The Texas Journal of Agriculture and Natural Resource 25:1-12 (2012) 1

Susceptibility of *Helicoverpa zea* to Commercial Insecticides Used in Green Bean Production on Texas High Plains[1]

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ABSTRACT

Susceptibility levels of adult *Helicoverpa zea* (Boddie), collected from green bean growing areas of Texas and New Mexico using sex-pheromone baited traps, were tested against some of the most common commercial insecticides that are extensively used in green bean production. Insecticide vial bioassays indicated variation in the levels of tolerance over the season with highest LC_{50} values during mid-season in 2002 and 2003. Studies included three active ingredients from two insecticide classes. Zeta-cypermethrin, bifenthrin, and methomyl were tested at nine different concentrations. Data from 2002 showed a statistically significant progressive decrease in susceptibility levels between the generations that were tested with bifenthrin and methomyl. In 2003, these variations in susceptibility levels were different though not significant. Differences in bifenthrin susceptibility were substantial between the locations in the middle of the 2003 season for third generation moths. Increased insecticide use in 2002 compared to 2003 might have accounted for higher tolerance levels in 2002.

KEY WORDS: *Helicoverpa zea*, corn, green beans, insecticide resistance

INTRODUCTION

Green beans, *(Phaseolus vulgaris* L.), also referred to as common, snap, wax, and field beans, are grown throughout the world for consumption as immature pods. In 2002, the United States alone had a market value for green beans of $127 million, with

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[1]Acknowledgements: We thank Dr. Buddy Laing for providing equipment and office space. Also, we are very thankful to Doug Dillon (Allen Canning Company) for providing previous efficacy and other empirical data.

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100,000 acres producing 6 million tons for the fresh market, while an additional 210,100 acres produced 831,260 tons for the processing market (USDA-NASS 2003). Texas ranked 14th in both fresh and processing markets in 2002. Its green bean production contributes around $429 million to the United States economy ($391 million and $38 million from fresh and processing market, respectively).

Helicoverpa zea (Boddie), referred to as cotton bollworm, corn earworm (CEW), or soybean pod borer, is considered an important pest of green beans because of its extensive tunneling of the pods, which leads to rejection at the processing plant. Insecticidal control is the primary pest management strategy for this pest in almost all cases. Bollworm has developed resistance to many insecticides (Winteringham and Hewlett, 1964), including organophosphates (methyl parathion in cotton) and organochlorines (Wolfenbarger 1971; Sparks 1981). Resistance is considered a key factor for the pest status of bollworm (Sparks 1981; Sparks et al., 1993). Tolerance to pyrethroids such as cypermethrin, permethrin, and methomyl were documented in *Helicoverpa zea* (Stadelbacher et al., 1990; Hsu and Yu 1991). Also, in South Carolina, widespread application of pyrethroids resulted in a drastic decline in efficacy against corn earworm (Brown et al., 1997). The excess use of insecticides, especially synthetic pyrethroids, might have triggered the development of higher tolerance in bollworm observed in Texas in 1986 (Kanga and Plapp 1996). Sparks (1981) reported that synthetic pyrethroids were effective against Texas bollworms. However, recent statewide bioassays have shown marked increase in tolerance in some areas, and pyrethroids have been abandoned during part of the growing season in some counties. In spite of recent changes in susceptibility to pyrethroids in field populations of *H. zea*, little information is available on possible mechanisms of pyrethroid resistance (Graves et al., 1993; Bagwell et al., 1996; Kanga et al., 1996). Because bollworm infestation in cotton often occurs along with tobacco budworm infestation, higher insecticide doses and more frequent applications used for budworm may cause an increase in the selection pressure on bollworm populations (Kanga et al., 1996). Thus, frequent monitoring of bollworm susceptibility to insecticides used in green bean production, along with efficacy assessment against bollworm populations may help in predicting possible outbreaks of resistance.

Reduced control of the bollworm populations because of the increase in tolerance might be relatively acceptable in cotton or other row crop production. However, green beans produced for the canning industry must be almost *H. zea* free, with an economic threshold of about one larva per 10,000 pods. Therefore, even moderate increase in insecticide tolerance poses a significant threat to green bean growers and the canning industry.

The objectives of this study are: (1) to determine the levels of susceptibility of *H. zea* to different insecticides used in green bean production; (2) to study the year to year variation in susceptibility levels; and (3) to discuss the likely efficacy of pyrethroids against bollworm in green beans as influenced by use of pyrethroids in the local cropping systems on Texas High Plains. Data from this study could be very useful to growers in making decisions based on the historic data on bollworms in the area.

MATERIALS AND METHODS

Studies were conducted in 2002 and 2003 in Parmer County, Texas, located on the Texas-New Mexico border (Figure 1). Most of the green beans grown in this area were under contract with a canning company based in Arkansas.

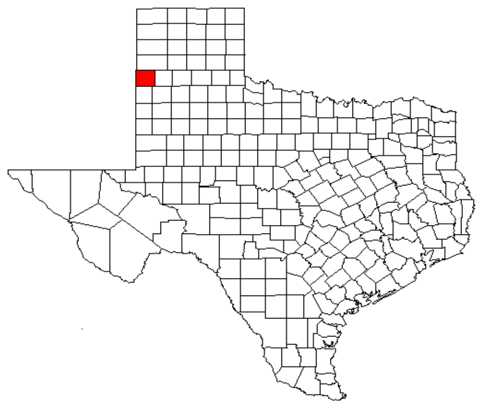

Figure 1. Location of Parmer County in Texas.

Two locations, Lazbuddie and Oklahoma Lane, were selected for the 2002 study. The nearby locations, Lariat and Hub, were selected in 2003 as crop sites used in the previous year were subjected to severe hail damage. All the study sites selected were within a 30-mile area (Figure 2).

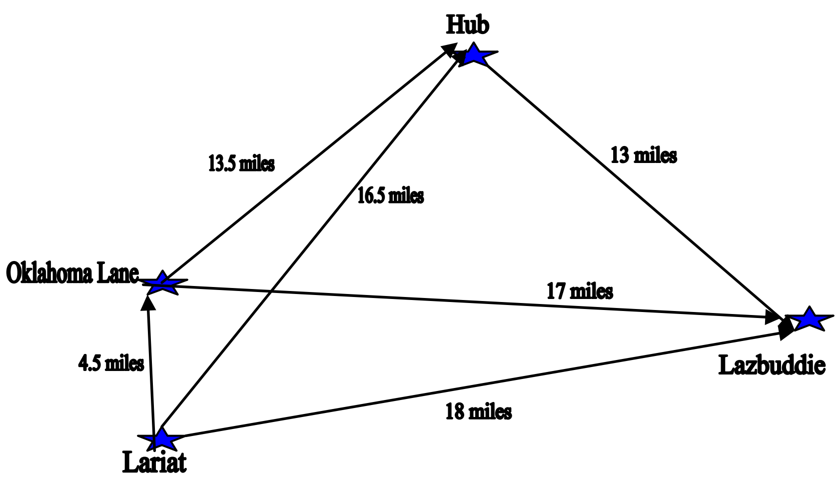

Figure 2. Relative distance between trap locations in 2002-2003, Parmer County, Texas.

Insecticides and insect treatment. Technical grade (>90% purity) bifenthrin, zeta-cypermethrin, and methomyl were used in the study. Vials with 9 different doses of each insecticide were prepared at New Mexico State University in Las Cruces and transferred to the test area. Concentrations (μg/vial) included for bifenthrin and zeta-cypermethrin were the same (0, 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.2, and 12.8) while methomyl differed from the other two in lacking concentration of 0.05 μg/vial and having an extra concentration of 25 μg/vial. The treatments in adult vial bioassay were conducted using 20 ml wide-mouth glass scintillation vials coated on the inside with insecticides (Kanga and Plapp 1992). A 0.5 m1 stock solution was added to the vials and the vials were rotated on a rack to get a uniform coating on the inner surface until they dried. Adult moths were collected using sex-pheromone baited Hartstack wire mesh traps (Hartstack et al., 1979), which were placed near cornfields surrounding green beans growing area. Fresh moths collected from the traps in the morning hours were treated by placing two moths per vial. Only healthy looking, non-rubbed adults of similar body size were included in the test (Figure 3). The vials containing the moths were then kept in a cooler with a small amount of ice to prevent excessively high temperatures.

Figure 3. Insecticide-treated vials representing different concentrations.

To avoid any influence of low temperatures on the efficacy of the insecticides, care was taken to avoid direct contact between the vials and ice; using Styrofoam and paper towels. Vials were transported as quickly as possible to the laboratory where they were held at room temperature until mortality data was obtained at 8 h post-exposure. Mortality was assessed by prodding the thoracic region with a pencil to check for survival.

Data analysis. Mortality rates were calculated by applying Abbott's (1925) formula. Data obtained from different generations were pooled and analyzed by means of probit analysis (Russell et al., 1977) using POLO-PLUS (LeOra Software 2003). The differences among the populations, in responses to insecticides, were considered not significant if the 95% confidence intervals of LC_{50} or LC_{90} overlapped (Robertson and Preisler 1992).

RESULTS

Insecticidal bioassay study showed variation in the levels of susceptibility. A significant decrease in susceptibility to both bifenthrin and methomyl was observed between the generations during the year 2002. However, populations from different generations in the year 2003 showed an increase in susceptibility levels.

Bifenthrin in Year 2002. The adult moths showed a significant decrease in susceptibility to bifenthrin as the 2002 growing season progressed (Table 1). LC_{50} values at Lazbuddie were 0.22 µg/vial in the June flight and 2.05 µg/vial in the August/September flight.

Table 1. Toxicity of bifenthrin to corn earworm moths in a bioassay conducted at Lazbuddie and Oklahoma Lane, Texas in 2002.

Generation	n[a]	Slope ± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lazbuddie		
June	280	1.79 ± 0.25	0.22 (0.15-0.30)	1.16 (0.79-2.08)	3.97
Aug/Sept	314	1.97 ± 0.20	2.05 (1.09-4.79)	9.2 (4.16-85.04)	33.47*
			Oklahoma Lane		
June	176	1.45 ± 0.27	0.24 (0.004-0.99)	1.86 (0.59-2.53)	11.23
Aug/Sept	380	2.12 ± 0.26	2.51 (1.59-3.75)	10.07 (6.17-25.8)	12.76*

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

Similarly, a significant reduction in susceptibility from 0.24 to 2.51 µg/vial was observed at Oklahoma Lane between the June and August/September flights. The differences in LC_{50} values between the generations at Lazbuddie (χ^2 = 3.97, 33.47, p < 0.05) as well as Oklahoma Lane (χ^2 = 11.23, 12.76; p < 0.05) were statistically significant.

Based on the overlapping confidence limits, the susceptibility level of moths tested at the two locations was not different between the two locations in the study.

Bifenthrin in Year 2003. The populations in 2003 showed a significant increase in susceptibility of the adults (Table 2). LC_{50} values declined significantly between first and the third generation (1.01 to 0.39 µg/vial) (χ^2 = 4.66, 3.95; p < 0.05) for moths collected at Lariat.

However, LC_{90} values from June-August (4.33 to 2.05 µg/vial) were not significantly different. Adult populations at Hub showed no significant variation in either LC_{50} or LC_{90} levels (χ^2 = 0.92, 0.97; p > 0.05). Comparison of the populations over the generations between the locations showed a significant difference in both LC_{50} and LC_{90} (non-overlapping of CI limits) values for third generation adults.

Methomyl in Year 2002. Adults tested for Methomyl showed a decreasing trend in susceptibility levels (Table 3). LC_{90} values increased between the generations and were also significantly different between Lazbuddie and Oklahoma Lane. LC_{50} values varied from 0.91-4.02 µg/vial and 1.46-5.38 µg/vial respectively (χ^2 = 6.68, 11.94 and 4.22, 2.28; $p < 0.05$). LC_{90} values followed similar increase (2.35-17.72 and 7.68-27.84 µg/vial respectively).

Comparison of the susceptibility levels at the two locations showed the LC_{50} values did not differ (overlapping CI limits). A significant difference was observed in the LC_{90} values just in first generation flights. The adult moths were found to be less susceptible in the latter half of the season.

Table 2. Toxicity of bifenthrin to corn earworm moths in a bioassay conducted at Lariat and Hub, Texas in 2003.

Generation	n[a]	Slope± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lariat		
June	320	1.79 ± 0.25	1.01 (0.73-1.30)	4.33 (3.19-6.67)	4.66*
July	320	1.97 ± 0.20	0.46 (0.09-0.95)	2.63 (1.29-11.52)	10.25
Aug	320	1.79 ± 0.24	0.39 (0.25-0.54)	2.05 (1.47-3.33)	3.95*
			Hub		
June	320	1.97 ± 0.22	0.92 (0.73-1.15)	4.15 (3.01-6.58)	3.06
July	320	2.19 ± 0.33	0.63 (0.40-0.88)	2.44 (1.77-3.82)	2.24
Aug	320	1.75 ± 0.21	0.97 (0.66-1.34)	5.23 (3.64-8.65)	3.98

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

Methomyl in Year 2003. Moths collected at Lariat and Hub showed no difference in susceptibility to methomyl among the collections in the June, July, and August (LC_{50} of 2.15-1.82 and 3.09-1.57 µg/vial) (Table 4).

All three generations were not significantly different in levels between generations as well as locations except that the second generation moths varied in susceptibility between locations (6.62 and 9.65 µg/vial; χ^2 = 4.26 & 6.37). Adults tested in 2003 adults were more susceptible as the season progressed.

Zeta-Cypermethrin in Year 2003. Moths tested for Zeta-cypermethrin in 2003 showed no significant variation in the susceptibility levels. LC_{50} and LC_{90} values were not different between generations or between locations tested. Overlapping CI limits evidenced the non-significance in the variations over the season with LC_{50} varying across the season (Lariat: 2.11-1.03; Hub: 1.93-1.2 µg/vial; p>0.05) between the June and August generations (Table 5).

LC_{90} values at Lariat declined from 6.18 in the first generation to 3.74 µg/vial (non-significant) in third generation. As this insecticide was not tested in the year 2002, the susceptibility levels were not compared between the years.

Table 3. Toxicity of methomyl to corn earworm moths in a bioassay conducted at Lazbuddie and Oklahoma Lane, Texas in 2002.

Generation	n[a]	Slope ± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lazbuddie		
June	320	3.10 ± 0.05	0.91 (0.56-1.25)	2.35 (1.66-4.48)	6.68
Aug/Sept	292	1.99 ± 0.28	4.02 (2.44-10.92)	17.72 (7.7-269.03)	11.94*
			Oklahoma Lane		
June	204	1.78 ± 0.31	1.46 (0.96-2.16)	7.68 (4.6-19.2)	4.22
Aug/Sept	342	1.79 ± 0.42	5.38 (3.79-9.8)	27.84 (13.5-158.8)	2.28

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

Table 4. Toxicity of methomyl to corn earworm moths in a bioassay conducted at Lariat and Hub, Texas in 2003.

Generation	n[a]	Slope ± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lariat		
June	320	1.93 ± 0.27	2.15 (0.71-3.81)	9.86 (5.56-9.98)	6.63
July	320	2.62 ± 0.47	2.15 (0.92-3.37)	6.62 (4.20-6.14)	4.26
Aug	320	2.63 ± 0.36	1.82 (0.91-2.77)	5.6 (3.61-13.60)	9.54
			Hub		
June	320	2.27 ± 0.47	3.09 (1.58-4.41)	11.31 (8.20-19.3)	2.98
July	320	2.36 ± 0.41	2.77 (1.10-4.34)	9.65 (6.20-22.61)	6.37
Aug	320	1.94 ± 0.20	1.57 (0.94-2.46)	7.19 (4.19-20.6)	11.25*

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

Table 5. Toxicity of zeta-cypermethrin to corn earworm moths in a bioassay conducted at Lariat and Hub, Texas in 2003.

Generation	n[a]	Slope ± SE	LC_{50}[b] (95% CI)[c]	LC_{90}[b] (95% CI)[c]	χ^2
			Lariat		
June	320	2.75 ± 1.03	2.11 (0.00-4.50)	6.18 (3.08-235)	24.42*
July	320	1.65 ± 0.27	1.1 (0.58-1.67)	6.51 (4.31-12.2)	4
Aug	320	2.29 ± 0.42	1.03 (0.63-1.4)	3.74 (2.75-6.22)	3.99
			Hub		
June	320	2.48 ± 0.31	1.93 (1.00-3.06)	6.31 (3.86-17.2)	10.92
July	320	1.95 ± 0.42	2.07 (1.02-3.04)	9.38 (6.35-19.34)	2.54
Aug	320	1.98 ± 0.21	1.20 (0.96-1.51)	5.34 (3.82-8.65)	3.44

[a] Number of corn earworms moths tested. [b] Lethal concentrations, expressed in micrograms of insecticide per vial. [c] 95% confidence limits shown beneath each LC_{50} and LC_{90}. * Significant.

DISCUSSION

Adult moth populations of *Helicoverpa zea* collected on the Texas-New Mexico border, a major green bean producing area, were subjected to adult vial tests to estimate the susceptibility levels to different insecticide chemistries, because of the intensive and continuous use of pesticides in these areas. Moths collected from pheromone traps over the course of the growing season showed variations in the levels of susceptibility. In both years' study, the susceptibility levels tended to decrease (though not always significantly) in the mid-season, followed by an increase later in the season. The decline in adult susceptibility over the season is, to some extent, due to the in-season insecticide use in both green beans and cotton. The increased application of insecticides in cotton and corn, in addition to green beans in this area during middle of summer, could be one reason for an increase in LC values from 0.63 µg/vial in July to 0.97 µg/via1 in August for bifenthrin during July and August. Conversely, exposure of adults to transgenic crops with *Bacillus thuringiensis* in the surrounding areas may be one reason for increased tolerance to insecticides. A study in *Helicoverpa armigera* has shown that the exposure of bollworm to sub lethal doses of *B. thuringiensis* produced a decline in tolerance to pyrethroids (Wang et al., 1994 and Tan et al., 1998). However, metabolic and non-metabolic resistance, cytochrome P450 dependent metabolism, and synthetic insecticides cannot be discounted as mechanisms for conferring resistance (Samir et al., 1993; Cohen et al., 1992; Wang and Hobbs 1995). Previous studies on *Helicoverpa armigera* indicated esterase and monooxygenase activity in resistance mechanisms (Gunning 1994 and Kranthi 1997). Also, research showed that the *H. zea* exposed to allelochemicals had decreased susceptibility to alpha-cypermethrin due to detoxification by a P450 mediated response (Li et al., 2000). Rose et al. (1992) identified an allelochemical-resistant strain of *H. virescens* with 2.0 to 2.5 fold resistance to quercetin, a flavonoid present in its host plants, exhibited similar levels of elevated resistance to the organophosphate methyl parathion, the carbamate methomyl, and the pyrethroid fenvalerate.

One key factor responsible for the change in the susceptible levels of the adults over the season is the constant mixing of the populations between areas. Polyphagy and migratory nature of this pest (Fitt 1989; Dent 1991) resulted in the immigration of moths with varying levels of tolerance into the region in the early part of the season, followed by a decrease due to the mating with the local populations. Although migration, transgenic crops, and alternative hosts were not investigated in this study, these factors warrant further study to determine their roles as a means of altering susceptibility levels in this pest. *H. zea*, a major pest of concern in most of the field crops today, has a broad host range and strong mobility to extend from southern to northern areas of the United States. The frequent movement of the adult moths between locations and fields is a major influence on this study.

A study conducted in 2004 across the entire state of Texas showed that the tolerance levels were relatively high in South Texas (Pietrantonio et al., 2004). The populations survived insecticidal concentrations of 30-60 μg/vial. Thus, given the migratory nature of this pest, this study highlights the possibility of population mixing in North Texas, resulting in lowered susceptibility in the beginning of the season. This migration plays a key role in carrying the resistant or susceptible alleles. Over the season, these moths mix with the other individuals resulting in dilution of the resistant alleles if they exist at all. The homozygous resistant adults mate with susceptible adults, thereby producing heterozygote offspring, which show tolerance. Discriminating doses should be based on this knowledge, so a more precise monitoring system can be developed. This would lead to more appropriate control strategies for this pest.

The frequency of the nerve insensitivity gene is expected to increase in the field populations if selection pressure to pyrethroid is continued (Kranthi et al., 2001). Since this mechanism is the most difficult to eliminate, thus appropriate management strategies need to be devised to further reduce selection pressure otherwise pyrethroid resistance may become more unmanageable in the future. A study conducted in Australia showed that the reduction in the pyrethroid selection pressure resulted in a shift in pyrethroid resistance mechanisms from nerve insensitivity to oxidative metabolism (Forrester et al., 1993). Hence, reduction of selection pressure could play a key role in diluting the contribution of nerve insensitivity to pyrethroid resistance.

Thus, it is highly advisable to avoid the use of pyrethroids against the first few generations and restrict their use to later generations of bollworm to prevent the potentially worsening resistance problem. Growers in the Texas high plains have already started shifting to zeta-cypermethrin (Porter, personal communication) due to lower efficacy of bifenthrin in 2001 and 2002. It is recommended to switch to another class of insecticides in the late season. These results need further investigation both at molecular and cellular levels to better understand the biochemical mechanisms (high esterase, nerve insensitivity, gene frequency, and cytochrome P450 monoxygenases) involved in tolerance development. Also, differences in the susceptibility levels between locations indicate the need for more frequent resistance monitoring at multiple locations. Extension entomologists, along with the processing industries and chemical industries are closely monitoring insecticide resistance to avoid control failures of this pest. The study indicated that bollworm control in green beans is not under direct threat, but needs the grower's attention and cooperation to keep the insecticide selection pressures low by adjusting the timing and frequency of applications.

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