. Typical late-season flight pattern for *H. zea* moths for selected southern Minnesota sites, 2005. Data collected using wire-mesh pheromone traps and zea-lure pheromone lures (photograph of *H. zea* adult from USDA-ARS).

Historically, the synthetic pyrethroids have provided excellent control of CEW in sweet corn and vegetable crops (2,10,11). However, since 2000, several researchers have observed reduced or inconsistent efficacy of pyrethroids in field trials (5). As illustrated in Fig. 2, there is also evidence of increasing tolerance to cypermethrin, a standard pyrethroid used for resistance monitoring purposes, in the southern US (7,14,17,19,20). Because CEW is not known to successfully overwinter at latitudes north of the 40th parallel, we believe that most of the CEW populations, particularly in the upper Midwestern regions of Minnesota, Wisconsin, and Michigan, likely result from migrating moths that have originated in the southern US (13,23). As shown in Fig. 1, most moth flights into Minnesota do not occur until late July or early August. Given the continued exposure of CEW to pyrethroids in the southern US in several crops (3,7,17,20), it is not surprising that moths immigrating into the northern US each year may carry genes for pyrethroid resistance. The purpose of this paper is to review the results of field efficacy trials for pyrethroid insecticides used against CEW in the Midwest, and the results of adult and larval assays to assess CEW resistance to selected pyrethroids, from 2003 to 2005.

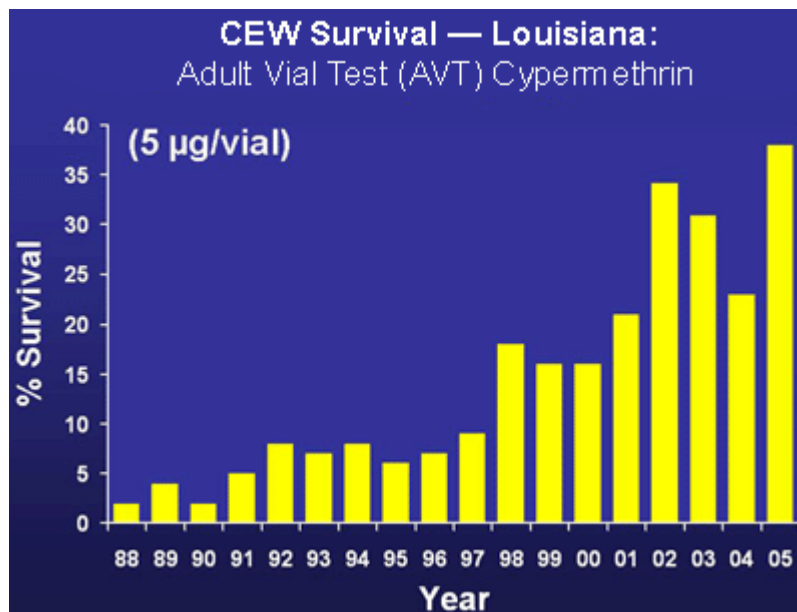


Fig. 2. Increasing survival of *H. zea* to cypermethrin, from 1988-2005 in Louisiana, based on results of adult vial tests (AVT) with cypermethrin (5 µg/vial).

### Pyrethroid Field Trials

Small-plot trials to evaluate the efficacy of selected insecticides against CEW in sweet corn have been conducted for several years in Midwest states using standard protocols, including four replications within a randomized complete block design (5,6). In these tests, sweet corn hybrids were planted relatively late in the season (mid- to late June), so that hybrids were reaching peak silk production in late July to mid-August, at the time of peak CEW flights (Fig. 1). Beginning at row tassel or early silk, insecticide treatments are applied, and continued every 5 to 7 days, typically for a total of 4 sprays. Under high CEW pressure, the spray interval may be shortened and more sprays will be applied. Applications are terminated as the ears approach the brown-silk stage. Typically, 25 to 30 primary ears per plot, are selected at random and examined for total number of CEW larvae per ear, kernel damage, and the number of small (instars 1 to 2), medium (instars 3 to 4) and large (instars 5 to 6) larvae per ear. In recent years, similar sweet corn hybrids (e.g., 'Jubilee' or 'Supersweet Jubilee,' Rogers Seeds/Syngenta, Nampa, ID), have been used to add uniformity to the multi-state trials. In many states, the same rates of selected pyrethroid insecticides have also been used to facilitate direct comparisons of results. Pyrethroid insecticides used in the Minnesota and Wisconsin studies, from 1996 to 2005, included two of the most commonly used products and rates: bifenthrin (Capture 2EC) applied at 0.04 lb ai/acre (2.6 fl oz/acre) and lambda-cyhalothrin (Warrior 1CS) applied at 0.025 lb ai/acre (3.2 fl oz/acre). Additional pyrethroids were evaluated in a multi-state trial in 2005, including: zeta-cypermethrin (Mustang Max 0.8EC, FMC Corp., Philadelphia, PA) applied at 0.021 to 0.025 lb ai/acre (3.4 to 4 oz/acre), and cyfluthrin (Baythroid 2EC, Bayer Corp., Pittsburgh, PA), applied at 0.025 to 0.044 lb ai/acre (1.6 to 2.8 oz/acre). In Minnesota, a back-pack sprayer (2 XRTJ 8002 nozzles) was used to deliver an average of 25 gal of water per acre to each 2-row plot; in Wisconsin, a high-clearance sprayer (9 XRTJ 80015 nozzles) was used to deliver 12.6 gal of water per acre to each 6-row plot. In 2005, an additional location at Le Sueur, MN was added (back-pack sprayer; 4 DGTJ-60 11002VS nozzles; 15 gal of water per acre; 2 rows), as well as one trial each from Illinois (high-clearance sprayer; 6 TXVS6 nozzles; 30 gal of water per acre; 4 rows), and Indiana (high-clearance sprayer; 12 TXTJ VS12 nozzles; 24.2 gal of water per acre; 4 rows).

Results of the 2003 pyrethroid efficacy studies, in Minnesota and Wisconsin (Table 1) confirmed earlier reports of reduced control of CEW, with mean control averaging only 45.5 and 33.5%, for bifenthrin and lambda-cyhalothrin, respectively. Similar protocols have been used in both states since 1996.

Historical data from these trials indicate that the decline in efficacy was most noticeable beginning in 2000 (Table 2), when mean control declined to 49% for bifenthrin and lambda-cyhalothrin combined. Most recently, during 2005, the efficacy of four commonly used pyrethroids, evaluated in Minnesota, Wisconsin, Illinois, and Indiana continued to provide poor efficacy, ranging from 19 to 37% control (Table 3). These results were surprising given the high levels of control observed during the 1990s (2,11).

Table 1. Efficacy of two standard pyrethroids in replicated, small-plot sweet corn trials in Minnesota and Wisconsin, 2003.

Treatment	Rate lb ai/acre (Prod./acre)	MN CEW/ear (% control)	WI CEW/ear (% control)	Mean control (%)
Bifenthrin	0.04 (2.6oz)	0.29 (68)	0.51 (23)	45.5
Lambda-cyhalothrin	0.025 (3.2oz)	0.72 (22)	0.36 (45)	33.5
Untreated check	—	0.92 (—)	0.66 (—)	—

MN: Planting date = 6/23/03; Variety = Jubilee; spray dates = 8/15, 8/21, 8/26, 9/2

WI: Planting date = 6/19/03; Variety = Super Sweet Jubilee Plus; spray dates = 8/13, 8/18, 8/25, 8/31

Based on standard sweet corn replicated RCBD protocol (5).

Table 2. Decreasing efficacy of bifenthrin and lambda-cyhalothrin estimated from replicated, small-plot sweet corn trials, in Minnesota and Wisconsin, 1996-2005.

Year	Mean % control				Mean
	Minnesota		Wisconsin		
	Bifenthrin	Lambda-cyhalothrin	Bifenthrin	Lambda-cyhalothrin	
1996	—	—	93	90	92
1997	88	98	—	—	93
1998	86	90	92	83	88
1999	—	—	83	78	81
2000	75	64	22	35	49
2001	18	31	17	0	17
2002	19	56	48	42	41
2003	68	22	23	45	40
2004	—	72	67	66	68
2005	79	71	8	0	40

Insecticides tested were used at standard rates of 0.04 and 0.025 lb ai/acre for bifenthrin and lambda-cyhalothrin, respectively, following standard randomized complete block design (RCBD); all instars combined for each trial; data based on mean number of larvae per ear, with average sample size of 100 ears/treatment per trial (25 ears/replication) as per conventional sweet corn protocol (5).

Table 3. Efficacy of pyrethroids from 2005 Midwest Multi-state *H. zea* pyrethroid efficacy trials.<sup>u</sup>

Insecticide	Rate		Percent control					Overall efficacy by product (x± SD; range)	n
	P/ acre	ai/ acre	MN / U of MN <sup>v</sup>	MN / Green Giant <sup>w</sup>	WI / U of WI <sup>x</sup>	IN / Purdue <sup>y</sup>	IL U of IL <sup>z</sup>		
Lambda-cyhalothrin	2.56-3.2 oz	0.02-0.025	71.4	15.4	0.0	9.9	0.0	19.3 ± 29.8 (0-71.4)	6
Bifenthrin	2.1-2.56 oz	0.033-0.04	78.6	36.7	7.7	26.2	—	37.3 ± 30.0 (7.7-78.6)	4
Zeta-cypermethrin	3.4-4 oz	0.021-0.025	75.0	64.1	0.0	9.2	18.8	33.4 ± 33.9 (0-75.0)	5
Cyfluthrin	1.6-2.8 oz	0.025-0.044	—	53.8	—	5.7	0.0	19.8 ± 29.6 (0-53.8)	3
Untreated check - CEW/ear	—	—	0.28	0.39	0.13	1.41	0.16	—	—

<sup>u</sup> Standard replicated sweet corn trials with randomized complete block design (4 replications) [e.g., (5)].

<sup>v</sup> University of Minnesota, W. Hutchison. Percent control is for all CEW instars; approximately 90% of larvae were late instar.

<sup>w</sup> General Mills/Green Giant, T. Rabaey. Percent control is for 3-6th instars.

<sup>x</sup> University of Wisconsin, B. Jensen. Percent control is for all CEW instars; approximately 75% of larvae were late instars.

<sup>y</sup> Purdue University, R. Foster. Percent control is for all CEW instars; approximately 80 to 90% of larvae were late instars.

<sup>z</sup> University of Illinois, R. Weinzierl. Percent control is for all CEW instars; majority was late instars (20).

### Adult Vial Tests (AVT) with Cypermethrin

To assess the level of resistance in CEW adults to pyrethroids, the standard Adult Vial Test (AVT), developed in the 1980s (7,14) was used to assess the level of susceptibility in CEW adults collected in Illinois, Minnesota, and Wisconsin from 2003 to 2005. This procedure has been used extensively in the southern US to monitor susceptibility of CEW and tobacco budworm, *Heliothis virescens* (F.), to pyrethroids (7,17,19,20). However, rather than collecting adults directly from pheromone traps, as in the southern US (20), we reared adults from field collected larvae, typically taken from small-plot insecticide trials (non-treated plots, as well as pyrethroid treated plots), or commercial sweet corn fields (previously treated with pyrethroids). Corn earworm larvae, usually 4th to 6th instars, were collected from late-season sweet corn in Minnesota, Illinois, and Wisconsin, during late August to early September from 2003 to 2005. Larvae were placed individually in 30-ml plastic diet cups containing artificial diet or fresh silk tissue for overnight shipment to Louisiana State University (LSU) for processing. At LSU, larvae were transferred to artificial diet, and colonies were established from each geographic collection. Either F<sub>1</sub> or F<sub>2</sub> adult moths were subsequently tested using the AVT, with either 5 or 10 µg of cypermethrin. Technical grade cypermethrin (FMC Corp.) was diluted in acetone and applied to 20-ml scintillation vials as per previous protocols (20). Although 5 µg of cypermethrin was initially selected (mid-1980s) as a discriminating dose that should kill 99 to 100% of a susceptible population, the 10 µg concentration has also been used since the 1990s, due to increasing survival in CEW collected in Louisiana (20). Thus, both concentrations were used for most of the Midwest locations. During 2003, 30 to 35 moths were assayed for each of the Illinois and Minnesota colonies at the 5 µg concentration. In 2004, sample sizes for both the 5 and 10 µg concentrations were: 30 for Illinois, 29 for Minnesota, and 20 for Wisconsin. During 2005, sample sizes for the 5 µg concentration were: 21 for Illinois, 129 for Minnesota, and 56 for Wisconsin. For the 10 µg concentration, sample sizes were: 20 for Illinois, 91 for Minnesota, and 47 for Wisconsin.

Survival of moths from selected Midwestern states during 2003 to 2005 using 5 and 10  $\mu\text{g}$  of cypermethrin in the AVT (Figs. 3 and 4) was generally higher than previous results observed in Louisiana (20), or in the Northeast (8), where the traditional method of collecting adults from pheromone traps is used. However, as noted by Temple et al. (20) and illustrated in Fig. 2, the Louisiana statewide average survival of CEW adults exposed to 5  $\mu\text{g}$  of cypermethrin, has been steadily increasing since the early 1990s, with the highest state average of 38% survival reported during 2005. Moreover, it is interesting that the significant increases in percentage survival occurred in 1990 and again in 2000 (Fig. 2). In each case, the statewide resistance levels remained relatively stable after each "stair-step" increase in survival. Our data for the Midwest states, to date, suggests that the resistance has been gradually increasing, or at least has been relatively stable from 2003 to 2005. The AVT results for Minnesota and Wisconsin at 10  $\mu\text{g}$  of cypermethrin, at 43 and 45% survival, respectively, are particularly concerning. Historically, > 25% survival at the 10  $\mu\text{g}$  concentration has been cause for concern at the field level, in Louisiana (20), and provides some explanation for the possible field control problems noted in the Midwest (see discussion below).

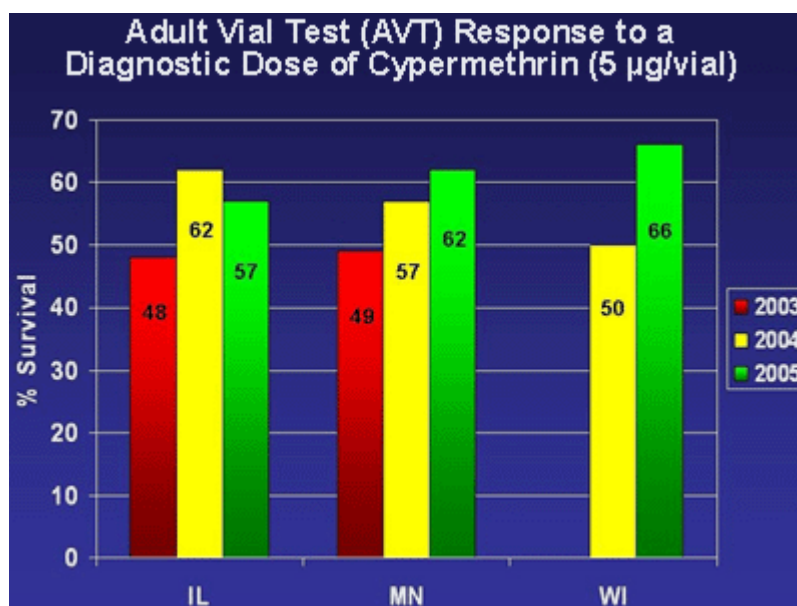


Fig. 3. Percentage survival of *H. zea* adults for Illinois, Minnesota, and Wisconsin from 2003-2005, based on adult vial tests (AVT) with cypermethrin (5  $\mu\text{g}/\text{vial}$ ).

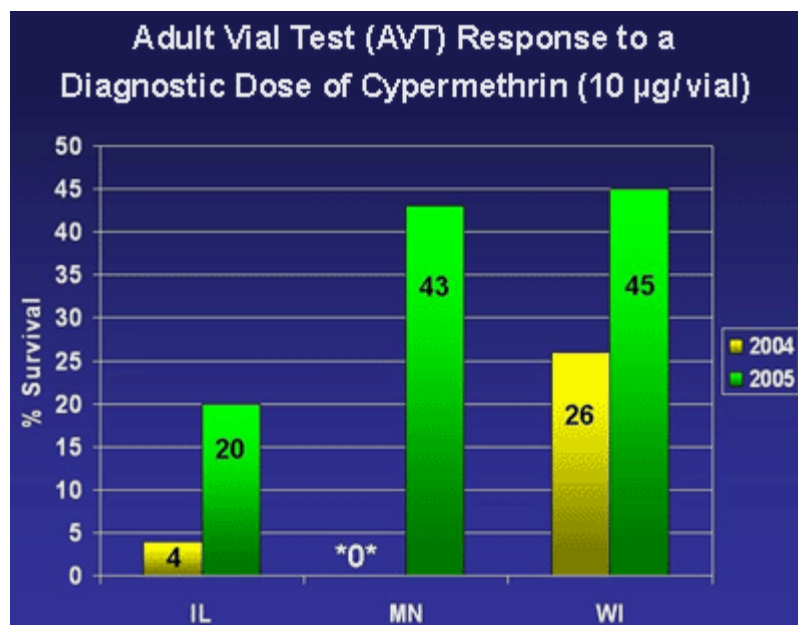


Fig. 4. Percentage survival of *H. zea* adults for Illinois, Minnesota, and Wisconsin from 2003-2005, based on adult vial tests (AVT) with cypermethrin (10 µg/vial).

#### Larval Assays with Cypermethrin and Lambda-cyhalothrin

As with the AVTs, well established protocols were also used for the larval assays at LSU (7,20). Following the establishment of Midwestern colonies at LSU, 3rd instars, ranging from 25 to 30 mg, were selected for assays, with appropriate dose ranges for cypermethrin and lambda-cyhalothrin necessary to estimate LD<sub>50</sub>'s. The LD<sub>50</sub>'s for each location were compared by evaluating overlap of the 95% confidence intervals (7).

Larval assays for cypermethrin indicated low to moderate resistance with some resistance ratios (RR) exceeding five fold, depending on the susceptible strain used for comparison (Table 4). However, larval assays with lambda-cyhalothrin (Table 5) suggest a greater diversity in the mortality response among populations, compared with cypermethrin (Table 4). The highest resistance ratio (RR = 39) was observed for the Wisconsin colony derived from a 2004 field collection. The 2004 Wisconsin colony also exhibited high adult survival (45% at 10 µg concentration of cypermethrin) in the AVT (Fig. 4). The elevated resistance ratios for lambda-cyhalothrin may be due to the continued widespread use of this pyrethroid on several crops throughout the US, or the possibility of different resistance mechanisms for lambda-cyhalothrin versus cypermethrin (15). Brown et al. (3,4) reported high levels of CEW resistance to lambda-cyhalothrin in both larvae and adults in South Carolina. For adults, 2.5 µg of lambda-cyhalothrin killed 100% of a susceptible CEW colony, but only 6.6% of the field-collected resistant colony. A subsequent cross of the susceptible and resistant colonies yielded 50% mortality at 2.5 µg. In addition, 10 and 30 µg was necessary to kill 100% of the hybrid (cross) and resistant colonies, respectively (4).

Table 4. Mortality response ( $LD_{50}$ ) of *H. zea* larvae to cypermethrin following larval collections from sweet corn during August-September in selected Midwestern US and Canadian sweet corn, as well as Louisiana locations, 2004, compared with previous reference colonies, 1993-1998.

Colony	n	LD50	95% CL	Slope	$\chi^2$	RR*
Louisiana-1998	160	0.049	0.022 – 0.066	1.86 ± 0.18	n/a	0-4
Illinois-93	n/a	0.096	0.054 – 0.171	1.16 ± 0.36	n/a	1-7
Arkansas (ARK-93)	n/a	0.159	0.121 – 0.212	2.02 ± 0.30	n/a	2-12
Champaign, Illinois-04	189	0.061	0.027 – 0.114	1.31 ± 0.25	6.59	1-5
Wisconsin-04	180	0.099	0.047 – 0.224	1.63 ± 0.23	17.81	2-8
Minnesota-04	120	0.039	0.021 – 0.062	1.20 ± 0.29	3.35	0-3
Ontario, Canada-04	180	0.020	0.007 – 0.033	1.41 ± 0.25	5.44	0-1
St. Joseph, LA-04	150	0.032	0.015 – 0.052	1.15 ± 0.22	4.93	0-3
Macon Ridge, LA-04	79	0.200	0.142 – 0.315	2.66 ± 0.51	1.16	3-15

\* Resistance Ratio (RR) calculated from  $LD_{50}$  data (0.013 to 0.065  $\mu\text{g}$ ) derived from MO and LA, respectively, 1988.

Table 5. Mortality response ( $LD_{50}$ ) of *H. zea* larvae to  $\lambda$ -cyhalothrin following larval collections from sweet corn during August-September 2004, compared with early (May) and late (August) collections in Louisiana, and a known pyrethroid-resistant colony from South Carolina (Estill-99).

Colony	n	LD50	95% CL	Slope	$\chi^2$	RR*
Champaign, IL	208	0.022	0.014 – 0.031	1.49 ± 0.22	2.22	11
Arlington, WI	150	0.078	0.059 – 0.103	2.18 ± 0.31	3.40	39
Ontario, Canada	210	0.018	0.009 – 0.026	1.29 ± 0.22	5.44	9
LA-Early-Macon Ridge	140	0.016	0.006 – 0.024	1.65 ± 0.34	6.03	8
LA-Late-St. Joseph	120	0.035	0.024 – 0.047	2.43 ± 0.43	4.13	17.5
SC-ESTILL-99	100	0.076	0.043 – 0.151	2.0 ± 0.46	n/a	38

\* Resistance Ratio (RR) calculated from  $LD_{50}$  data (0.002  $\mu\text{g}/\text{larva}$ ) derived from the Clemson, SC lab, based on field-collected *H. zea* larvae in S. Carolina cotton, as of 1999 (3).

### Implications for *H. zea* Resistance Monitoring and Management

The decline in susceptibility of CEW to synthetic pyrethroids, including cypermethrin and lambda-cyhalothrin are not surprising given the continued and long-term exposure of CEW to these insecticides throughout many cropping systems in the southern (7,17,20) and northern US (22). Prior to our findings, a CEW population also collected from sweet corn in Illinois during 1991, was found to be resistant to another pyrethroid, permethrin (1). Similar levels of permethrin resistance (five fold in larvae) were observed in the South Carolina strain, Estill96 (3,4), that also had high levels of resistance to lambda-cyhalothrin. As CEW is not known to overwinter at latitudes > 40° (9), the annual late-season migrations into the Midwest are assumed to originate from the southern US (13,23), with source populations also possibly originating in northern Mexico (17,18). Thus, the gradual increase in tolerance to cypermethrin from 1998 to 2005 in Louisiana (Fig. 2), as well as the recent trends for resistance in Texas (17), provide a few examples of resistance development in two states that are believed to harbor "source" populations of CEW that subsequently migrate north.

The field data reported here are limited to replicated, small-plot pyrethroid efficacy trials (Tables 1, 2, and 3). Although small-plot trials (treated by ground application) are not equivalent to large-scale commercial fields (treated aerially), the consistently low level of control observed in small plots is cause for concern about the potential for continued pyrethroid resistance development in

the Midwest. This is of particular concern to the smaller, fresh-market growers who rely on ground applications of insecticides. In small plot trials, insecticide activity is measured primarily against CEW eggs and larvae, and does not include the additional mortality effect on adults that occurs in commercial fields, where insecticides are applied aerially (2). However, as demonstrated by our AVT results from 2003 to 2005 (Figs. 3 and 4), adult resistance to cypermethrin also occurs and processors may not be able to rely on adult mortality to reduce infestations.

Survival of adults from the Midwest in the AVT (Figs. 3 and 4) may have reached higher levels since the moths tested were reared from field-collected larvae. In most cases, for the Midwest locations, larvae were collected from plots or commercial fields that had received one or more pyrethroid applications (usually bifenthrin or lambda-cyhalothrin). Thus, the field-collected larvae represent survivors of additional local selection pressure. Adults emerging from these colonies would be expected to exhibit higher levels of resistance versus moths collected directly from pheromone traps, where the source of moths is unknown. Fleischer et al. (8) recently found in the Northeast that AVT survival was also higher at 5 µg cypermethrin, for moths developing from field collected larvae versus moths taken directly from pheromone traps. Regardless of potential differences in these two AVT assays, the pattern of resistance, as indicated by consistently high survival rates in the Midwest, is indicative of the field control problems observed in the small-plot trials (Table 3). In large commercial sweet corn fields, poor control was also reported in Ontario, Canada in 2003 and in a replicated aerial trial in 2006 in southern Minnesota, where only 50% control with bifenthrin (Discipline, Amvac Corp., Commerce, CA) was observed (T. L. Rabaey, *unpublished data*). Given all the results to date, including the decline in field performance as well as AVT and larval assays, the level of resistance can be described as moderate. Regardless of how the resistance level is characterized, efficacy in standard, replicated small plot trials has declined since 2000 and reports of control problems in sweet corn fields, including the smaller fresh-market as well as larger processing fields have also increased since 2003 (W. D. Hutchison, *unpublished data*).

As noted by Weinzierl (22), there are currently very few alternatives to the pyrethroids for consistent CEW control for the variety of Midwestern crops affected. The most promising alternative includes transgenic sweet corn, engineered to express the Cry1Ab toxin from the bacterium, *Bacillus thuringiensis* (6); however, this is currently limited to fresh-market growers. Pyrethroid resistance in CEW is also known to be inherited as incomplete dominance (2,3), and several mechanisms for resistance are likely operative in US populations, including enhanced metabolism of pyrethroids (metabolic resistance) via P450 enzymes or target-site insensitivity (15). Finally, with high value horticultural crops, the tolerance for CEW damage and/or contaminates in final product is extremely low. Assuming that pyrethroid selection pressure in CEW continues in multiple crops in the southern US (20), genes for pyrethroid resistance continue to be conserved in southern overwintering populations (19), selection continues in multiple life stages (adults and larvae), and incomplete dominance (versus a recessive trait), the prospects for successful management of CEW in the northern US is not promising.

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