EFFECTIVENESS OF BT COTTON TOWARDS BOLLWORMS AND BENEFIT OF SUPPLEMENTAL OVERSPRAYS

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Abstract

Since the introduction of Bt cotton in the United States in 1996, management of the bollworm, *Helicoverpa zea*, has become much less problematic. However, there are still incidents where unacceptable fruit injury is experienced and insecticidal oversprays are utilized to prevent yield loss. There has been much speculation surrounding the reasons for control failures among Bt cotton technologies including Bt resistance or tolerance, inadequate expression of Bt toxins expression due to plant phenology or environmental stressors. The objective of this project was to evaluate the efficacy of second and third generation Bt cotton at four locations within the Mid-South for efficacy towards bollworms and to determine if overspraying Bt cotton results in a reduction in damage and increased yields relative to non-Bt cotton. Additionally, we report an incidence where bollworms were able to survive and damage Widestrike 3 cotton, and our attempt to demonstrate susceptibility of these larvae on Widestrike 3 leaf tissue. Our data demonstrates that all currently available Bt cotton technologies may suffer unacceptable bollworm injury and survival. Widestrike appears to be most sensitive to experiencing control failures and benefits from insecticide oversprays targeting bollworms. Bollgard II, Twinlink and Widestrike 3 may also suffer control failures, but are less common than Widestrike. Larvae that survive to late instar in Bt cotton may possess the ability to produce offspring capable of feeding and surviving on Bt cotton. Whether this indicates resistance to one or more Bt toxins, or is simply a reflection of naturally occurring population variability is not clear. Regardless, bollworm larvae that do survive Bt cotton appear to suffer non-lethal effects and severe fitness costs. The ability of these survivors to propagate and maintain the ability to survive on Bt cotton appears doubtful.

Introduction

The introduction in 1996 of transgenic cotton containing genes expressing *Bacillus thuringi*ensis (*Bt*) proteins ushered in a new era in cotton insect pest management. The first Bt cotton introduced in the U.S. was Bollgard I which expressed the Cry1Ac endo-toxin. This toxin was highly effective towards tobacco budworm, *Heliothis virescens*, but moderately toxic towards bollworm, *Helicoverpa zea*. Insecticide applications targeting tobacco budworm were completely eliminated, while those targeting bollworm were greatly reduced. To increase efficacy and for resistance management, dual and multi-Bt gene cotton varieties have since been introduced including Bollgard II, Widestrike and Widestrike 3. Although these introductions have increased the efficacy of transgenic cotton targeting lepidoteran pests, including bollworm, there are still incidents where unacceptable fruit injury is experienced and insecticidal oversprays are required to preserve yield.

The objective of this project was to evaluate the efficacy of second and third generation Bt cotton for efficacy towards bollworms and to determine if overspraying Bt cotton results in a reduction in damage and increased yields relative to non-Bt cotton. Additionally, we report an incidence where bollworms were able to survive and damage Widestrike 3 cotton, and our attempt to demonstrate susceptibility of these larvae on Widestrike 3 leaf tissue.

Materials and Methods

Tests were conducted at five locations (four reported here) across the Mid-South to determine the impact of foliar insecticide applications targeting lepidopteran pests on injury and yields in second and third generation Bt cottons. The Bt cotton technologies evaluated included: TwinLink™ (TL; Cry1Ab, Cry2Ae), Bollgard II® (BG2; Cry1Ac, Cry2Ab), WideStrike® (WS; Cry1Ac, Cry1F), and WideStrike 3® (WS3; Cry1Ac, Cry1F, Vip3A). A non-Bt variety (NBT) was included as a check. All tests were 5 x 2 factorials with factor A being the cotton technology and factor B being entries sprayed for bollworms with Prevathon at 20 fl-oz/ac, or non-sprayed. Plots were 4 rows wide x 40-60 ft in length. Each factorial combination was replicated 4 times. Foliar applications were made in accordance with the occurrence of larvae in the non-Bt cotton plots at each individual location.

Insect densities, and square and boll injury were determined prior to foliar treatment and weekly thereafter using a modified whole plant sampling procedure of 20-25 plants per plot. The top 4-5 nodes plus one white or pink bloom and one small to medium-sized boll were be sampled on each plant. If larvae or fresh injury was observed, the entire plant was sampled. Total counts of larvae, total numbers of damages squares, and total numbers of damaged bolls (including flowers) were recorded in each plot. For purposes of this report, all fruit injury was pooled and averaged across the season and larval counts were excluded. All plots were harvested and yields were determined. All field data were analyzed using ANOVA and means were separated using an F protected LSD ($P < 0.05$).

In addition to the Bt efficacy and overspray tests, a collection of late instar bollworms was made from a field of Widestrike 3 cotton (PHY 495 W3RF) near Winnsboro, LA in 2014. The F1 and F4 progeny from these larvae (WLA population) and those from a known susceptible population acquired from the USDA-ARS in Stoneville, MS (USDA population) were evaluated for their ability to survive on greenhouse grown Widestrike 3 and non-Bt leaf tissue. New, fully expanded leaves were removed from the plants and individually placed abaxial side down into 8 well trays with the bottom of each tray covered with agar to maintain leaf moisture. Each bioassay consisted of 4 replicates of 5 larvae per replicate. One neonate (<24 hr.) was placed on an individual leaf and then sealed and left to feed on the leaf tissue. Trays were kept under 12:12 L:D photoperiod and ~27°C environment. Mortality was recorded at two, four, six and eight days after infestation (DAI). At 8 DAI, surviving larvae were weighed. Bioassay data were analyzed using a two-way ANOVA and repeated measures analysis ($P < 0.05$).

Additionally, leaf, square and boll tissue collected from the bollworm infested Widestrike 3 field were evaluated for Cry1Ac, Cry1F and Vip3A toxin expression using lateral flow ELISA indicator strips (Environlogix, Portland, ME). Leaf tissue from the greenhouse grown plants used in the F1 bioassay were also quantitatively evaluated by an independent laboratory for the expression of Cry1Ac, Cry1F and Vip3A.

Results and Discussion

Field tests

Bollworm pest pressure was characterized as high at the Arkansas location and subsequently, mean seasonal fruit injury exceeded 50% in the non-sprayed, non-Bt plots (Figure 1). However, there were no differences in injury among the non-sprayed Bt entries. Twinlink was the only entry where significantly more fruit injury occurred in the non-sprayed plots versus plots that were treated with Prevathon. None of the sprayed Bt entries yielded significantly more than there non-sprayed counterparts (Figure 2).

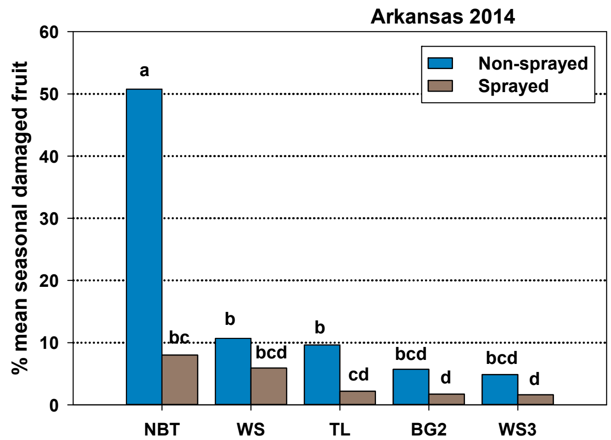

Figure 1. Seasonal mean fruit injury response of sprayed and non-sprayed Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) at the Arkansas location. Means in a column with the same letter are not significantly different based on an F protected LDS ($P < 0.05$).

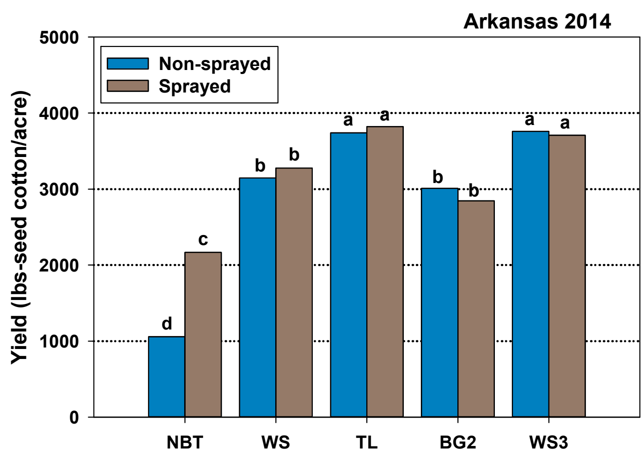

Figure 2. Yield response of sprayed and non-sprayed Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) at the Arkansas location. Means in a column with the same letter are not significantly different based on an F protected LDS ($P < 0.05$).

At the Louisiana test site, bollworm pressure was characterized as moderate; however, the bollworm infestation was fairly concise so the seasonal mean fruit injury was relatively low (Figure 3). The non-Bt and Widestrike entries were the only entries to significantly benefit from a Prevathon application, and the difference in injury was reflected in the differences observed in yield (Figure 4).

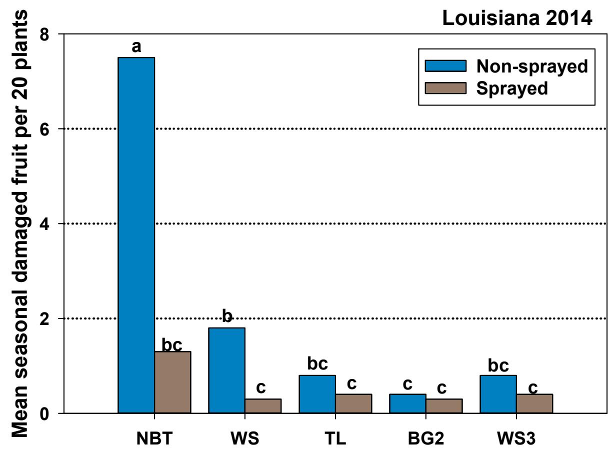

Figure 3. Seasonal mean fruit injury response of sprayed and non-sprayed Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) at the Louisiana location. Means in a column with the same letter are not significantly different based on an F protected LDS (*P* < 0.05).

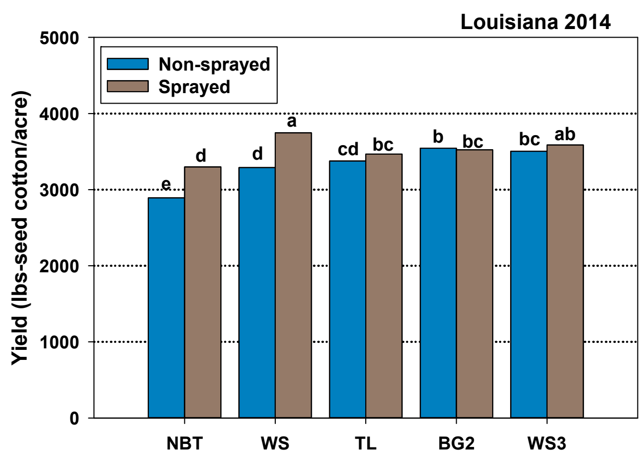

Figure 4. Yield response of sprayed and non-sprayed Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) at the Louisiana location. Means in a column with the same letter are not significantly different based on an F protected LDS (*P* < 0.05).

The test in Mississippi was characterized as having moderate bollworm pressure. At this location the non-Bt, Widestrike and Twinlink entries all suffered significantly less fruit injury when treated with Prevathon relative to the non-sprayed entries (Figure 5). All of the non-sprayed Bt entries suffered fewer damaged fruit than the non-Bt. This location also saw significant increases in yield among all entries when treated with Prevathon (Figure 6).

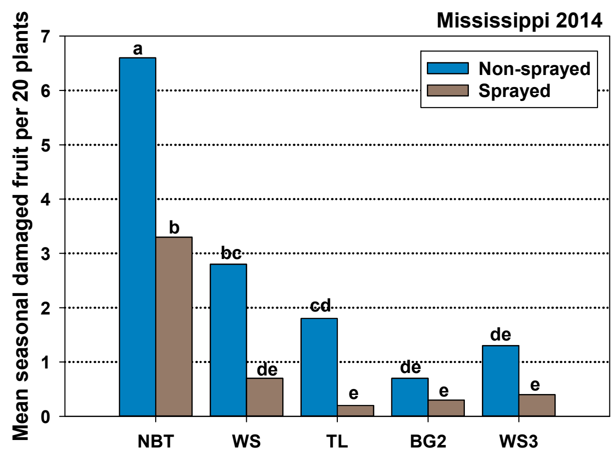

Figure 5. Seasonal mean fruit injury response of sprayed and non-sprayed Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) at then Mississippi location. Means in a column with the same letter are not significantly different based on an F protected LDS ($P < 0.05$).

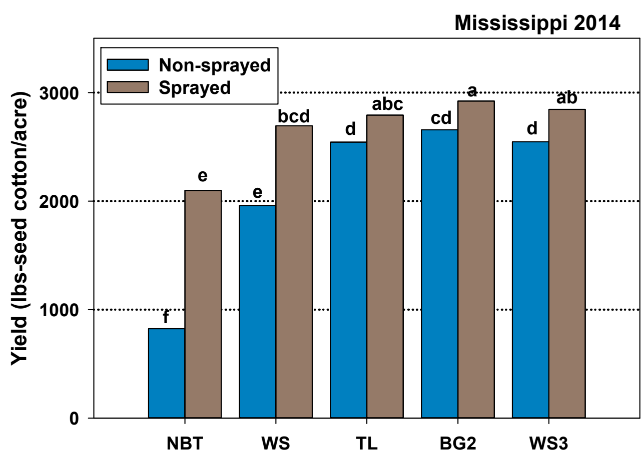

Figure 6. Yield response of sprayed and non-sprayed Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) at the Mississippi location. Means in a column with the same letter are not significantly different based on an F protected LDS ($P < 0.05$).

The Tennessee location was characterized as having low bollworm pressure that arrived late in the season. All of the Bt entries suffered less injury than the sprayed and non-sprayed Bt plots, but the Prevathon treated Bt entries did not have significantly fewer damaged fruit than their non-sprayed counterparts (Figure 7). Yields were variable at the Tennessee site, and no differences were detected among any of the entries (Figure 8).

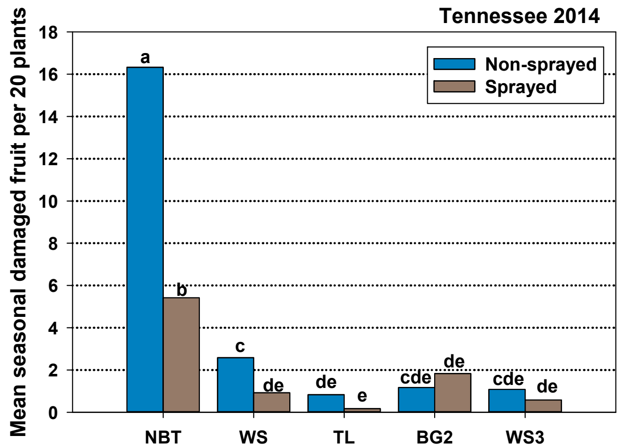

Figure 7. Seasonal mean fruit injury response of sprayed and non-sprayed Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) at the Tennessee location. Means in a column with the same letter are not significantly different based on an F protected LDS ($P < 0.05$).

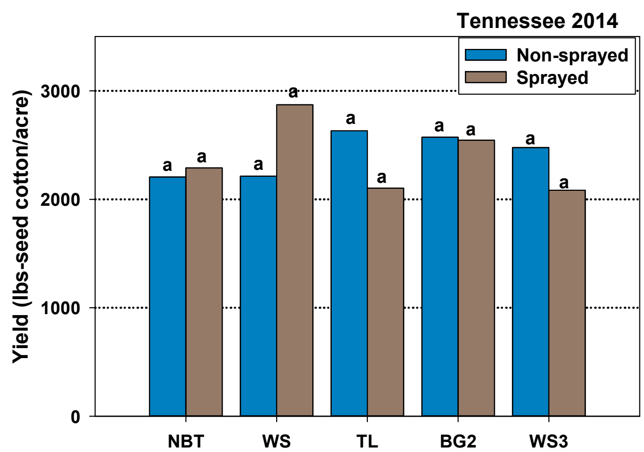

Figure 8. Yield response of sprayed and non-sprayed Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) at the Tennessee location. Means in a column with the same letter are not significantly different based on an F protected LDS ($P < 0.05$).

Across all locations, non-Bt cotton treated with Prevathon averaged a 69% increase in yield over non-sprayed non-Bt cotton (Figure 9). At the four test sites included in this report, 3 of locations observed >5% yield increase from treating non-Bt cotton. Among the Bt entries, Widestrike benefitted the most from foliar oversprays, with an average yield increase of 21% over non-sprayed Widestrike cotton. Similar to the non-Bt, Widestrike also saw a >5% yield increase in 3 of the 4 test locations. The benefit of treating Bollgard II, Twinlink or Widestrike 3 cotton for bollworms with Prevathon was negligible with the exception of the Mississippi location.

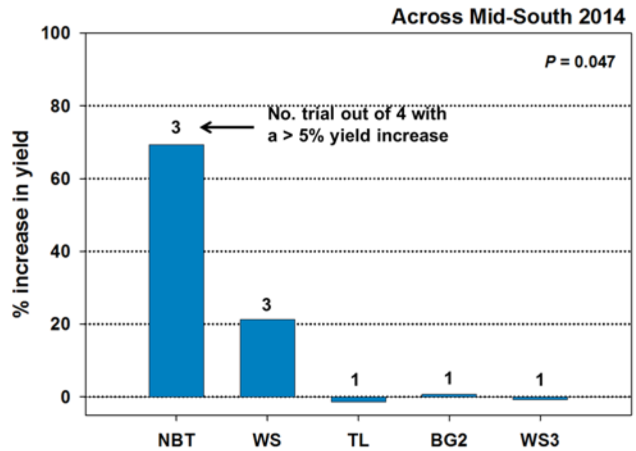

Figure 9. Percentage increase in yield from treating Bt and non-Bt cotton (NBT = non-Bt, WS = Widestrike, TL = Twinlink, BG2 = Bollgard II, and WS3 = Widestrike 3) with Prevathon at 20 fl-oz/ac across locations.

Bioassays

In 2014, there were numerous reports of bollworms surviving and injuring commercially available Bt cotton technologies. As supported in the previously mentioned field studies, these reports are common among Widestrike cotton varieties, and occasionally in Bollgard II and Twinlink. Widestrike 3 was first introduced as the commercial variety PHY 495 W3RF in 2014. This Bt technology is unique in that it possess genes expressing the Vip3A toxin. Widestrike 3 technology was planted on very limited acres in 2014, and because of the uniqueness of this technology, incidences of injury associated with bollworm feeding were unexpected. However, there were two documented reports of unacceptable bollworm injury in Widestrike 3 cotton in 2014; one in Louisiana and another in Arkansas. Late instar bollworms were collected from each of these locations, and a colony was successfully established from the Louisiana location (WLA population).

At the Louisiana location, terminal feeding was noted and the incidence of injured fruit recorded (Figure 10). Injury was greatest on August 8, when it reached approximately 8.5% damaged fruit. At the time larvae were collected, leaf, square and boll tissue was tested for expression of the Bt toxins using lateral flow ELISA indicator strips. These strips simply indicate a presence or absence of the Bt toxin, and is thus not a qualitative indicator. The strip tests demonstrated that all of the toxins were being expressed. However, during the period when injury occurred the crop had reached cutout, and it is possible that the tissues were not expressing the Bt toxins at optimal levels.

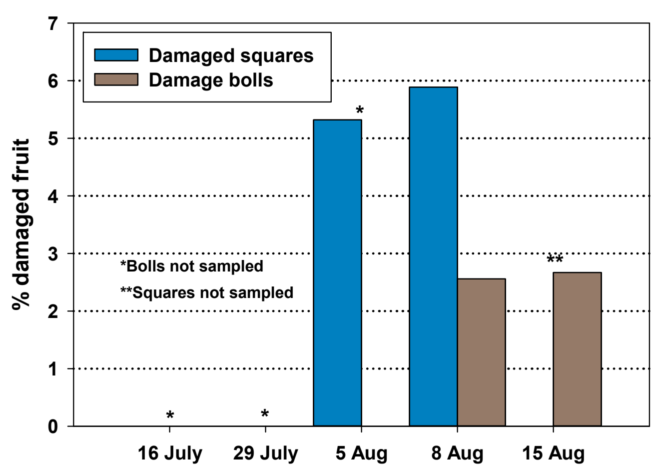

Figure 10. Percentage bollworm injured fruit within a Widestrike 3 cotton field.

The F1 generation from the field collected larvae were evaluated for their ability to survive on greenhouse grown Widestrike 3 leaf tissue, relative to non-Bt leaf tissue. Additionally, a known Bt susceptible population (USDA) was acquired from the USDA-ARS in Stoneville, MS. This population was evaluated for its response to Widestrike 3 and non-Bt cotton as a check.

When reared on non-Bt leaf tissue, at 8 days after infestation with neonates, the WLA population exhibited 30% mortality but was not statistically different from the USDA population which suffered 15% mortality (Figure 11). The WLA population responded very similarly when fed Widestrike 3 cotton leaf tissue, suffering 38% mortality while the USDA population suffered 100% mortality (Figure 12). Most of the WLA larvae that survived 8 days feeding on Widestrike 3 cotton leaf tissue achieved third instar; however, larval weight was greatly affected (Figure 13). WLA larvae feeding on non-Bt cotton (FM 966 RF) averaged approximately 23 mg per larva while those feeding on Widestrike 3 leaves only weighed 7 mg. Larvae that survived the Widestrike 3 bioassay were transferred to artificial diet and a colony of these larvae were established. However, adult fecundity of this colony is low and successful colony maintenance is uncertain.

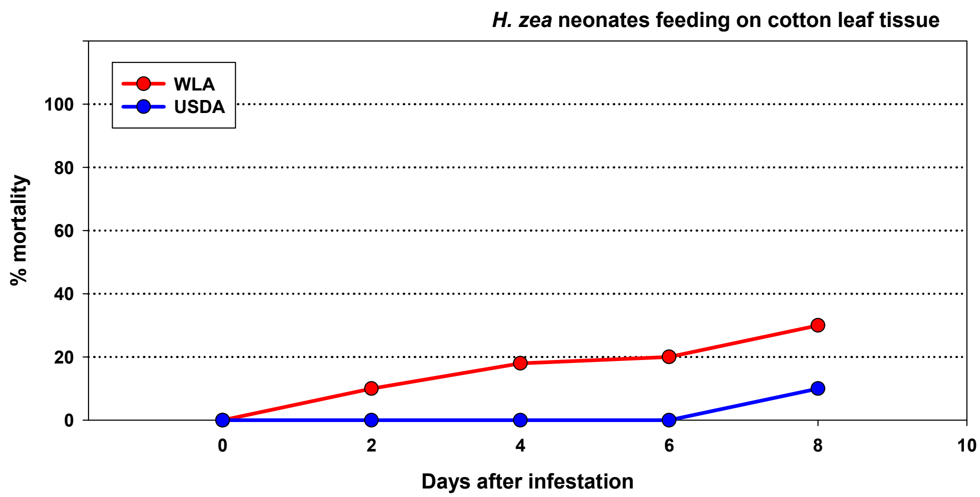

Figure 11. Response of the F1 generation of bollworms (WLA) collected from Widestrike 3 cotton in Winnsboro, LA feeding on non-Bt cotton, relative to a known Bt susceptible population (USDA).

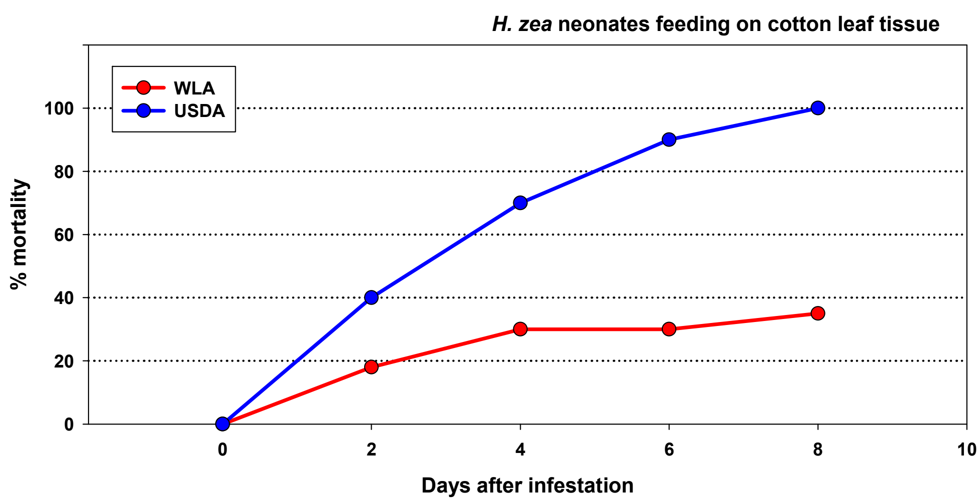

Figure 12. Response of the F1 generation of bollworms (WLA) collected from Widestrike 3 cotton in Winnsboro, LA feeding on Widestrike 3 cotton, relative to a known Bt susceptible population (USDA).

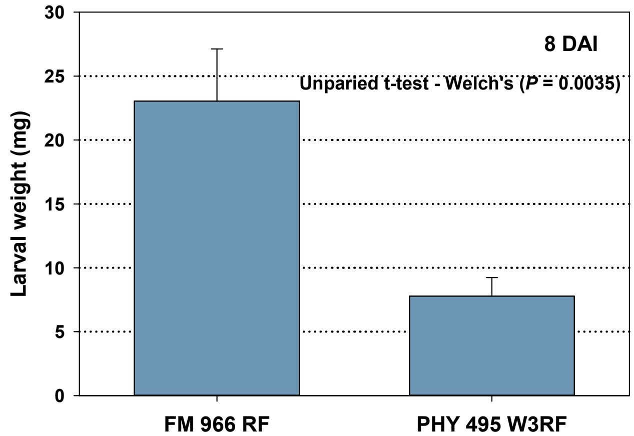

Figure 13. Larval weight of the F1 generation of WLA bollworms that survived a Widestrike 3 (PHY 495 W3RF) leaf feeding bioassay relative to larvae surviving on non-Bt cotton (FM 966RF).

To insure that the Widestrike 3 leaves used in the bioassays were expressing Bt toxins at acceptable levels, tissue from the greenhouse grown Widestrike 3 (PHY 495 W3RF) and Widestrike (PHY 499 WRF) were collected and sent to an independent laboratory for expression analysis. The results from these evaluations suggested that the Widestrike 3 plants were expressing Cry1F, Cry1Ac and Vip3A, and were at levels that should result in high bollworm mortality (Figures 14, 15 and 16).

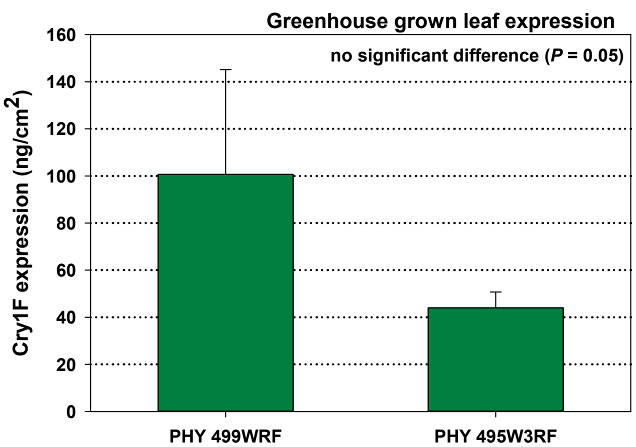

Figure 14. Quantitative expression of Cry1F toxin from leaves of greenhouse grown Widestrike (PHY 499 WRF) and Widestrike 3 (PHY 495 WRF) cotton.

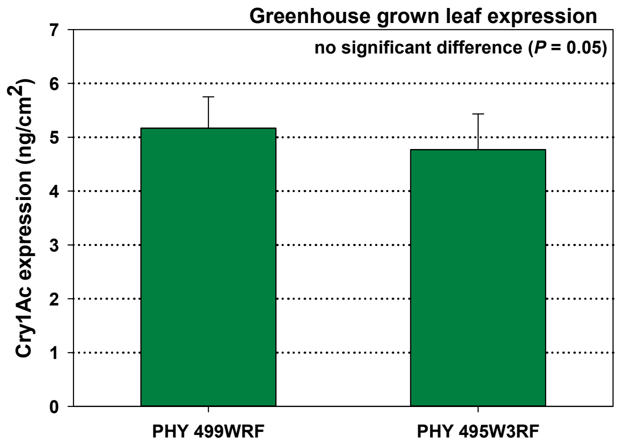

Figure 15. Quantitative expression of Cry1Ac toxin from leaves of greenhouse grown Widestrike (PHY 499 WRF) and Widestrike 3 (PHY 495 WRF) cotton.

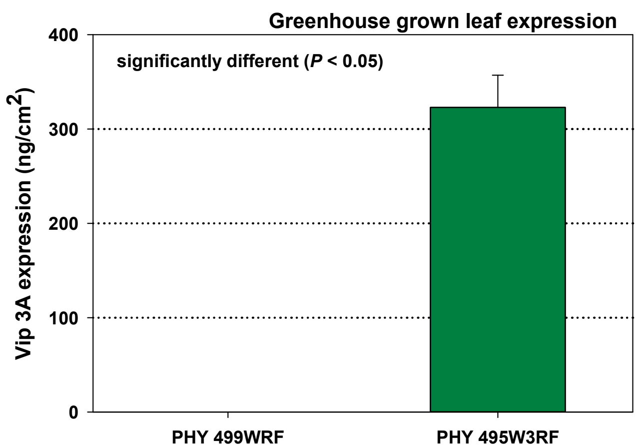

Figure 16. Quantitative expression of Vip3A toxin from leaves of greenhouse grown Widestrike (PHY 499 WRF) and Widestrike 3 (PHY 495 WRF) cotton.

To determine stability of the tolerance/resistance to Widestrike 3, the WLA population was reevaluated on Widestrike 3 leaf tissue at the F4 generation and compared to the USDA population. There were no differences in response of between the populations; both suffered 100% mortality at 8 days after infestation (Figure 17).

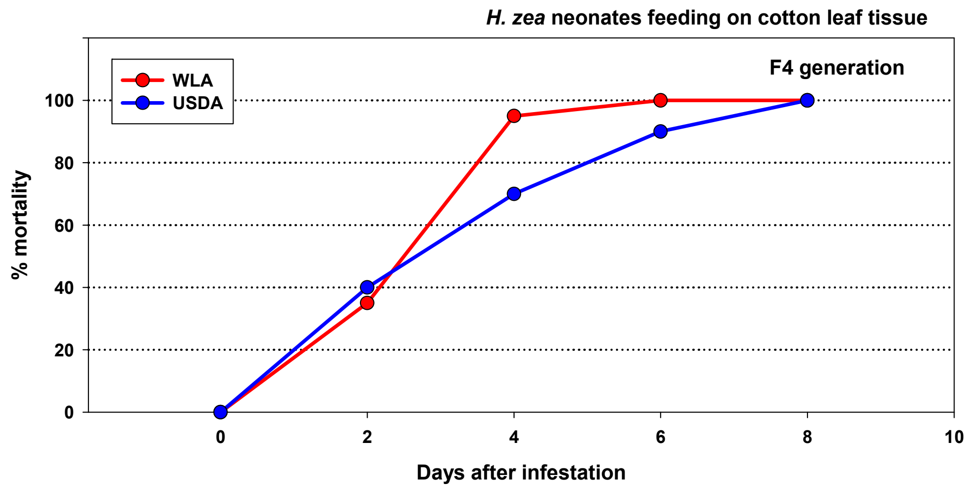

Figure 17. Response of the F4 generation of bollworms (WLA) collected from Widestrike 3 cotton in Winnsboro, LA feeding on Widestrike 3 cotton, relative to a known Bt susceptible population (USDA).

Our data demonstrates that all currently available Bt cotton technologies may suffer unacceptable bollworm injury and survival. Widestrike appears to be most sensitive to experiencing control failures and benefits from insecticide oversprays targeting bollworms. Bollgard II, Twinlink and Widestrike 3 may also suffer control failures, but are less common than Widestrike.

Larvae that survive to late instar in Bt cotton may possess the ability to produce offspring capable of feeding and surviving on Bt cotton. Whether this indicates resistance to one or more Bt toxins, or is simply a reflection of naturally occurring population variability is not clear. Regardless, bollworm larvae that do survive Bt cotton appear to suffer non-lethal effects and severe fitness costs. The ability of these survivors to propagate and maintain the ability to survive on Bt cotton appears doubtful.