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SEASONAL SHIFTS IN SUSCEPTIBILITY OF HELICOVERPA ZEA TO CRY1AC: EVIDENCE OF FIELD SELECTION?

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Abstract

Susceptibilities of 73 bollworm, *Helicoverpa zea* field populations to Cry1Ac toxin were measured in diet incorporated assays at the University of Arkansas in 2005. Overall regression estimates of combined data across all colony categories and individual experiments for lethal concentration mortality (LC_{50}) and molt-inhibition plus mortality (MIC_{50}) were developed. Susceptibility ratios were calculated to examine variation in susceptibility of populations from different host plants, geographic locations, life stages of insects at collection and seasonal time of collection. Susceptibilities of bollworm to Cry1Ac varied among field and laboratory reference colonies. Susceptibility of field colonies was influenced by life stage of insect at collection and host plant. Susceptibility was affected by laboratory colonization. Resistance ratios decreased with increased number of generations in laboratory culture. Higher LC_{50}s were associated with colonies established from collections made later in the season.

Introduction

Susceptibility of heliothines to Cry1Ac varies widely across geographic regions (Stone and Sims 1993, Luttrell et al. 1999, Hardee et al. 2001, Wu et al. 1999, 2002, Jalali et al. 2004, Ali et al. 2005). Research has shown that the bollworm, *Helicoverpa zea* (Boddie) and the tobacco budworm, *Heliothis virescens* (F.) have the genetic capacity to evolve resistance to Bt toxins in the laboratory (Stone et al. 1989, Gould et al. 1992, 1995, Burd et al. 2003, Fengxia et al. 2003, Lu et al. 2004). Bt cotton represents a majority of the current cotton acreage in Arkansas, while Bt corn expressing similar Bt toxins is also grown in this diverse production system. As a result, there is significant potential for selection of resistance and cross-resistance to the Bt toxins expressed.

From 2002 to 2004, we have found that susceptibility of field-collected bollworm to Cry1Ac varies within Arkansas (Ali et al. 2005). Variability in field populations of bollworm seems to be associated with within season selection. Higher LC_{50}s are usually associated with larvae collected from Bt crops and larvae collected late in the season. With increased reliance on these Bt crops for insect control, the potential for resistance development to Bt toxins becomes important and practical. It is an issue for concern for producers and technology regulators. To better understand the status of Bt resistance in heliothines in Arkansas, we initiated research in 2005 to further define selection and characterize the reason for the seasonal shifts in concentration-mortality responses observed in earlier field collections. We considered the potential influence of host plant, geographic location, insect life stage at collection and seasonal time of insect collection on measured susceptibility to Cry1Ac in the laboratory.

Materials and Methods

Insects

Over 125 colonies of bollworm were collected from different regions of Arkansas during 2005. Of these, we established 73 colonies for laboratory assessment of Bt susceptibility. Field collections were targeted in the four corners of the state. The northern corner was in Washington County, the site of the University of Arkansas research farm in Fayetteville. This is an urban region with limited agricultural crops, most of which are associated with the university farm. The site in the Southwest Arkansas was on Matteson Farms in Little River County. This site is a large grain farm dominated by corm and soybean production with no commercial cotton production. The Southeast site was located on two large planting companies in Drew and Desha Counties, Tillar and Company and R. A. Pickens and Sons. Large acreages of cotton are grown, but the area also includes a diversity of field crops including corn, rice and soybean. Collections from Northeast Arkansas were made on Wildy Farms in Mississippi County. This production area is almost entirely devoted to cotton acreage.

The total number of moths, eggs and larvae collected across all fields in 2005 were 327, 1048 and 4596, respectively. This represents 25, 26 and 80 colonies established from moths, eggs and larvae, respectively (Fig. 1). Among the larvae, the greatest numbers were collected as 3rd instars (31.2%), followed by 4th instars (25.1%), 2nd

instars (20.0%), 5th instars (18.3%) and 1st instars (5.3%). The numbers of colonies established from collections on corn, clover, Bt corn, chickpea and cotton were 35, 18, 14, 12 and 9, respectively (Fig. 2). Across months, the most colonies were established from collections made in July (30) followed by June (27), August (25), May (23), September (15) and April and October (3) (Fig. 3).

Bioassays

Among these field collections, 73 laboratory colonies of bollworm were established and susceptibilities of their progenies (1-3 generation) to Cry1Ac were measured in a diet incorporation bioassay (Ali et al. 2005). University of Arkansas (UALab) laboratory susceptible colony of bollworm was used as the control references in all Bt assays. Additionally, one laboratory colony was received from Monsanto Company, St. Louis, MO. Colonies were maintained on a pinto bean artificial diet (Burton, 1969) in the Margaret M^{c}Clendon Insect Rearing Facility, Department of Entomology, University of Arkansas, Fayetteville, AR in a walk-in temperature-controlled room at 26°C, 70% RH and 14:10 (L:D) photoperiod. Progenies of resulting colonies were used for bioassays.

Neonate bollworms were individually exposed to Bt toxin in wells of bioassay trays (C-D International) containing appropriate amounts of lyophilized MVPII (Cry1Ac) (provided by Monsanto Company) incorporated into pinto bean diet. There were 48 to 112 larvae used for each concentration and 3 to 8 replications were conducted on different days. Larval mortality and mortality plus those that failed to molt to second instars were recorded after 7 days of exposure to the treated diet. Overall regressions (using combined data set from individual experiments for each colony) for lethal concentration mortality (LC_{50}) and molt-inhibition plus stunting (MIC_{50}) were developed by probit analysis (SAS 1998) for each geographic location of collection, host plant, stage of insect at collection, month and season of collection and generation in laboratory colonization. Susceptibility ratios for each category of field population were determined by dividing LC_{50} or MIC_{50} of the field population by LC_{50} or MIC_{50} of the reference UALab colony. The 95% CI for each population category was calculated according to the Robertson and Preisler (1992) to determine if variation in susceptibility of populations was associated with the different crops, geographic locations, life stages of insect at collection and seasonal time of insect collection. Since our historical baseline data is LC_{50} estimates (Luttrell et al. 1999), results are limited to LC_{50} estimates in this report.

Results and Discussion

Overall susceptibility of field populations of bollworm to Cry1Ac varied up to 20-fold as compared to the reference laboratory colony. Susceptibility of the Monsanto laboratory colony (MonLab) was similar to those of field colonies. Colonies collected from Carolina geranium and black light collected moths were significantly more susceptible to Cry1Ac than those collected from Bt corn, clover, corn, cotton, chickpea and pigeon pea. Colonies collected from sorghum were less susceptible than those from other field colonies. Gore et al. (2003) also reported that progenies of moths emerged from larvae fed sorghum were less susceptible to Cry1Ac than those fed corn. Colonies from black light-collected moths were more susceptible than those established from collections of eggs and larvae on host plants (Fig. 4 and Fig. 5).

Susceptibility of colonies assayed in the second generation was greater than those assayed in the first or third generation (Fig. 6). The lower LC_{50}s in the second generation suggests that susceptibility affected by laboratory colonization. The reason for a slight decrease in susceptibility from second to third generation was not known, but it may be due to the small number of regressions for third generation assays as compared to the first and second generations. Further research is being conducted to understand the effects of laboratory colonization on measurements of Cry1Ac susceptibility.

Across geographic locations, LC_{50}s of colonies varied. Colonies from Southwest Arkansas were more susceptible than colonies from Southeast Arkansas, while colonies from Northwest Arkansas were less susceptible than those from Southwest and Southeast Arkansas (Fig. 7). Difference in susceptibility of colonies from Southwest and Southeast Arkansas may be due to the crops grown in these locations. Major crops in Southeast and Southwest Arkansas are cotton and corn, respectively. Colonies from corn were more susceptible than those from cotton to Cry1Ac (Ali et al. 2005). Gore et al (2003) have also reported that progenies of moths emerging from larvae fed cotton were less susceptible to Cry1Ac than to those larvae fed corn. Storer et al. (2003) suggested that selection for Bt resistance may depend upon local deployment levels of Bt crops and the probability of developing resistance may

be greater on Bt cotton. The reason for the lower susceptibility of colonies from Northwest Arkansas is not known, but it is likely due to the small number of colonies that were available from this location.

Across months, susceptibility of colonies collected in September and October were less susceptible than those collected in April, May, June, July and August (Fig. 8). When grouped by relative time of season, colonies collected in mid season (June, July and August) were less susceptible than those collected in early season (April and May). Colonies collected in late season (September and October) were less susceptible than those collected in early and mid season (Fig. 9). Our results clearly show that larvae collected later in the season are less susceptible to Cry1Ac than those collected earlier in the season. This suggests ongoing selection in the field environment. Our assay results seem to be sensitive enough to detect these seasonal shifts in susceptibilities. Additional research is needed to understand the genetic implications of these variable responses.

Acknowledgements

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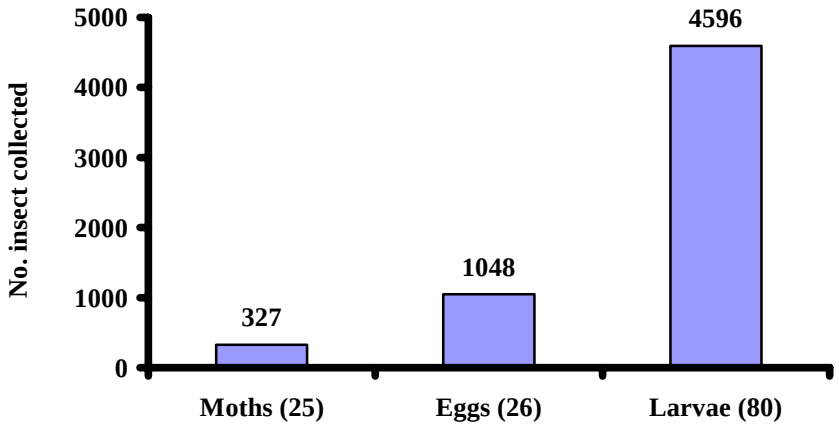

Figure 1. Number of insects collected from the field in different life stages during 2005. Numbers in parentheses are the number of laboratory colonies established from these field collections.

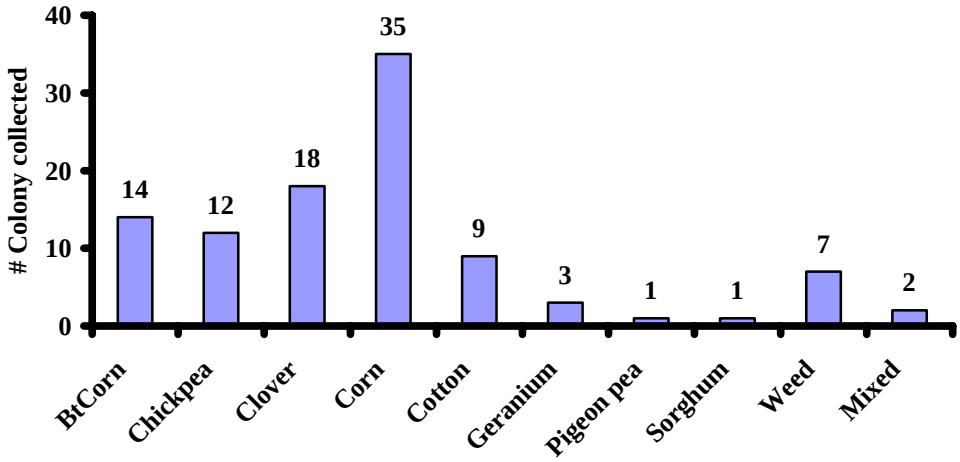

Figure 2. Number of colonies established from field collection on different host plants in 2005.

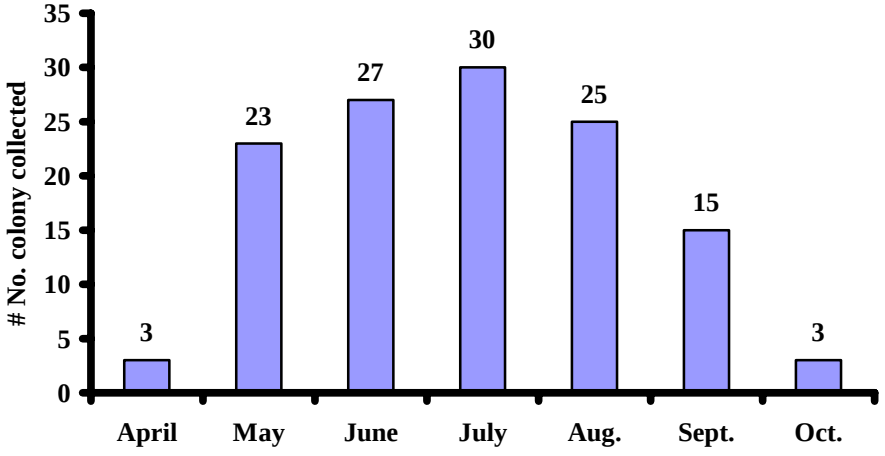

Figure 3. Number of field colonies collected in different months in 2005.

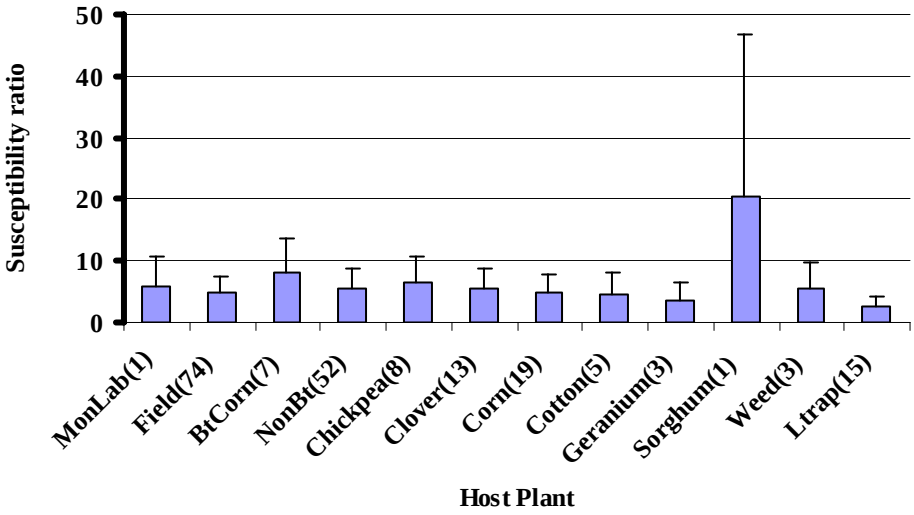

Figure 4. Susceptibility ratios of bollworm populations collected on different host plants exposed to Cry1Ac in diet-incorporated assays. Numbers in parentheses are the number of colonies included in the overall regression.

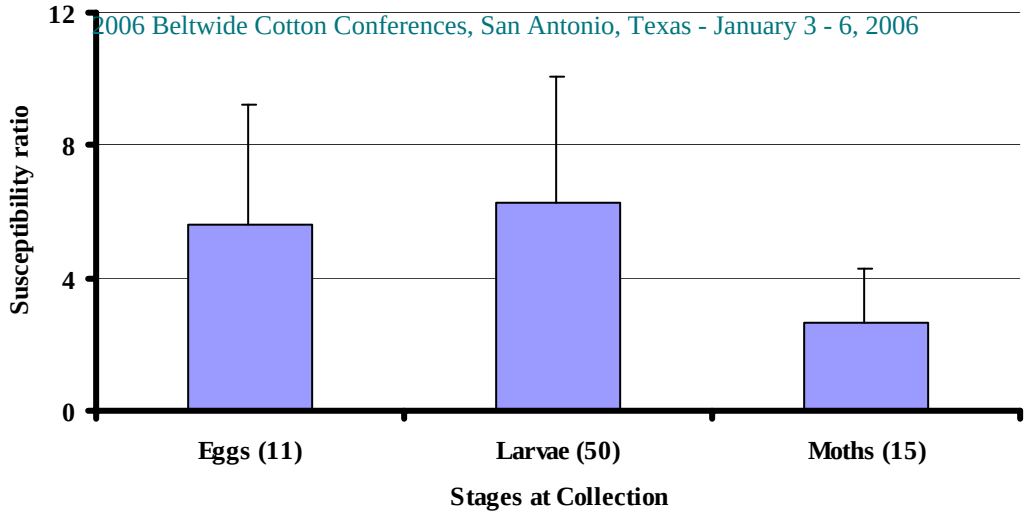

Figure 5. Susceptibility ratios of bollworm populations collected as different life stages exposed to Cry1Ac in diet-incorporated assays. Numbers in parentheses are the number of colonies included in the overall regression.

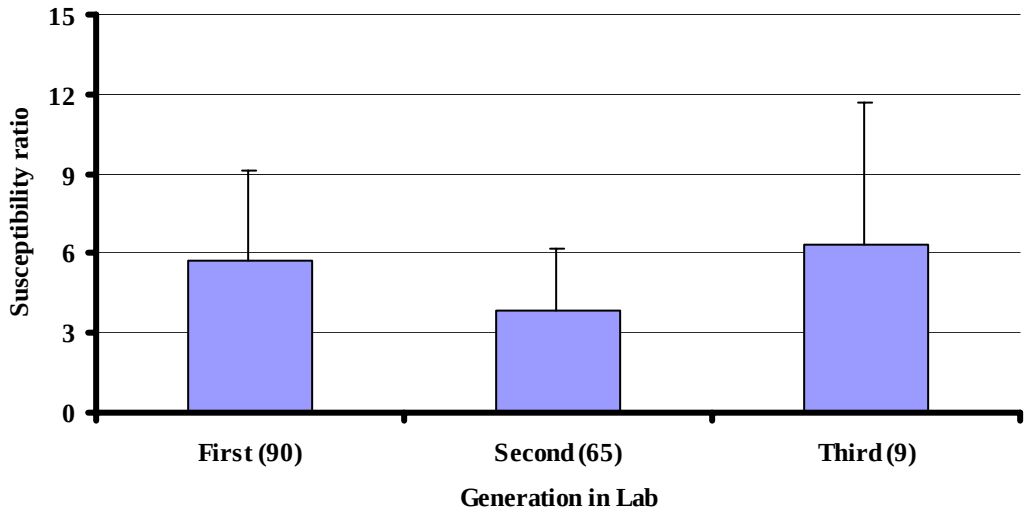

Figure 6. Susceptibility ratios of bollworm populations to Cry1Ac in diet-incorporated assays conducted against different generations in the laboratory. Numbers in parentheses are the number of regressions in the overall regression.

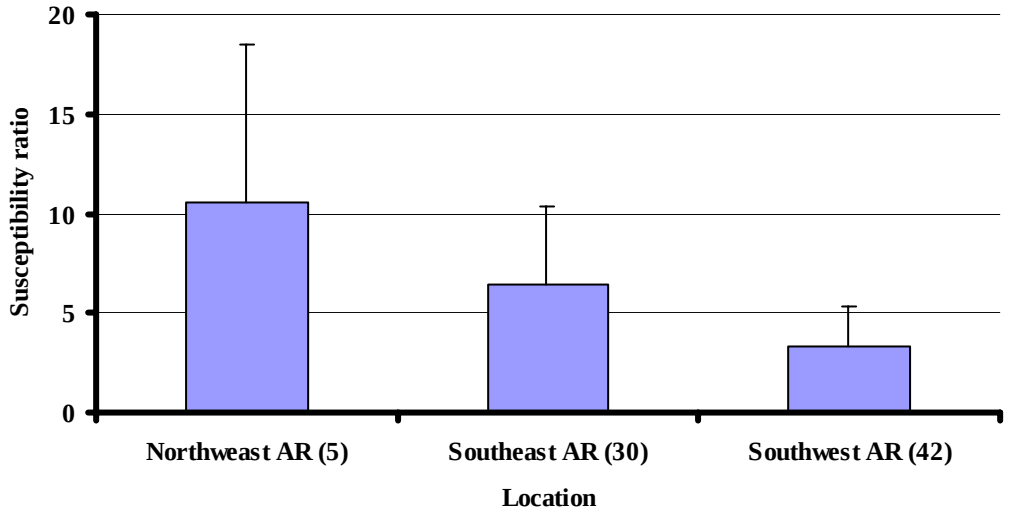

Figure 7. Susceptibility ratios of bollworm populations collected across different geographic locations of Arkansas exposed to Cry1Ac in diet-incorporated assays. Numbers in parentheses are the number of colonies included in the overall regression.

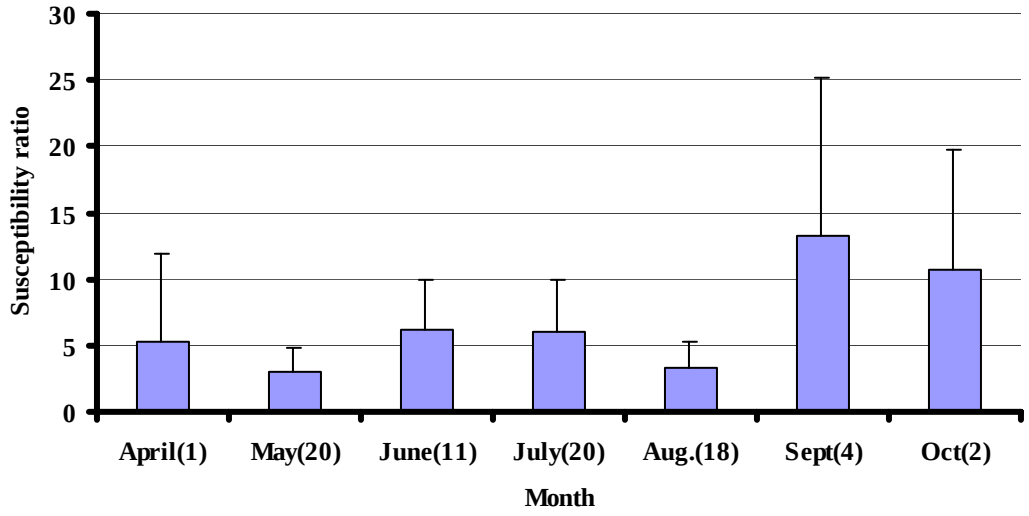

Figure 8. Susceptibility ratios of bollworm populations collected in different months exposed to Cry1Ac in diet-incorporated assays. Numbers in parentheses are the number of colonies included in the overall regression.

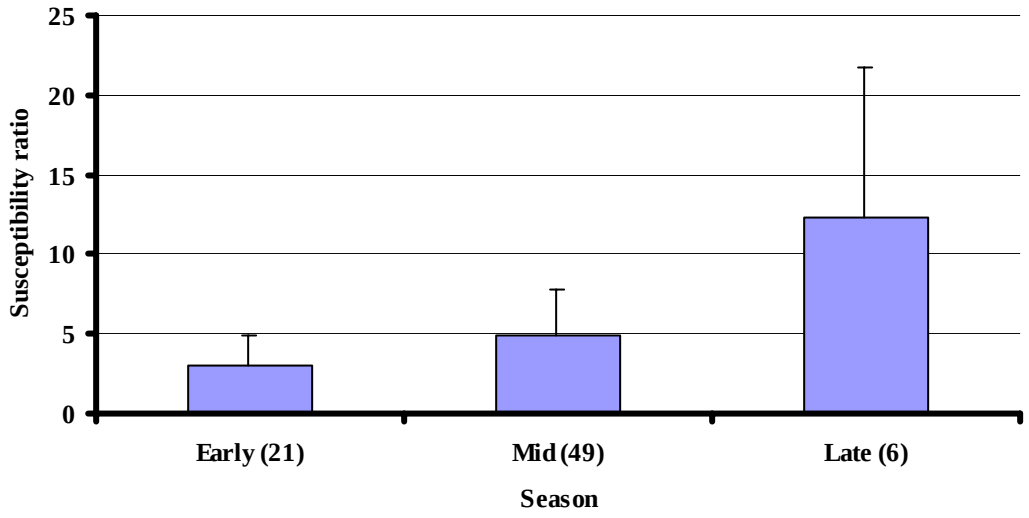

Figure 9. Susceptibility ratios of bollworm populations groped by relative time of season exposed to Cry1Ac in diet-incorporated assays. Numbers in parentheses are the number of colonies included in the overall regression.