CONTROLLING TARNISHED PLANT BUG, *LYGUS LINEOLARIS* L., IN COTTON, *GOSSYPIUM HIRSUTUM* L., THROUGH A TRANSGENIC APPROACH

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Abstract

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is a major pest of cotton, *Gossypium hirsutum* L., in the midsouthern United States. It is exclusively controlled with foliar insecticide applications, many of which, resistance has already been confirmed. A new *Bacillus thuringiensis (Bt)* trait has been introduced in cotton that will be used to control hemipteran and thysanopteran pest species. The objective of this research was to conduct experiments to determine the impact of this new transgenic variety of cotton on tarnished plant bug populations and to assess future treatment thresholds in these varieties. Experiments were conducted in Sidon, MS and in Stoneville, MS. Experiments were designed to compare the interactions between the *Bt* traited plots and non-traited plots and the insecticide spray treatments, which consisted of different thresholds comparing early season (pre-flowering) and mid-late season (flowering) applications. At the Sidon location, there were significantly lower tarnished plant bug nymph numbers in the traited plots versus the non-traited plots, as well as significant differences in the treatments. At the Stoneville location there were significant differences between treatments, but not traited plots versus non-traited plots. There were significant differences in yield when comparing traited plots versus non-traited plots in the current threshold, late season, double threshold, and untreated control treatments at the Sidon location. Significant differences in yield were observed between traited plots and non-traited plots in the early season, late season, double threshold, quadruple threshold, and untreated control treatments. In both locations traited treatments yielded higher than non-traited treatments. Implementing this transgenic approach, the number of insecticide applications necessary to effectively control tarnished plant bugs will be reduced compared to non-transgenic isolines.

Introduction

Cotton is considered a major commodity for producers in many areas of the southern United States. In 2016, an estimated 10.0 million acres of cotton were planted in the U.S. Of this 10.0 million acres, Mississippi planted 450,000 acres, representing a large portion of our row crop acreage (NASS 2010).

The tarnished plant bug is the most economically important insect pest of cotton in the midsouthern region of the U.S. (Bachman et al. 2017). Although tarnished plant bug can feed on cotton at any growth stage, they cause the majority of the economic damage from first-square through early bloom (Burris et al. 1997). This damage can include delayed maturity, deformed cotton plants, fruit abscission, and direct damage to cotton bolls (Burris et al. 1997). Several sampling methods are used to assess tarnished plant bug populations, as well as different thresholds depending on the growth stage of the cotton. For the first two weeks of cotton squaring, the threshold is 8 tarnished plant bugs per 100 sweeps with a sweep net and 1 tarnished plant bug per 6 row feet when using a drop cloth (Musser et al. 2009). During the third week of squaring through bloom, the threshold increases to 15 tarnished plant bugs per 100 sweeps with a sweep net and 3 tarnished plant bugs per 6 row feet with a drop cloth (Musser et al. 2009). There is also a plant-based sampling technique that is commonly used for monitoring square retention. The square retention threshold is to treat when less than 80% of first position squares remain on the plant prior to first bloom (Stewart et al. 2009). Another plant-based sampling method used in this research was the dirty square threshold. This method is used later in the growing season and examines "dirty" squares. A "dirty" square is a square with an exposed bud that has been discolored due to plant bug feeding. The threshold for this technique is 10% dirty squares, focusing samples on medium-sized squares (Musser et al. 2009).

In 2004, Mississippi Delta farmers began to see tarnished plant bugs develop insecticide resistance to organophosphates, carbamates, and pyrethroids. As a result of insecticide resistance, growers incurred an increase in control costs for tarnished plant bugs in cotton. In 2003, average control costs for tarnished plant bug were $25.50 per acre. In 2004, costs spiked to $43.11 per acre and by 2011 control costs had escalated to $97.02 per acre

(Gore et al. 2015). This increase in control costs intensified the search for a method to control these pests with a more cost-friendly alternative. Currently, cotton that utilizes a *Bt* trait to control tarnished plant bugs, similar to the *Bt* trait commonly used in other crops to control lepidopteran pests, is being evaluated. Additionally, many cultural control tactics such as early planting, use hairy leaf varieties, blocking cotton away from corn, and lower nitrogen rates are also recommended (Gore et al. 2015).

The objective in this study was to evaluate thresholds for tarnished plant bugs on cotton modified to be resistant to *Lygus* and determine the value of early season (pre-flowering) protection compared to mid-late season (flowering) protection.

Materials and Methods

Field trials were conducted in Stoneville, MS at the Delta Research and Extension Center and at Sidon, MS on Sidon Plantation. Plot size was 4 rows, 12.19 meters long planted on 96.5 cm row width. Plant population was 135,900 seed/hectare. The trial was arranged in a randomized complete block design with a two by seven factorial arrangement of treatments. Factor A was traited (Cry51Aa2) compared to non-traited. Traited and non-traited entries were in similar backgrounds. Factor B was threshold treatment (Table 1). Data were analyzed with a general linear mixed model analysis of variance (PROC GLIMMIX, SAS ver. 9.4).

Table 1. List of threshold treatments for traited and untraited cotton.

Treatment #	Spray Treatment	Time of Season
1	Unsprayed	-
2	Current Threshold	Full Season
3	2X Current Threshold	Full Season
4	4x Current Threshold	Full Season
5	Weekly Threshold	Full Season
6	Current Threshold	Early Season
7	Current Threshold	Late Season

Data collection methods for tarnished plant bugs varied throughout the year depending on the cotton's growth stage. From first-square until first bloom, square retention was taken weekly. First position squares from the upper 3 nodes of 25 plants per plot were examined to determine square retention. During the first three weeks of squaring, sweep net samples were conducted twice per week. Fifteen sweeps were conducted per plot. Beginning at bloom, two drop cloth samples per plot were taken once weekly. To preserve the plants from unnecessary physical damage, dirty square sampling was also taken once a week starting during the 4th week of squaring through bloom three to four days after the drop cloth sampling. For the dirty square samples, 25 squares per plot were visually observed to determine if there was any tarnished plant bug damage, with applications being triggered at 10% dirty squares found. All treatments were sprayed independently with foliar insecticides when the threshold for a particular treatment was reached.

Results and Discussion

For tarnished plant bug densities, there was a significant difference in the main effects of trait and treatment at the Sidon, MS location. Average number of tarnished plant bug nymphs were significantly higher in the non-traited plots than the traited plots. Significant differences were also observed among treatments (Figure 1). At the Stoneville location there was not a difference in the trait main effect but treatments were significantly different (Figure 2). At both locations, square retention was significantly higher in the traited plots compared to non-traited plots. At both locations, square retention stayed well above the 80% treatment threshold for the traited plots compared to non-traited plots (Figures 3 and 4). Traited plots ranged from 90-95% during the sampling period compared to 70-75% in non-traited plots. On the last sample date, non-traited plots averaged 84% because many of the threshold treatments had received foliar applications of insecticide.

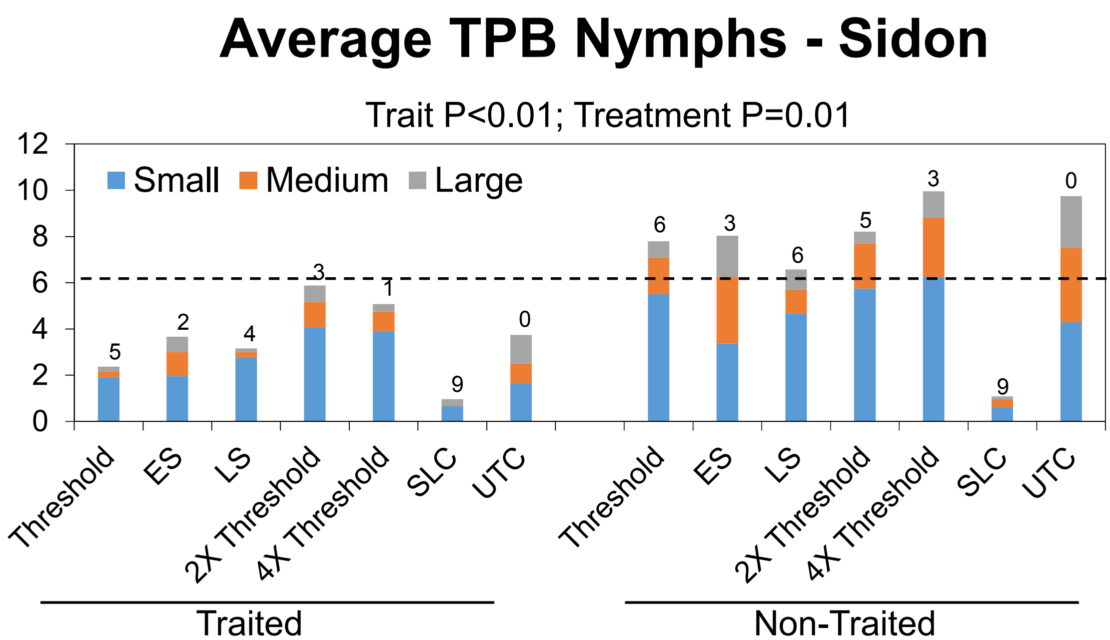

Figure 1. Average number of tarnished plant bug nymphs per 10 row ft. in traited and non-traited plots in Sidon, MS and individual insecticide applications/treatment. Means followed by the same letter do not differ significantly at *P* = 0.05.

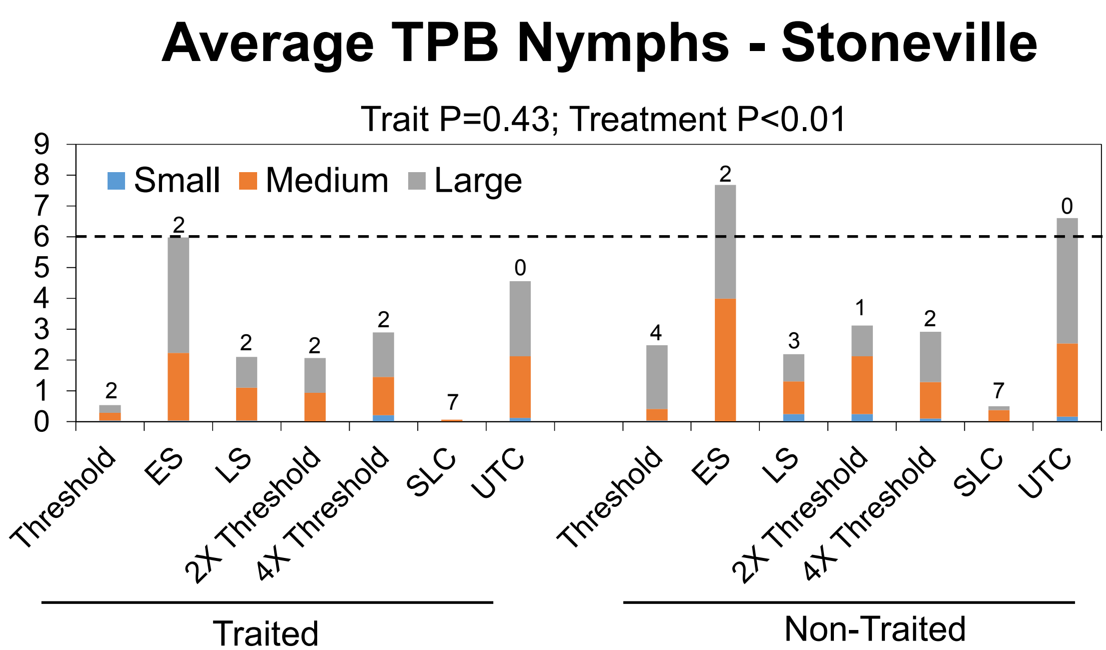

Figure 2. Average number of tarnished plant bug nymphs per 10 row ft. in traited and non-traited plots in Stoneville, MS and individual insecticide applications/treatment. Means followed by the same letter do not differ significantly at *P* = 0.05.

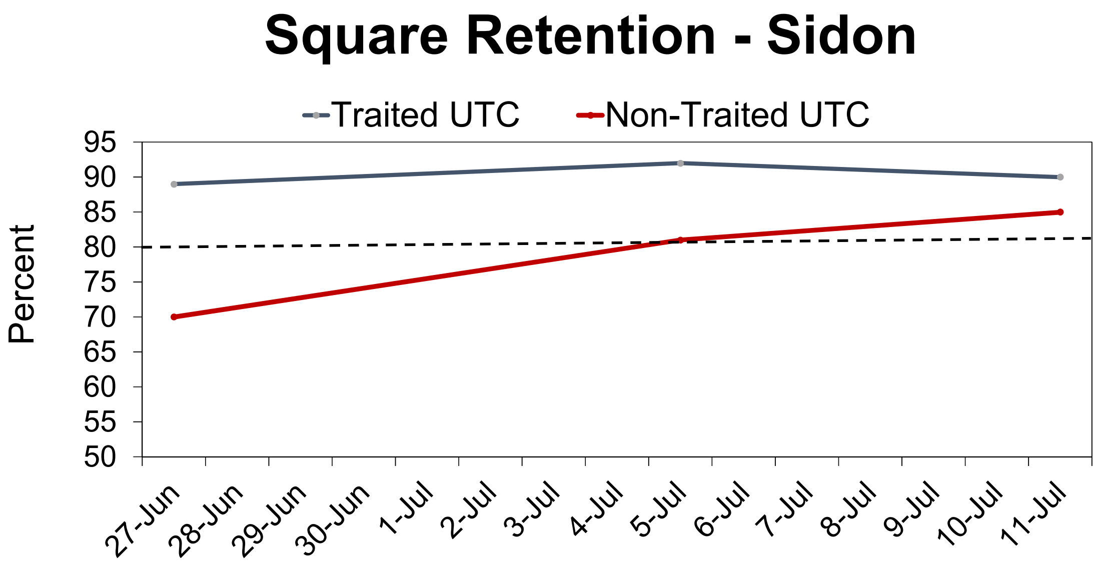

Figure 3. Square retention in traited and non-traited plots in Sidon, MS 2016.

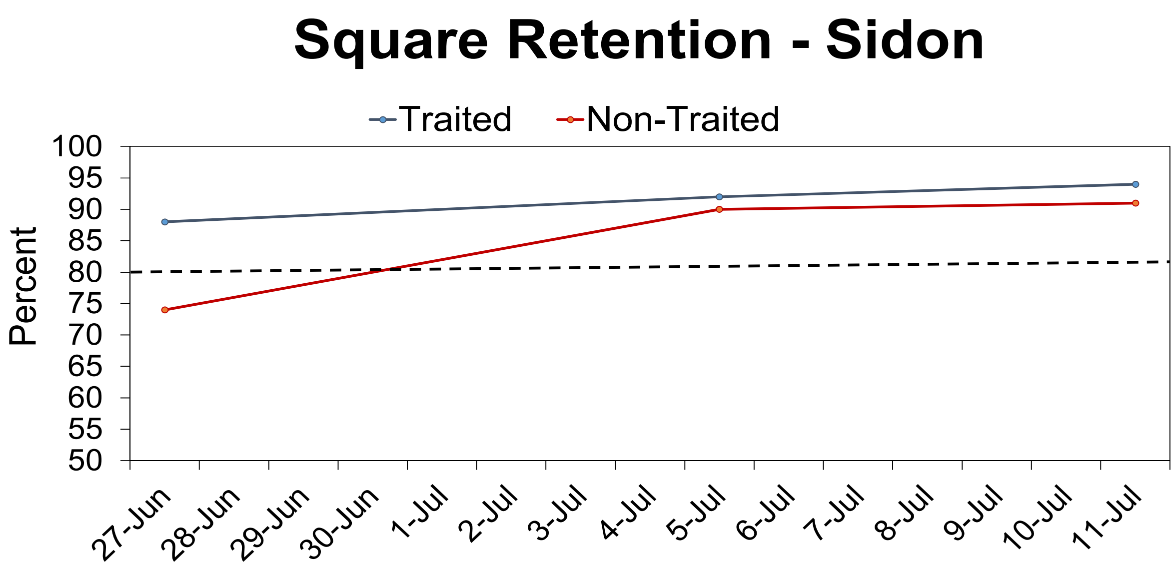

Figure 4. Square retention in traited and non-traited plots in Stoneville, MS 2016.

There was a significant trait by spray treatment interaction for cotton yields at the Sidon, MS location (Figure 5). Cotton yields from the traited plots were significantly higher than the non-traited plots for the current threshold (threshold), late season only control (LS), double threshold (2X), and the untreated control (UTC) treatments. In contrast, no differences between traited and non-traited were observed for the early season only (ES), quadruple threshold (4X), and season long control (SLC) treatments.

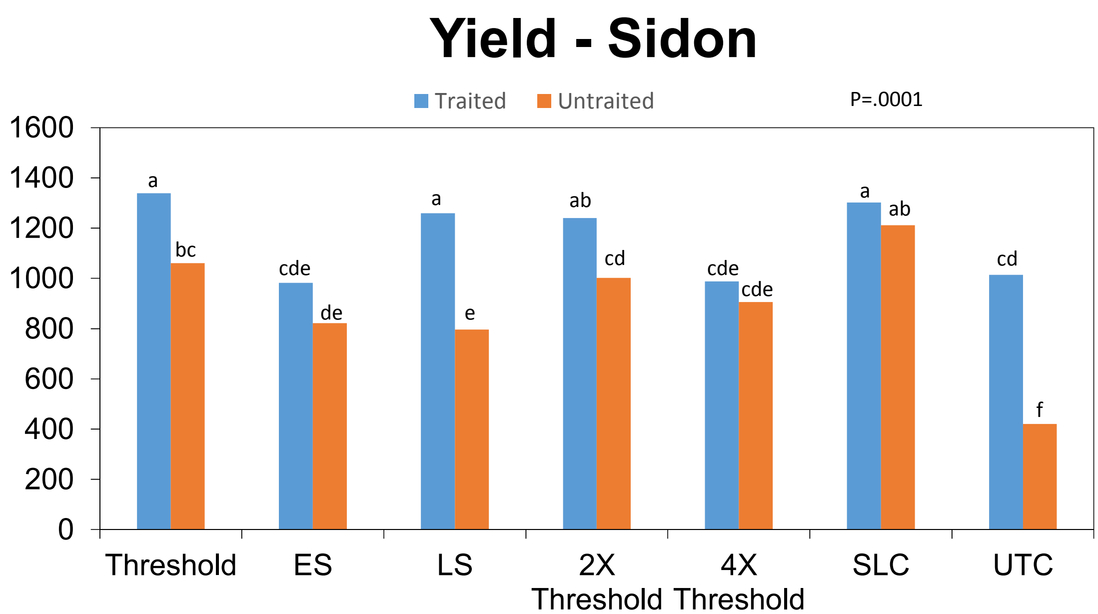

Figure 5. Yield data in Sidon, MS. Yield expressed in lbs./lint acre. Means followed by the same letter do not differ significantly at $P = 0.05$.

There was a significant trait by spray treatment interaction for cotton yields at the Stoneville, MS location (Figure 6). Cotton yields from the traited plots were significantly higher than the non-traited plots for the early season only control (ES), late season only control (LS), double threshold (2X), and untreated control (UTC) treatments. In contrast, no differences in yield were observed between the traited and non-traited cotton for the current threshold (threshold) and season long control (SLC) treatments.

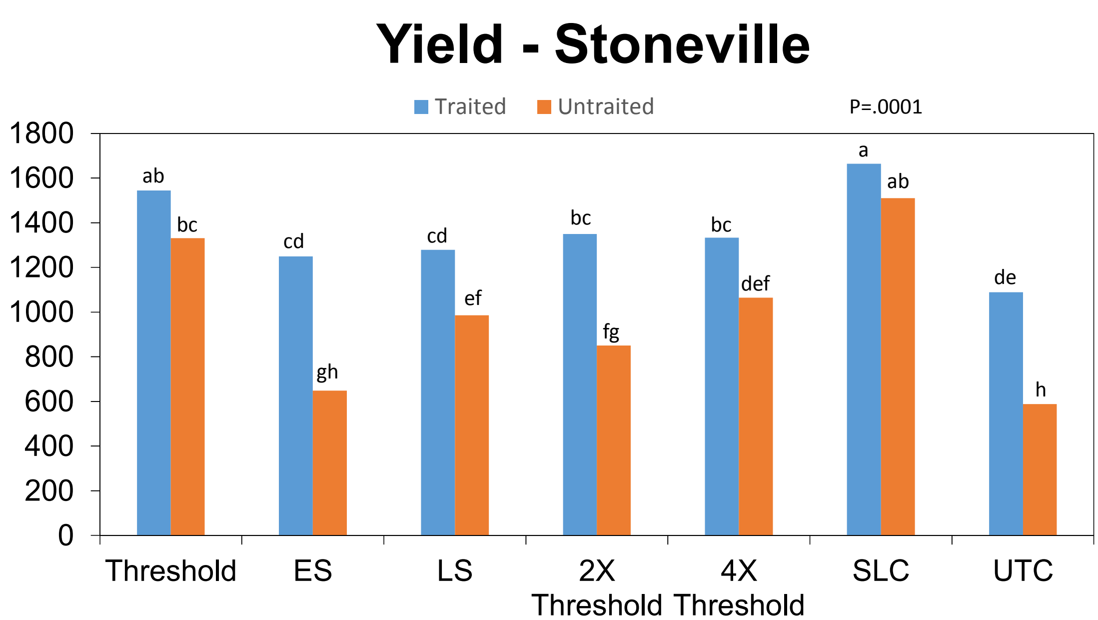

Figure 6. Yield data in Stoneville, MS. Yield expressed in lbs./lint acre. Means followed by the same letter do not differ significantly at $P = 0.05$.

Across both locations more insecticide applications were triggered in the non-traited plots compared to the traited plots for all of the threshold treatments (Figure 7). Traited treatments triggered an average of 2.5 applications across both locations and all threshold treatments compared to an average of 3.5 applications in non-traited treatments. This resulted in an average of 1.0 less applications in traited treatments compared to non-traited treatments across both locations.

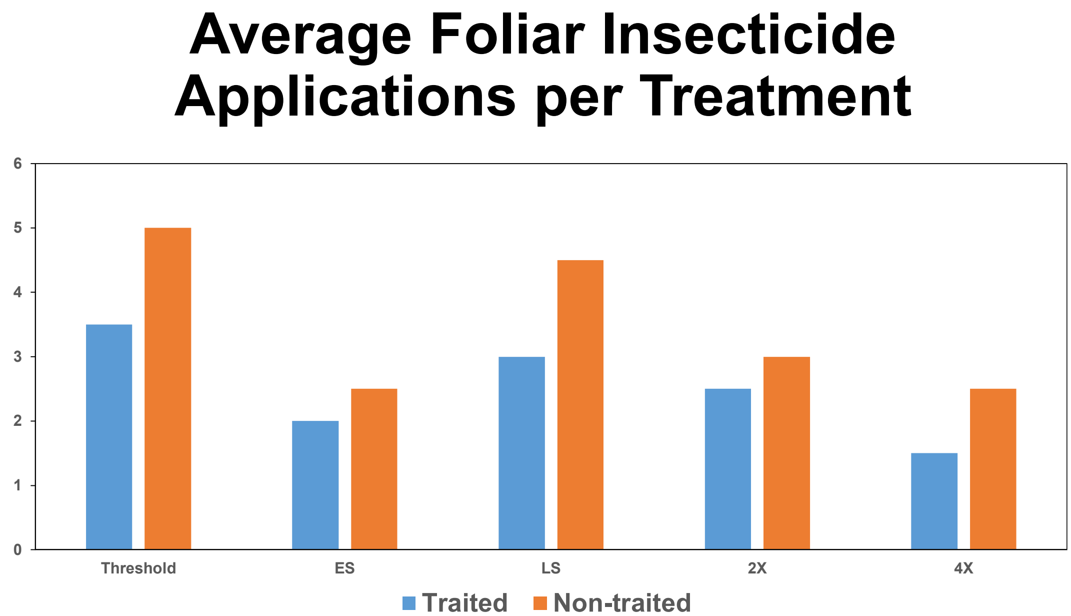

Figure 7. Number of foliar insecticide applications per treatment averaged across both Sidon, MS and Stoneville, MS locations.

Summary

Lygus Bt traited cotton at the Sidon location resulted in significantly fewer tarnished plant bug nymphs and fewer damaged squares in the unsprayed control (UTC) plots. Additionally, higher square retention and higher yields were observed for the *Lygus* traited cotton in four of the seven comparative treatments. Fewer insecticide applications were triggered in the traited cotton compared to the untraited cotton. At the Stoneville location, the *Bt* trait resulted in significantly fewer damaged squares in the unsprayed control (UTC) plots, and higher square retention and higher yields in five of the seven comparative treatments, and fewer insecticide applications triggered.

These data indicate that this new transgenic cotton variety will save cotton growers at least one to two insecticide applications for tarnished plant bugs per growing season. These preliminary data suggest that the *Lygus Bt* trait may allow for an increased threshold for tarnished plant bugs, but an additional year of research is needed. Square retention data also indicated that cotton growers can expect to retain a higher number of squares which may result in fewer sprays and higher yields.

Any technology allowing farmers to produce higher yields while saving money on insecticide applications is a tremendous advantage in the current agricultural industry. Because the *Bt* trait was not 100% effective on *Lygus* in these trials, more research is needed to develop the most efficient threshold for managing tarnished plant bugs. It is also crucial to educate agricultural consultants, salesmen, and farmers in the industry on this product so that timely, effective application decisions are made in order to maximize the potential of this new technology.

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