STATUS OF MONITORING FOR TOLERANCE TO CRYIAc IN POPULATIONS OF *HELICOVERPA ZEA* AND *HELIOTHIS VIRESCENS*: THREE-YEAR SUMMARY

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Abstract

Field populations of *Helicoverpa zea* (Boddie) and *Heliothis virescens* F. from the eastern half of the U. S. cotton belt were monitored (1996-1998) for tolerance to the *Bt* toxin CryIAc. The tolerances of field populations of *H. zea* increased during the three-year period. Areas producing the greatest increase in tolerance to CryIAc had a greater percentage of their acreage planted in *Bt* cotton. In general, tolerances of *H. virescens* populations did not change, with the single exception being the third generation of *H. virescens* collected from the MS Delta. The small changes in tolerance reported herein suggest that populations may be more tolerant of CryIAc but do not show that the current tolerance is at a level to cause control failures in the field.

Introduction

The conservation of susceptibility to the delta endotoxin proteins of *Bacillus thuringiensis* (*Bt*) in field populations of insect pests of cotton has received considerable interest. First, resistance to *Bt* proteins has been documented in laboratory and field populations of several insect pests (Stone et al. 1989, Gould et al. 1992, 1995, Moar et al. 1995, Tabashnik et al. 1990, 1997). In addition, the recent registration and deployment of transgenic cotton expressing the *Bt* protein, CryIAc, and the development of resistance to many conventional insecticides by Lepidopterous pests of cotton have made the preservation of susceptibility to CryIAc an important goal of pest management in cotton-growing areas. To manage resistance effectively, it is necessary to monitor insect pests of cotton for changes in their tolerances of CryIAc. Tolerances to CryIAc in field populations of *Heliothis virescens* F. (tobacco budworm, TBW) and *Helicoverpa zea* (Boddie) (cotton bollworm, CBW) were monitored for three years (1996-1998) in an attempt to detect any decline in susceptibility to the CryIAc toxin. We present the status of our monitoring efforts to date in this paper.

Methods and Materials

Entomologists and consultants from nine states within the U. S. cotton belt collected eggs or larvae produced by field populations of TBW and CBW. The collected individuals (F_1's) were shipped to USDA-ARS, Stoneville, MS, reared to pupation on artificial diet (29 ± 3 °C, 55-60% RH, 14:10 (L:D) h photoperiod) and offspring (F_2) of the F_1 adults were evaluated for tolerance to CryIAc. Field populations producing fewer than 50 adults were discarded to diminish the likelihood of inbreeding effects.

A spray chamber bioassay was used during 1996 to evaluate all field populations and laboratory strains (USDA-ARS, Stoneville, MS) of TBW and CBW. Details about the methods and materials used in the spray chamber bioassays are reported in Elzen et al. (1990). MVPII, the biological insecticide closest in properties to the CryIAc protein expressed in transgenic cotton, was applied to cotton terminals placed in florist's water wicks. A single 3rd-instar larva (30 ± 3 mg) was placed on each cotton terminal 30 minutes after the application of MVPII. Numbers of moribund and dead larvae were determined after 72 h. Only populations from Washington County, MS were evaluated via the spray chamber bioassay during 1997 and 1998.

During 1997 and 1998, all field and laboratory populations of TBW and CBW were evaluated for tolerance to CryIAc via agar overlays containing a freeze-dried formulation of MVPII powder (J. T. Greenplate, Monsanto, unpublished). Concentrations of CryIAc in the agar overlay were 0.05 and 5.0 µg/ml for TBW and CBW, respectively. The concentrations were based on EC98 data for the two species (Sims et al. 1996). One neonate was added to each well containing the agar overlay of CryIAc and the larva was allowed to feed on the toxin for 5 d (29 ± 3 °C, 55-60% RH, 14:10 (L:D) h photoperiod). For each test, 64-128 larvae/colony were assayed on the Bt toxin, as well as non-toxic diet. The numbers of larvae dead or stunted (<3rd instar) were recorded. Individuals that reached the 3rd instar were considered tolerant. Larvae from the laboratory colonies of TBW and CBW were evaluated for each test to act as a control for diet and growing conditions.

Results

In all spray chamber bioassays, the MVPII killed significantly more larvae than the water-control treatment (all tests $P < 0.0005$). The mortality of CBW populations from Washington County, MS treated with MVPII varied among years (Figure 1; G test, $P = 0.019$). CBW larvae were more tolerant of MVPII in 1998 relative to 1996, and each year the percentage mortality decreased. Unlike populations of CBW, there were no significant differences among years for mortality of TBW populations from Washington County (Figure 2; $P = 0.431$). However, only 24.4% of TBW larvae from the third generation of 1998 were killed by the MVPII treatment (see below).

There were no significant differences for the percentages of <3rd instar larvae between field colonies of TBW and CBW and their respective laboratory colonies for tests on non-

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toxic diet ($P > 0.4$ for all tests from 1997 and 1998). However, when treated with CryIAc, significantly more larvae from CBW field colonies reached the 3rd instar than those from the laboratory control strain [paired t-test; 1997: $0_{Difference}$ (±SE) = 1.50±0.29%, $P < 0.0001$; 1998: $0_{Difference}$ = 6.20±1.71%, $P = 0.0002$]. The differences between field strains and the control strain were significantly greater in 1998 than 1997 (t-test assuming unequal variances: t = 2.703, 30.7 df, $P = 0.0111$).

To better compare the results of 1997 and 1998 we pooled populations from the regions sampled in both years. Only the MS Delta was sampled in both years during the first and second generations. Therefore, to control for host-plant/generation effects we only compared the tolerances among regions and between years for the third and fourth generations. We found significant differences among regions for the % = 3rd instar larvae (Range = 1.71-13.37%, $P < 0.0001$, Figure 3). There were also significantly more tolerant larvae in 1998 (9.50%) than 1997 (1.85%) ($P < 0.0001$). Differences among regions were dependent on year. During 1997 there were no significant differences among regions for the % = 3rd instar larvae ($P = 0.138$). However, significant differences among regions in the tolerance of larvae to CryIAc were found during 1998 ($P < 0.0001$). 1997 populations and populations collected in TX and AR during 1998 did not significantly differ in their percentage of tolerant larvae (Figure 3). 1998 populations from southern AL and the panhandle of FL (AL/FL) were significantly more tolerant than all other regions (Figure 3).

Because CBW larvae were collected during all four generations in Washington Co., MS, we used larvae from these populations to compare the host-plant effects on the tolerance of CBW larvae during 1997 and 1998 (G tests of independence). The tolerances of CBW larvae from Washington Co. collected during 1998 were significantly greater than the tolerances of larvae collected during 1997 (Figure 4; $P < 0.0001$). Host plant also significantly affected the tolerances of collected larvae ($P < 0.0001$). However, the effect of host plant was dependent on the year of collection (Figure 4). During 1997, no significant host-plant effects were observed ($P = 0.167$). The host plant that CBW larvae were collected from significantly influenced tolerance to CryIAc during 1998 ($P < 0.0001$). Populations of CBW collected from *Bt* cotton during 1998 were significantly more tolerant of CryIAc than all than all other groups. Larvae collected from corn during 1998 were also significantly more tolerant of the CryIAc than 1997 populations and 1998 larvae collected from wild hosts and non-*Bt* cotton.

When compared to the laboratory strain, field populations of TBW were more tolerant of CryIAc during 1997 and no different in 1998 (1997: $0_{Difference}$ (±SE) = 1.46±0.49%, $P = 0.007$; 1998: $0_{Difference}$ = 1.82±1.27%, $P = 0.189$). The average difference between the tolerances of field and lab colonies in 1998 was not significantly different from those in 1997 [P = 0.833; Range (% Tolerance): 1997 0 - 6.25%; 1998 0 - 13.4%]. For regions where TBW was collected during 1997 and 1998, there were significant differences among regions (Figure 5). The differences among regions were a consequence of the higher tolerances of third-generation populations of TBW from the MS Delta (Figure 5). TBW larvae from this population were the most tolerant of CryIAc in both overlay (13.4% tolerant) and spray chamber (24% mortality) bioassays.

Discussion

The data presented above suggest that current field populations of CBW are slightly more tolerant of CryIAc than populations at the beginning of the study. However, it should be noted that our measure of tolerance is based on a sub-lethal dose of CryIAc and should not be interpreted as resistance. Our results do indicate that factors improving resistance may already be increasing in the field. Southern AL and the panhandle of FL are areas where the most Bt cotton was grown. It is of anecdotal interest that CBW populations collected from this region show the greatest change in tolerance to CryIAc. Furthermore, populations collected from *Bt* cotton in the MS Delta exhibited the greatest tolerances relative to neighboring populations collected from non-Bt cotton. Based on the small change in tolerance, the genetic basis of the detected tolerance does not appear to be a major recessive gene. It is less difficult to measure the effects of quantitative genes with sub-lethal doses and more research is needed to determine the importance of minor genes in the development of resistance under field conditions.

Previous research found populations of TBW more susceptible of CryIAc than populations of CBW (Stone and Sims 1993). Gould et al. (1992) found resistance in TBW to be governed by a major recessive gene with a moderately low frequency in the field (Gould et al. 1997). As a consequence, populations of CBW may be expected to respond more quickly to selection exerted by transgenic cotton expressing CryIAc than populations of TBW. We observed no major changes in the tolerances of TBW larvae, with the exception of Generation 3 from the MS Delta. This change was too small to be the major recessive gene reported in earlier studies (see CBW discussion above). Our current research is examining the genetic basis of this small change.

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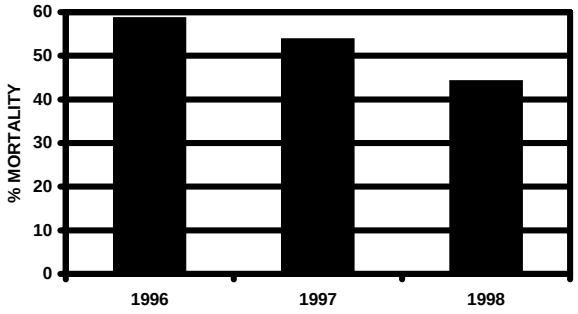

Figure 1. Mortality of CBW larvae from Washington Co., MS when treated with spray chamber applications of MVPII.

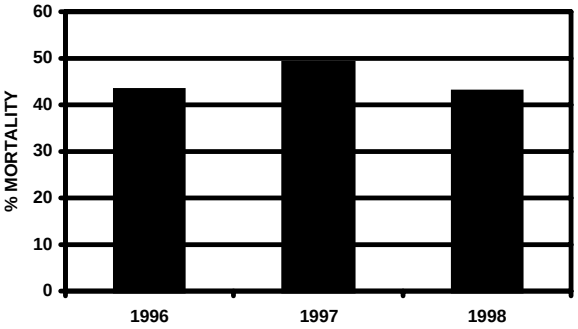

Figure 2. Mortality of TBW larvae from Washington Co., MS when treated with spray chamber applications of MVPII.

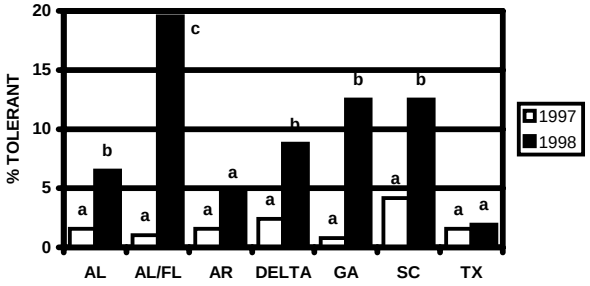

Figure 3. Percentage of CBW larvae = 3rd instar (tolerant) after five days of feeding on CryIAc. Percentages denoted by the same letter are not significantly different as determined by tests of homogeneity.

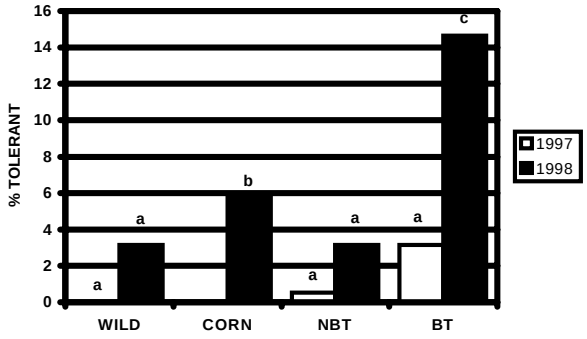

Figure 4. Percentage of CBW larvae from Washington Co., MS = 3rd instar after five days of feeding on CryIAc. Percentages denoted by the same letter are not significantly different as determined by tests of homogeneity.

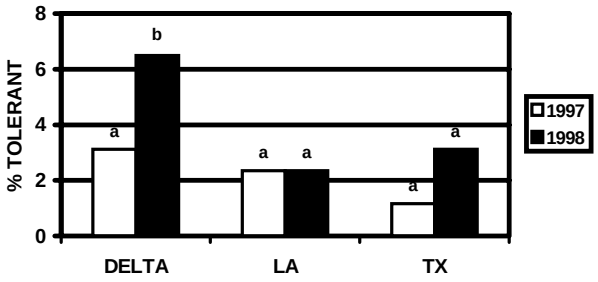

Figure 5. Percentage of TBW larvae tolerant of CryIAc during 1997 and 1998 (Generation 3 and Generation 4). Percentages denoted by the same letter are not significantly different as determined by tests of homogeneity.