# FACTORS POTENTIALLY INFLUENCING THE SURVIVALOF *HELICOVERPA ZEA* ON BOLLGARD® COTTON

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## Abstract

In the years surrounding the 1996 commercial introduction of Bollgard® cotton (Bollgard® is the gene that encodes the CryIAc insecticidal toxin from the soil microbe *Bacillus thuringiensis* var. *kurstaki*.), studies have been performed to explore several factors which may influence survival of the cotton bollworm (*Helicoverpa zea*) in plantings of *B.t.* transgenic cotton. These factors include: *H. zea* susceptibility to the *B.t.* toxin, levels of *B.t.* toxin expressed in the plant and its temporal and spatial distribution, and the behavioral responses of *H. zea* larvae to *B.t.* toxin. Previous $LC_{50}$ comparisons indicate that *H. zea* neonate larvae are 4 to 60 times less susceptible to CryIAc than are *Heliothis virescens*, and that geographically diverse populations of *H. zea* can exhibit $LC_{50}$ values that vary as much as 16-fold. CryIAc expression studies show that toxin present in economically important fruiting positions varies from about 10-15 µg/g fresh weight (fw) at 40 days after planting (DAP) to 1-2 µg/g fw at 120 DAP; CryIAc levels in terminal foliage change similarly over time from around 20 µg/g fw to around 5 µg/g fw at 40 and 120 DAP, respectively. Furthermore, evaluation of component parts of blooms show that expression of CryIAc is not uniform; very little toxin is apparent in the pollen. Laboratory studies suggest that *H. zea* larvae can avoid diet with CryIAc and will preferentially feed upon non-toxic alternatives including untreated diet and, at higher population densities, other larvae. These data and others suggest scenarios whereby *H. zea* populations, inherently less susceptible to CryIAc than *H. virescens* and highly variable in their susceptibility, may be able to take advantage of several factors to survive in numbers and produce economic damage to Bollgard® cotton plantings. These factors include: lower levels of CryIAc later in the growing season, non-uniform expression of toxin, especially in blooms, larval avoidance of toxin, and cannibalism.

## Introduction

In its first commercial season, Bollgard® cotton enjoyed great success, both from market-penetration and value-added perspectives (Barton, 1997). Over 5700 growers used Bollgard® on nearly 13% of US cotton acres (1.8 million acres); average yield improvements of just over 7% were realized. This translated to an economic advantage of roughly $33 per acre after an equivalent outlay for insect control, including the Bollgard® technology fee. Sixty percent of Bollgard® growers were able to totally eliminate insecticide treatments for the control of *Heliothis virescens*, *Pectinophora gossypiella*, and *Helicoverpa zea*. The only one of these three pests that did generate supplemental sprays on Bollgard® was the bollworm, *H. zea* (Barton, 1997).

Bollworm damage to Bollgard® cotton in 1996 was reported after July 1st (Carter et al. 1997; Greenplate, 1997; Lambert, 1997; Layton et al. 1997; Roof & DuRant, 1997) and was viewed by some as a failure (Kaiser, 1996; Mellon, 1996) in spite of the economic/environmental benefits mentioned above and published data by Mahaffey et al. (1995) demonstrating the potential for high populations of *H. zea* to inflict substantial damage to Bollgard® cotton. Subsequently, bollworm damage to Bollgard® cotton has been evaluated from several perspectives and previously published data has been revisited. Three main topics pertinent to bollworm survival on Bollgard® cotton are: 1) *H. zea* susceptibility to the Bollgard® *B.t.* toxin (CryIAc), 2) levels of CryIAc in appropriate cotton tissues, and 3) the ability of *H. zea* to avoid the CryIAc toxin.

*H. zea* susceptibility to CryIAc is highly variable and considerably lower than that of *Heliothis virescens*. Stone and Sims (1993) showed a 16-fold difference in $LC_{50}$'s among 15 geographically diverse populations throughout the southern US. In the same study, *H. zea* populations displayed $LC_{50}$ values that were 4 to 60 times higher than the mean $LC_{50}$ for 12 geographically distinct *H. virescens* populations. Resistance monitoring over the last two years indicates that populations of *H. zea* (many from damaged Bollgard® fields) have not changed in their susceptibility to CryIAc (Hardee et al. 1997; Hardee & Adams, 1997; Greenplate, 1997).

In response to early damage to Bollgard® fields in the Brazos valley of Texas in 1996, levels of CryIAc were found to be equivalent in cotton plants from damaged and adjacent undamaged fields, suggesting that large differences in levels of expressed CryIAc were not the reason for the damage (Greenplate, 1997). Greenplate (1997) also reported roughly equivalent levels of CryIAc in fruiting structures of varying maturities from Bollgard® plants in damaged fields. When component parts of white blooms were evaluated, however, relatively low levels of CryIAc were measured in pollen (Greenplate, 1997); subsequent experiments (Greenplate, unpublished) have supported this with some Bollgard® pollen showing levels of CryIAc below the sensitivity of the quantitative assays used (both bioassay and ELISA).

This study will report upon seasonal levels of CryIAc expression in Bollgard® plants. It will also explore the ability of *H. zea* larvae to detect and avoid *B.t.* toxin, and take advantage of relatively non-toxic food sources (either

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"clean diet" or conspecific larvae). These results along with the studies mentioned above may suggest scenarios whereby *H. zea* larvae can survive and damage Bollgard® cotton.

## Materials and Methods

To evaluate seasonal levels of CryIAc *in planta*, a sensitive laboratory bioassay was developed to quantify CryIAc expressed in transformed cotton using as samples lyophilized plant tissue (Greenplate, in preparation). The assay involved the application of powdered lyophilized cotton tissue in suspension over synthetic insect diet. Eggs of *Heliothis virescens* (tobacco budworm) were added and they hatched on the diet. The degree of larval development (the proportion of larvae reaching 3rd instar after 7 days) correlated inversely with the log of the CryIAc concentration in the powder suspension. This was validated with several standard curves employing purified CryIAc added to a control cotton powder suspension. The assay sensitivity range fell between 0.1 and 50 ng CryIAc/mL of suspension. Using this assay as a quantitative method, CryIAc activity was monitored in Bollgard® cotton line 531 (in Coker 312 background) at four field sites in 1994 and six field sites in 1995 with respect to a spatial component within plants and a temporal component throughout the growing season. Sample collection in the field roughly spanned the period from the appearance of first squares (40-60 days after planting) through first bloom (65-85 days) and open bolls (105-125 days). This study is described generally herein; a detailed description and presentation of results is in preparation (Greenplate, in preparation).

In laboratory bioassays, *H. zea* neonates were exposed to either CryIAc or the closely related, but less toxic, CryIAb (Hofte & Whiteley, 1989; MacIntosh et al. 1990). The toxins were incorporated into synthetic insect diet (Multi-species lepidoptera diet, Southland Products Inc., Lake Village, AR) and dispensed into 1 mL wells of bioassay trays (CDI Inc., Pitman, NJ) as previously described (MacIntosh et al. 1990). Diet choice arenas were set up in the 1 mL wells of diet trays by removing half the gelled treated diet and replacing it with untreated diet so that each well contained equal amounts of *B.t.*-treated diet and clean diet. In studies to evaluate the possible contribution of cannibalism to *H. zea* survival in the presence of *B.t.* toxin, wells were infested with 1, 2, 3, or 5 neonate larvae. In all studies each treatment (or concentration in concentration-response studies) involved the infesting of 16 to 32 wells. *H. zea* diet bioassays were incubated for 7 days at 26° C. Data collected included numbers surviving, wells with survivors (in cannibalism studies), numbers preferring treated or untreated diet (based upon estimated volumes consumed), and individual larval weights.

## Results

CryIAc expression studies conducted during 1994 and 1995 (Figure 1) showed that toxin present in economically important fruiting positions (Primary position at nodes 7, 9, 11, & 13) dropped from about 10-15 µg/g fresh weight (fw) at 40 days after planting (DAP) to 1-2 µg/g fw at 120 DAP; CryIAc levels in terminal foliage changed similarly over time from around 20 µg/g fw to around 5 µg/g fw at 40 and 120 DAP, respectively.

Diet choice studies indicated that *H. zea* neonate larvae were able to discriminate between untreated diet and diet containing *B.t.* toxin (in this case CryIAb). When half the diet contained greater than 4 µg/mL, the larvae fed preferentially upon the untreated diet with nearly 4 times as many preferring untreated diet when the treated diet contained 32 µg CryIAb per mL (Figure 2). When mean larval weights were recorded, larvae given a choice were 2 to 6 times larger than larvae given no choice at CryIAb concentrations of 2 to 32 µg/mL, respectively (Table 1). These weight data also support the presence of a feeding preference based upon the ability of *H. zea* larvae to detect and avoid *B.t.* toxin.

Density-dependent responses to B.t. toxin were evaluated using both CryIAc and CryIAb. The presence of two or three larvae increased the number of wells that contained surviving larvae at every concentration of CryIAc; at concentrations of 12.5 µg/mL or less, the treatment with 3 larvae per well contained 20-30% more wells with survivors (Figure 3). It should also be noted that at these concentrations (12µg/mL or less), wells with survivors almost exclusively contained single surviving larvae. When surviving larvae from this study were weighed (Figure 4), a trend appeared showing consistently larger larvae (nearly 2-fold) in wells starting out with 3 neonates. In studies using CryIAb nearly all wells had single survivors, although at 32 µg/mL up to 1/3 of the wells had multiple surviving larvae which were severely stunted. Weight data resembled those for CryIAc as concentrations from 2 to 32 µg/mL showed increased weight in surviving larvae associated with higher larval densities (Figure 5); 2 to 4-fold increases were seen in wells infested with 3 neonates, 2-fold increases in wells infested with 5 neonates. These data suggest that certain *H. zea* larvae can benefit in terms of survival and vigor by cannibalization in the presence of *B.t.* toxin.

## Discussion

Two years of field studies have revealed that average levels of CryIAc found in important primary fruiting positions have dropped below 5 µg/g by 80 - 90 days after planting (Figure 1). In 1996, when *H. zea* damage was first reported (Brazos Valley) it occurred at approximately 90-100 days after planting (Greenplate, 1997); indeed, most cases of *H. zea* damage to Bollgard® cotton have been reported mid- to late-season. In instances where *H. zea* survived in significant numbers, subsequent measurements of CryIAc levels in damaged blooms and bolls revealed approximate values of 2 µg/g fresh weight or less (Greenplate, 1997), suggesting that, in these tissues, values under 3 µg/g fresh

weight may indicate vulnerability to *H. zea*. No similar situations have been reported for either *Heliothis virescens* or *Pectinophora gossypiella*, supporting the idea that *in planta* levels of CryIAc are adequate for control of these pests in Bollgard® cotton. It is obvious to assume that higher levels of CryIAc will lead to greater efficacy; it also has been shown that cotton terpenoids enhance the *in planta* activity of *B.t.* in engineered cotton (Sachs *et al*. 1996). The precise relationship between levels of CryIA(c) and the *in planta* bioactivity will likely be influenced by other factors in the plant which may include the type and age of the tissue in question.

This report has demonstrated the ability of *H. zea* neonate larvae to discriminate between *B.t.*-treated and untreated diet and to preferentially feed upon the clean diet (Table 1; Figure 2). This adaptive behavior may find an outlet in Bollgard® cotton blooms where levels of CryIAc are extremely low in the pollen (Greenplate, 1997). The ability to avoid *B.t.* may also influence the tendency of *H. zea* to cannibalize conspecifics; the opportunity to feed upon other larvae resulted in the enhanced vigor of survivors (Figure 3; Figure 4; Figure 5). Densities of 3 larvae per well in the presence of *B.t.* produced data that was nearly identical to that produced when single larvae were presented with a choice between treated and untreated diet (Figure 6). This suggests that high densities may provide a reservoir of "untreated diet" that may allow not only survival of some larvae, but increased vigor as well. Although the direct relationship between levels of *B.t.* toxin in synthetic diet bioassays and field efficacy of Bollgard® cotton cannot be precisely quantified, it is important to note that these behaviors were demonstrated at levels of *B.t*. which are close to those found in Bollgard® cotton when it does sustain *H. zea* damage (Figure 1; Greenplate, 1997).

In summary, a number of factors may interact to allow significant survival of *H. zea* in Bollgard® cotton and lead to subsequent economic damage; those factors include:

1) Late season levels of CryIAc in fruiting structures of Bollgard® cotton.
2) Non-uniform expression of CryIAc in blooms.
3) Wide variability of CryIAc susceptibility among *H. zea* populations.
4) Ability of *H. zea* to avoid *B.t.* toxin.
5) Locally high larval densities which may encourage cannibalism and escape from toxin.

These preliminary data may provide avenues for more valuable future studies involving on-plant observations of *H. zea* larvae either in the greenhouse or the field.

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Table 1. Mean *H. zea* larval weights (mg/larva) under either no-choice (All Treated) or choice (Half Treated) conditions in the presence of CryIAb toxin. Each value represents the mean and SEM of 16 individual larvae; **bold values** are significantly different from the corresponding All Treated value (Dunnet's test; $P < 0.05$).

| CryIAb | Half Treated | SEM | All Treated | SEM |
|---|---|---|---|---|
| 0.5 µg/mL | 64.2 | 6.3 | 75.5 | 13.7 |
| 2 µg/mL | **32.6** | 4.3 | 17.6 | 5.2 |
| 8 µg/mL | **19.5** | 2.8 | 6.1 | 2.4 |
| 32 µg/mL | **7.0** | 1.4 | 1.2 | 0.3 |

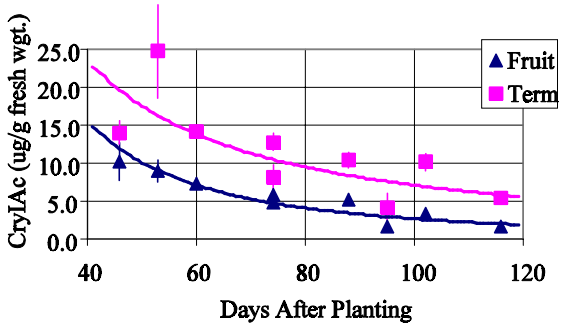


Figure 1. CryIAc activity in main terminals and fruiting structures (primary positions on nodes 7, 9, 11, & 13) of Bollgard® cotton in Coker 312 background. Data is combined for 1994 (4 field sites) and 1995 ( 6 field sites).

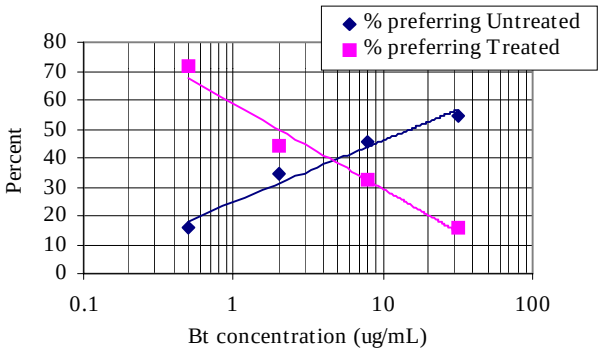


Figure 2. Feeding preferences of *H. zea* larvae in choice conditions.

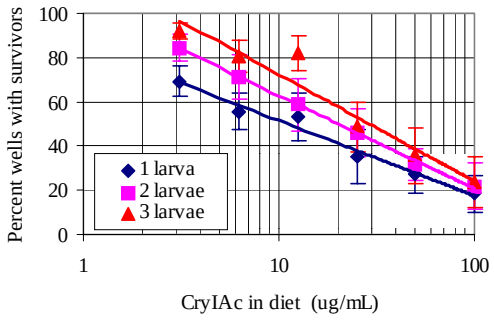


Figure 3. Density-dependent survivorship of *H. zea* neonate larvae in the presence of CryIAc toxin.

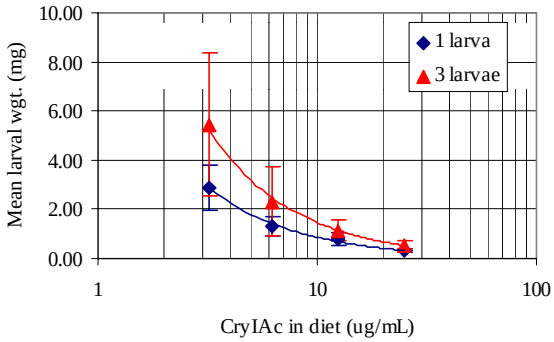


Figure 4. Density-dependent development of *H. zea* larvae in the presence of CryIAc toxin.

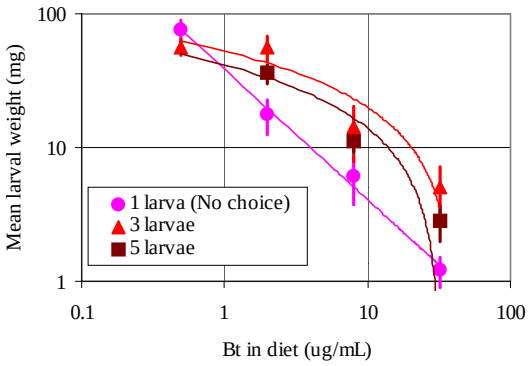


Figure 5. Density-dependent development of *H. zea* larvae in the presence of CryIAb toxin.

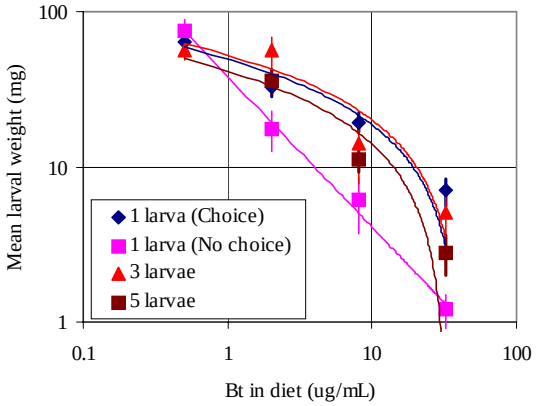


Figure 6. Density- and choice-dependent development of *H. zea* larvae in the presence of CryIAb toxin.