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Field Study Investigating Cry51Aa2.834_16 in Cotton for Control of Thrips (Thysanoptera: Thripidae) and Tarnished Plant Bugs (Hemiptera: Miridae)

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Abstract

Field experiments were conducted in 2016 and 2017 in Tennessee to evaluate the effects of a novel *Bacillus thuringiensis* (Bt)-transgenic toxin, Bt Cry51Aa2.834_16, on thrips (Thysanoptera: Thripidae) and tarnished plant bug, *Lygus lineolaris* (Palisot De Beauvois) (Hemiptera: Miridae), in cotton. Protection from thrips injury with the Bt trait was as good or better than an insecticide-based approach. The use of the Bt trait resulted in reduced numbers of immature tarnished plant bug, particularly large nymphs, and partial protection from plant bug injury. Cotton that expressed Bt Cry51Aa2.834_16 had greater yields than the non-Bt isolate when insecticides were not used. Although Bt Cry51Aa2.834_16 reduced the need for insecticide applications, foliar-applied insecticide applications were needed to provide adequate plant protection from tarnished plant bug. The currently recommended treatment thresholds for tarnished plant bug performed similarly well for Bt Cry51Aa2.834_16 and non-Bt isolines. Insecticide applications for tarnished plant bug increased fiber quality, while Bt Cry51Aa2.834_16 had minor effects. The Bt-transgenic toxin Cry51Aa2.834_16 is expected to reduce the need for insecticide applications targeting thrips and tarnished plant bug and could be a valuable addition to an overall insect management program in cotton.

Key words: thrips, tarnished plant bug, Bt cotton, Cry51Aa2.834_16, insecticide

Cotton, *Gossypium hirsutum* L., is one of the top three cash crops grown in the Mid-South (USDA NASS 2017). Over the last 10 yr, thrips (Thysanoptera: Thripidae) have ranked among the top three insect pests in cotton based on costs to producers (Cook 2018). Among the thrips species that attack seedling cotton, tobacco thrips, *Frankliniella fusca* (Hinds), are the most common species found in the Mid-South and Southeast (Layton and Reed 2002, Reed and Jackson 2002, Reed et al. 2006, Stewart et al. 2013). Thrips injury can lead to stunted growth, delayed maturity, reduced stands, and yield loss (Layton and Reed 2002, Stewart and Lentz 2010). Currently, thrips are primarily managed using prophylactic, at-planting insecticide applications, such as seed or in-furrow treatments. The neonicotinoid seed treatments, namely imidacloprid and thiamethoxam, are widely used by cotton growers (Cook et al. 2011) and can provide protection for several weeks after planting (Graham et al. 1995), but there have been growing concerns about thrips resistance to neonicotinoids. Documented control issues in the mid-south (Darnell et al. 2015, 2016) and the southeast (Huseth et al. 2016) have led to the need for new ways to combat this pest.

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), typically ranks as the most important insect

pest of cotton in the Mid-South (Cook 2018). This pest can feed on plants at any growth stage, but most economic damage is done from the onset of squaring (flower buds) through the blooming period (Layton 2000). Tarnished plant bug can cause the abscission of squares and bolls, resulting in substantial yield loss (Scales and Furr 1968, Scott et al. 1985, Layton 1995, Russell 1999). Foliar-applied insecticide applications are often used to manage infestations of tarnished plant bug. Insecticides are often rotated and/or tank mixed to help maintain effective control (Catchot et al. 2014), but the tarnished plant bug has developed resistance to several insecticides (Cleveland and Furr 1979, Snodgrass and Scott 1988, Snodgrass 1996, Snodgrass and Scott 2000, Snodgrass 2006, Snodgrass et al. 2009, Parys et al. 2017). This has made management of tarnished plant bug increasingly difficult, leading to the need for new methods to manage this pest.

The use of transgenic cotton expressing *Bacillus thuringiensis* (Bt) has been widely adopted (Fernandez-Cornejo et al. 2014). These cotton varieties express toxins targeting the control of lepidopteran pests, including corn earworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.) (Siebert et al. 2008).

The adoption of these Bt varieties caused a shift from lepidopteran pests to other pests, especially the tarnished plant bug in the Mid-South. (Musser et al. 2009). Current Bt crops do not provide control of hemipteran insects like the tarnished plant bug. However, Monsanto Company (St. Louis, MO) has developed a new Bt protein, Cry51Aa2.834_16, referred to as Bt Cry51Aa2 from here on. This toxin has insecticidal activity against several insect pests in the family Miridae, including *Pseudatomoscelis seriatus* (Reuter), *Lygus hesperus* (Knight) (Hemiptera: Miridae) and tarnished plant bug (Baum et al. 2012). In 2016, Gowda et al. reported the incorporation of this Bt trait into cotton (Gowda et al. 2016). In addition to insecticidal activity against mirids, this protein also has activity against at least some thrips (Bachman et al. 2017). Thus, the objective of this study was to evaluate the efficacy and potential impact of this novel Bt toxin on the management of thrips and tarnished plant bug, both being important pests of cotton in the Mid-South.

Materials and Methods

Plot Establishment

Experiments were conducted in 2016 and 2017 at the West Tennessee Research and Education Center (WTREC) in Jackson, TN and the Research and Education Center at Milan (MREC) in Milan, TN. Prior to planting, seeds were treated with a fungicide seed treatment (Trilex Advanced; Bayer Crop Science, Raleigh, NC) at a standard recommended rate. Trials in 2016 were planted on 5 May at WTREC and on 9 May at MREC. In 2017, trials were planted on 9 May at WTREC and on 10 May at MREC. A seeding rate of ~13.2 seeds per meter was used in all cases and all tests were planted into no-till production fields. Plots at WTREC were irrigated with a lateral irrigation system and plots at both locations were fertilized and managed for weeds according to University of Tennessee Extension recommendations (Steckel et al. 2016, Savoy and Joines 1996).

Treatment Factors

Treatments were arranged in a split-split-plot experimental design within a randomized complete block with four replications. Main plots were 16 rows wide (0.97 m centers at WTREC and 1.02 m at MREC) and 10.7 m long and consisted of three foliar-applied insecticide regimens for tarnished plant bug including 1) no applications, 2) applications based on currently recommended thresholds, and 3) automatic/aggressive applications made weekly. Sub-plots were eight rows of either Bt Cry51Aa2 or non-Bt. Seed for each was provided by Monsanto Company (St. Louis, MO) and were near-isogenic lines of the same variety (DP 393). Sub-subplots were four rows wide and consisted of fungicide-only treated seed or seed treated with a fungicide and an insecticide seed treatment (IST). Aeris (Bayer CropScience, Raleigh, NC), a combination of imidacloprid and thiodicarb, was used as the IST and applied at a standard rate of 0.75 mg ai/seed. Cotton treated with Aeris was also treated with 271.81 g ai/ha of acephate (Orthene 97S, Amvac Chemical Company, Walnut Creek, CA) at the first true-leaf stage.

The treatment threshold and foliar insecticides used to control tarnished plant bug were those recommended in the *Insect Control Recommendations for Field Crops* for Tennessee (Stewart et al. 2017). For plots treated according to threshold, the decision to treat was made independently for each *B. thuringiensis* by IST factor combination based on average pest density in the four sub-subplots. During the squaring period, the threshold was defined as eight bugs per 100 sweeps or when square retention fell below 80%. At the initiation of bloom, a threshold level of three bugs per 1.52 row m,

based on drop cloth sampling was used to make treatment decisions. Automatic applications of insecticides for tarnished plant bug in the aggressive treatment regimen were made weekly beginning the first week squares were observed. The insecticides used varied depending upon the time of season and other hemipteran pest species such as clouded plant bug, *Neurocolpus nubilus* (Say) (Hemiptera: Miridae), and stink bug (Hemiptera: Pentatomidae) infestation levels. Foliar tank-mixed applications of imidacloprid (Admire Pro, Bayer Crop Science, Raleigh, NC) at a rate of 68.4 g ai per ha and thiamethoxam (Centric 40WG, Syngenta Crop Protection, Inc., Greensboro, NC) at a rate of 35.0 g ai per ha were primarily used control tarnished plant bugs prior to bloom. Around the first week of bloom, novaluron (Diamond 0.83EC, ADAMA USA, Raleigh, NC) was added to the tank mixture at a rate of 43.6 g ai per ha. Once blooming began, we used treatments of sulfoxaflor (Transform WG, Dow AgroSciences, Indianapolis, IN) at a rate of 52.5 g ai per ha or tank-mixtures of acephate (Orthene 97, Valent USA, Walnut Creek, CA) and bifenox (Brigade 2EC, FMC Corporation, Princeton, NJ) at 727.9 g and 87.5 g of ai per ha, respectively. Since tested lines did not express a Bt toxin for control of lepidopteran pests, applications of chlorantraniliprole (Prevathon, DuPont Crop Protection, Newark, DE) were made at 75.3 g ai per ha to minimize any potential effects of lepidopteran pests while having minimal effects on tarnished plant bug populations.

Thrips Sampling

Cotton was sampled at the 1.5 and 3.5 leaf stage to estimate the density of thrips. Five plants were sampled from each sub-subplot. Samples at the 1.5 leaf stage were collected 5–6 d after the foliar application of acephate was made to cotton with an IST. Plants were cut at the ground level and placed in 16 oz jars containing 70% ethyl alcohol. Each plant was taken out of the jar and rinsed with 70% ethyl alcohol over a glass container topped with a sieve (150 µm) to collect the thrips. The jar was then rinsed with 70% ethyl alcohol over the sieve to collect any remaining thrips left inside. The sieve was then rinsed with 70% ethyl alcohol into a grid-ded 100 × 15 mm Petri dish and the thrips counted underneath a microscope. Thrips were counted and categorized as either adult or immature. Adult thrips were classified as either tobacco thrips, soybean thrips, *Neohydatothrips variabilis* (Beach), or other thrips. The same procedure was used for collections at the 3.5 leaf stage, except plants were placed in a 32 oz plastic bag, without alcohol, in order to collect plant biomass data prior to washing thrips from the plants. The fresh weight of each sample, minus the weight of the bag, was recorded and the samples were placed in a refrigerator until thrips were counted within the next 24 h.

Whole-plot, visual ratings of thrips injury and vigor were taken at the same growth stages. Vigor ratings were made on a 0–5 basis, with 0 representing no living plants in the plot and 5 representing maximum vigor. Thrips ratings were also on a 0–5 scale, with 0 representing no injury to any plant in the plot and 5 no living plants in the plot. Also, total squares per 1-m row were counted in each sub-sub plot at the first week of squaring to help assess thrips injury effect on cotton maturity.

Tarnished Plant Bug Sampling

Methods of estimating tarnished plant bug infestations and injury varied depending upon the growth stage of the cotton. Weekly sampling of tarnished plant bug began at first-square. Prior to flowering, samples were taken from the center two rows with a 38.1-cm diameter sweep net by taking 25 sweeps in each sub-subplot. Square

retention was monitored by examining the first position fruiting sites on the top two nodes, excluding the terminal node, of plants until 25 sites were examined in each sub-subplot. The number of retained squares was recorded. A square was considered missing if it abscised when touched or the bracts were flared. Beginning at the third week of squaring and through much of the blooming period, tarnished plant bug densities were estimated using a black drop cloth. Samples were taken by laying the cloth between two cotton rows near the center of the plot and vigorously shaking all of the plants from each row. One sample resulted in 1.52 m of row being sampled. Tarnished plant bug nymphs and adults were recorded separately. Tarnished plant bug nymphs were visually separated based on size as either small (first or second instars) or large (third, fourth, and fifth instars). Sampling was terminated when cotton reached five nodes above white flower plus 350 heat units (DD60s°F). Numbers of clouded plant bugs and stink bugs were also recorded at each sample date, as these pests could potentially be impacted by this technology and have influence on fruit injury and yield.

To determine overall fruit retention during the squaring period, a total of 10 plants per sub-subplot was sampled from the middle two rows to estimate percent retention of first position fruiting structures at the first week of bloom. Plants were examined at each fruiting branch and the number of present first position fruit per node were recorded.

Yield

In all trials, the center two rows of each sub-subplot were harvested and seed cotton weights were recorded. At the WTREC location, seed cotton samples (≈ 1.0 kg) from each sub-subplot were collected in $15.2 \times 9.5 \times 27.3$ cm paper sacks to be sampled in a table-top gin to determine lint turnout and fiber quality. Because this Bt Cry51Aa2 is under USDA regulation, great care was made to keep collected seed cotton samples in a contained and restricted environment until ginned, and all seed from the ginning process were devitalized before disposal.

Data Analysis

Sample date was not included in the model because insecticides were applied to each treatment and in each test independently. Each treatment received insecticide applications a different number of times and at different timings during the season, making interpretation of data difficult. Therefore, numbers of thrips, thrips injury ratings, plant vigor, pre-bloom square retention, tarnished plant bug

numbers in sweep nets, and tarnished plant numbers on drop cloths were analyzed across all sample dates to show the overall impact of each management strategy on those variables. All data were analyzed using a general linear mixed model of analysis of variance PROC GLIMMIX of SAS (Version 9.4, SAS Institute, Cary, NC). IST, Bt Cry51Aa2 (trait) and spray regimen (spray) were designated as fixed effects. Year, location, year by location, and replication nested within year by location were designated as random effects to allow inferences to be made over a range of environments (Carmer et al. 1989, Blouin et al. 2011). Degrees of freedom were estimated using the Kenward-Roger method (Kenward and Roger 2009). Means were estimated using LSMEANS and separated based on Fisher's protected least significant difference (LSD) ($\alpha = 0.05$). Three-way interactions were not included in the model for thrips related data because foliar applications for tarnished plant bug had not been initiated at this time.

Results

Thrips

A significant interaction of IST by trait on the average total number of adult and immature thrips ($F = 70.16$; $df = 1, 364$; $P < 0.001$) was observed. The majority of thrips found were immatures (78.4%). Based on adults, tobacco thrips composed nearly 71% of the population. The most thrips, $78.1 \pm 4.95/5$ plants (mean \pm SE) were found on non-Bt cotton that did not have an IST (Fig. 1, Supp. Table S1). No significant difference between total thrips on Bt Cry51Aa2 cotton without an IST and non-Bt cotton treated with an IST and a foliar insecticide application was observed. The fewest number of thrips ($7.8 \pm 0.66/5$ plants) was found on Bt Cry51Aa2 cotton treated with an IST and a foliar insecticide application (Fig. 1, Supp. Table S1).

For thrips injury, an interaction of IST by trait ($F = 444.1$; $df = 1, 356$; $P < 0.001$) was observed. Non-Bt cotton without an IST had more thrips injury (3.02 ± 0.08) than all other treatments (Fig. 1, Supp. Table S1). Bt Cry51Aa2 cotton without an IST had less thrips injury than non-Bt cotton treated with an IST and a foliar insecticide application, and Bt Cry51Aa2 cotton treated with an IST and a foliar application of acephate had the least injury (0.64 ± 0.03) (Fig. 1, Supp. Table S1).

There was no effect of trait on average aboveground biomass of seedlings ($F = 0.04$; $df = 1, 146.8$; $P = 0.843$). However, an effect of IST was observed ($F = 43.89$; $df = 1, 146.8$; $P < 0.001$) where cotton treated with an IST had greater biomass than cotton without an IST (Fig. 1, Supp. Table S1). No interaction between IST and trait was

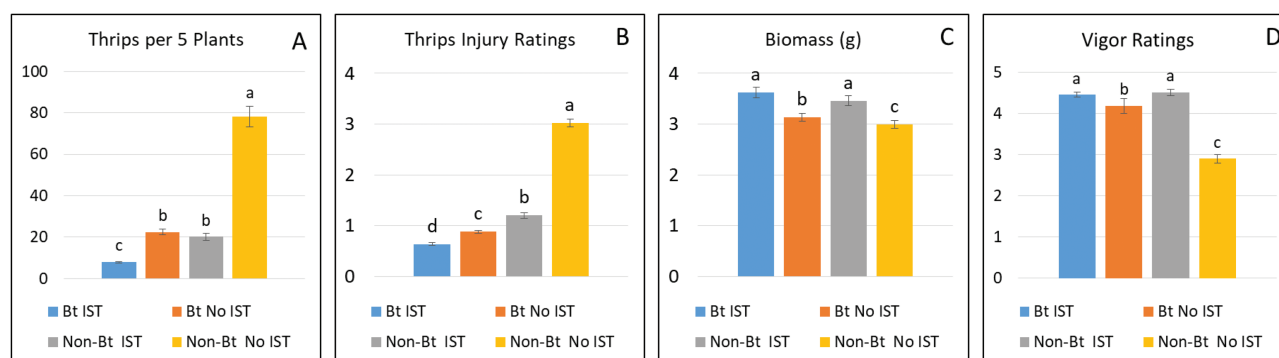


Fig. 1. Average total number of (A) thrips per five plants, (B) average thrips injury rating, (C) average aboveground biomass per five plants, and (D) average vigor ratings for Bt Cry51Aa2 and non-Bt cotton, with and without an IST averaged across 2 yr and two locations. Error bars represent 95% confidence intervals. Common letters above bars indicate treatments are not different (Fisher's protected LSD, $\alpha = 0.05$). Visual rating of thrips injury on a 0–5 scale where 0 represents no injury to any plant in a plot. Visual rating of plant vigor on a 0–5 scale where 0 indicates no living plants in a plot.

found, although it did approach significance ($F = 3.78$; $df = 1, 146.8$; $P = 0.054$). There was an interaction of IST by trait on whole plot vigor ratings ($F = 35.80$; $df = 1, 85$; $P < 0.001$). Similar to biomass, cotton treated with an IST had higher vigor ratings, regardless of trait. However, the Bt Cry51Aa2 cotton without an IST had more vigor (4.18 ± 0.18) than non-Bt cotton that did not have an IST (2.90 ± 0.10) (Fig. 1, Supp. Table S1).

The use of an IST ($F = 1.15$; $df = 1, 173$; $P = 0.284$) did not affect the total number of squares present during the first week of squaring. However, there was an effect of trait ($F = 5.17$; $df = 1, 173$; $P = 0.024$), with more squares in Bt Cry51Aa2 cotton (26.29 ± 1.44) than in non-Bt cotton (24.20 ± 1.23). No interaction of IST by trait was found ($F = 0.15$; $df = 1, 173$; $P = 0.067$).

Tarnished Plant Bug

Based on current threshold recommendations for tarnished plant bug, 1 to 7 insecticide applications were needed to manage tarnished plant bug depending on the year and test location (Fig. 2, Supp. Table S2). In both years, more insecticide applications were needed at the Jackson location than in Milan, and more applications were required on non-Bt cotton than the Bt Cry51Aa2 cotton. On average across the four trials, the Bt Cry51Aa2 cotton required 1.25 fewer insecticide applications for tarnished plant bug than non-Bt cotton when treated according to threshold recommendations.

Unless indicated, three-way interactions were not significant ($P > 0.05$) and are not discussed. No two-way interactions were found for IST by trait ($F = 0.00$; $df = 1, 825.7$; $P = 0.959$), IST by spray ($F = 1.26$; $df = 2, 825.7$; $P = 0.284$), or trait by spray ($F = 1.67$; $df = 2, 827.7$; $P = 0.189$) on the average number of tarnished plant bug adults found prior to bloom. IST did not have significant effect on the average number of tarnished plant bug adults found on cotton prior to bloom ($F = 0.49$; $df = 1, 825.7$; $P = 0.483$), but there was a significant effect of trait ($F = 14.94$; $df = 1, 825.7$; $P < 0.001$) and of spray regimen ($F = 21.54$; $df = 2, 825.7$; $P < 0.001$). About 23% more adults were found on non-Bt cotton (2.68 ± 0.13) than on Bt Cry51Aa2 cotton (2.07 ± 0.11). Also, there were significantly more adult tarnished plant bug found in cotton that was not sprayed with insecticides (3.00 ± 0.18) compared with the other treatment regimens, and more adult tarnished plant bug were found in cotton

managed using the threshold approach (2.39 ± 0.14) compared with the more aggressive treatment regimen (1.73 ± 0.11).

Overall, square retention stayed above 80%, regardless of the treatment factors (Fig. 6). However, a three-way interaction of IST by trait by spray regimen on average square retention was observed ($F = 3.53$; $df = 2, 824.4$; $P = 0.029$). Square retention in Bt Cry51Aa2 cotton stayed above the average retention of the trial treatments ($91.04 \pm 0.86\%$), regardless of IST or spray regimen (Fig. 6). Square retention was greater in the Bt Cry51Aa2 cotton than the non-Bt cotton when no insecticide applications were made. Unless the non-Bt cotton had an IST and was sprayed automatically, its retention was below the average retention of the trial treatments (Fig. 6). At first bloom, first position square retention was mapped to determine the overall retention during the squaring period of the season. There was no interaction of IST by trait ($F = 0.01$; $df = 1, 165$; $P = 0.918$) or IST by spray regimen ($F = 2.26$; $df = 2, 165$; $P = 0.108$). There was an interaction of trait by spray regimen ($F = 3.33$; $df = 2, 165$; $P = 0.039$). In non-Bt cotton, there was significantly higher square retention in the threshold approach ($81.77 \pm 1.25\%$) compared to unsprayed ($74.97 \pm 2.04\%$). In the Bt Cry51Aa2 cotton, there was no difference between the threshold approach ($85.08 \pm 0.76\%$) and cotton unsprayed for tarnished plant bug ($82.91 \pm 1.05\%$). Significantly higher square retention was observed in Bt Cry51Aa2 cotton sprayed aggressively with insecticides ($90.39 \pm 0.86\%$) compared to all other treatments, followed by non-Bt cotton sprayed aggressively ($87.14 \pm 0.98\%$). Non-Bt cotton that was not treated for tarnished plant bug had the lowest overall square retention.

The majority of tarnished plant bug observed from samples taken during the blooming period were nymphs (92.4%). There was an interaction of IST by spray regimen on the average number of tarnished plant bug nymphs found in drop cloth samples ($F = 7.58$; $df = 2, 847.1$; $P < 0.001$). Significantly more nymphs were found in cotton not treated with foliar insecticides for tarnished plant bug than all other treatments, followed by cotton treated with an IST but not treated for tarnished plant bug (Fig. 3, Supp. Table S3). Significantly more nymphs were observed in cotton without an IST compared to cotton treated with an IST when no insecticide applications were made for tarnished plant bug. However, when insecticides were used for tarnished plant bug, there was no difference in the

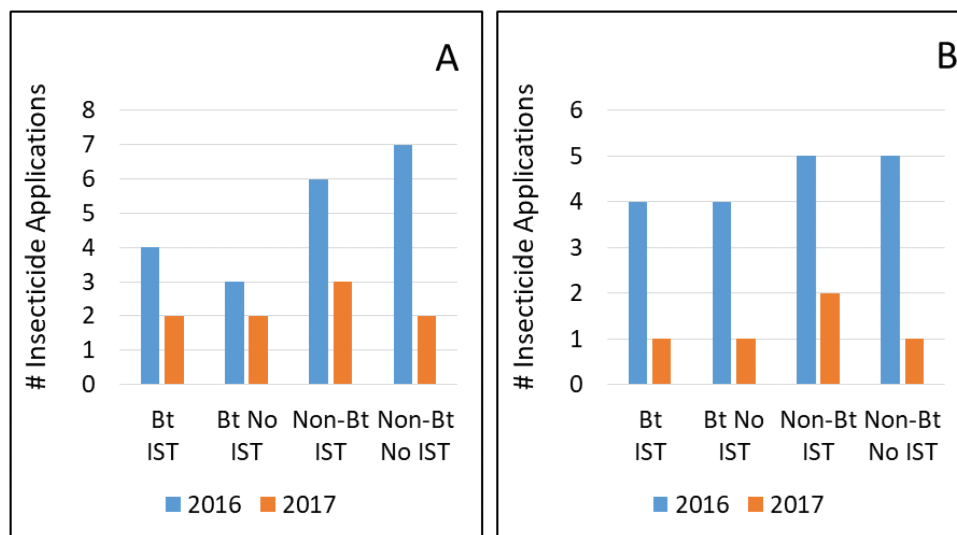


Fig. 2. Total number of foliar insecticide applications made to manage tarnished plant bugs in Bt Cry51Aa2 and non-Bt cotton managed using a threshold approach, with and without an IST in (A) Jackson and (B) Milan, TN.

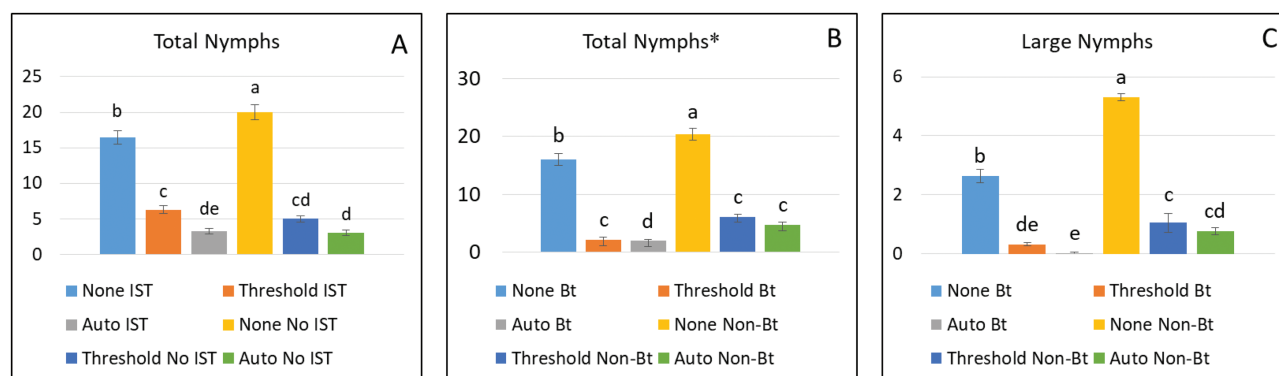


Fig. 3. Average total number of tarnished plant bug nymphs found per 3.02 m row (SEM) of blooming cotton averaged across 2 yr and two locations. (A) Cotton with and without an IST and managed for tarnished plant bug with different spray regimens (automatic [auto], threshold or none). (B) Bt Cry51Aa2 and non-Bt cotton managed for tarnished plant bug with different spray regimens. Error bars represent 95% confidence intervals. Common letters above bars indicate treatments are not different (Fisher's protected LSD, $\alpha = 0.05$). *Interaction of trait and spray regimen for total nymphs approached significance ($P = 0.0591$).

number of nymphs observed regardless of using an IST (Fig. 3, Supp. Table S3). There was an interaction of trait and spray regimen on tarnished plant bug nymphs ($F = 3.06$; $df = 2, 846.7$; $P = 0.047$). In plots unsprayed or sprayed automatically for tarnished plant bug, significantly more nymphs were found in non-Bt cotton than the Bt Cry51Aa2 cotton. No difference in the number of nymphs was observed between Bt Cry51Aa2 cotton and non-Bt cotton when managed for tarnished plant bug using the threshold approach (Fig. 3, Supp. Table S3).

For the average number of large tarnished plant bug nymphs found per 3.05 row m during bloom, no interactions of IST by trait were found ($F = 3.14$; $df = 1, 831.8$; $P = 0.077$) or IST by spray regimen ($F = 0.19$; $df = 2, 831.7$; $P = 0.824$). However, an interaction of trait by spray regimen was observed ($F = 21.23$; $df = 2, 830.5$; $P < 0.001$). There were more large nymphs in non-Bt cotton not sprayed for tarnished plant bug (5.31 ± 0.33) followed by Bt Cry51Aa2 cotton not sprayed for tarnished plant bug (2.64 ± 0.23). However, there was no difference in the number of large nymphs observed in non-Bt cotton sprayed on threshold or automatically (Fig. 3, Supp. Table S3). There were fewer large tarnished plant bug nymphs found in the Bt Cry51Aa2/threshold treatment than the

non-Bt/threshold treatment (Fig. 3, Supp. Table S3). For all spray regimens, significantly fewer nymphs were observed on Bt Cry51Aa2 cotton than on non-Bt cotton.

There were no significant main or interaction effects of IST, trait, or spray regimen for stink bugs or clouded plant bugs (Supp. Table S4). In untreated plots, an average of 0.13 ± 0.02 stink bugs and 0.23 ± 0.01 clouded plant bugs were found per 10 row feet during bloom.

Yield

For seed-cotton yield interactions of IST by trait ($F = 6.49$; $df = 1, 177$; $P = 0.012$) and trait by spray regimen were found ($F = 4.36$; $df = 2, 177$; $P = 0.014$). Yields were higher when there was some type of thrips control, either an IST or the Bt trait, compared with non-Bt cotton without an IST (Fig. 4, Supp. Table S5). Cotton treated aggressively for tarnished plant bug out-yielded the other spray regimens and there was no difference between the Bt Cry51Aa2 ($4,190 \pm 157.07$ kg/ha) and non-Bt cotton ($4,006 \pm 194.78$ kg/ha). However, the Bt Cry51Aa2 cotton yielded approximately 17% more than the non-Bt cotton when it was not sprayed for tarnished plant bug (Fig. 4, Supp. Table S6). There was not a significant difference in

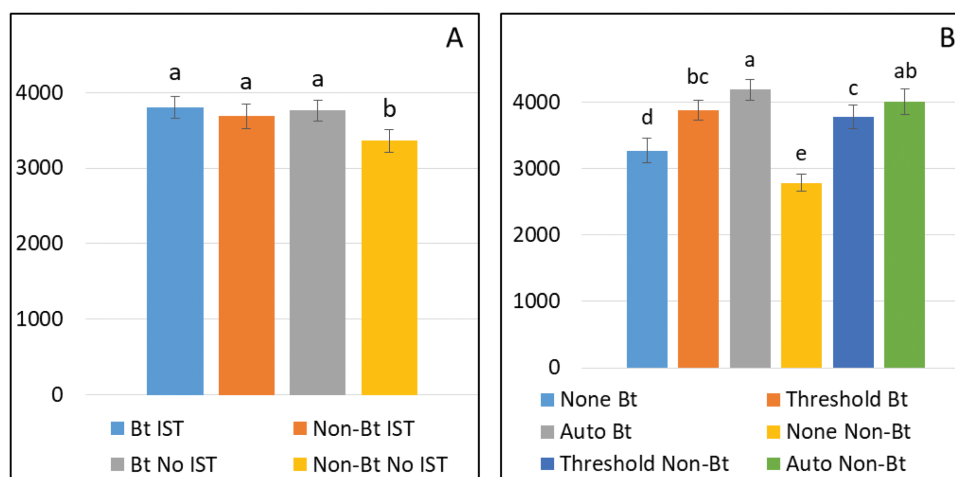


Fig. 4. (A) Average total kilograms of seed-cotton per hectare for Bt Cry51Aa2 and non-Bt cotton treat with and without an IST averaged across 2 yr and two locations. (B) Average total kilograms of seed-cotton per hectare for Bt Cry51Aa2 and non-Bt cotton managed for tarnished plant bug with different spray regimens (automatic [auto], threshold or none) averaged across 2 yr and two locations. Error bars represent 95% confidence intervals. Common letters above bars indicate treatments are not different (Fisher's protected LSD, $\alpha = 0.05$).

yield between the non-Bt cotton managed with automatic insecticide applications and the Bt Cry51Aa2 cotton managed using the threshold approach, and there was also no difference between the yields of Bt or non-Bt cotton managed using the threshold approach for tarnished plant bug management (Fig. 4, Supp. Table S6).

Percent gin turnout averaged 42.3% and was not affected by any of the model parameters (Table 1). Trait had an effect on fiber length and uniformity (Table 1), with the average length of non-Bt cotton (3.05 ± 0.01 cm) being longer than Bt Cry51Aa cotton (3.02 ± 0.01 cm), and non-Bt ($84.37 \pm 0.11\%$) cotton had higher uniformity than Bt Cry51Aa2 cotton ($84.01 \pm 0.13\%$). The IST did not affect any fiber properties. For fiber length, when no seed treatment was used, fiber length was 3.05 cm compared to when an IST was used, fibers were 3.03 cm long. Non-Bt cotton generally had higher fiber strength than the Bt Cry51Aa2 cotton, but an interaction of trait and spray regimen on fiber strength was found where non-Bt cotton that was not sprayed for tarnished plant bug was stronger ($=34.35 \pm 0.31$) than all other treatments (Table 1). The non-Bt/automatic spray regimen ($=33.48 \pm 0.37$) was not different than non-Bt/threshold ($=33.11 \pm 0.25$) or Bt Cry51Aa2/threshold ($=32.89 \pm 0.24$), but the non-Bt/automatic spray was stronger than the Bt Cry51Aa2/not sprayed ($=32.62 \pm 0.32$) and the Bt Cry51Aa2/automatic ($=32.55 \pm 0.34$) treatments (Table 1).

Micronaire was significantly affected by both trait and spray regimen (Table 1, Fig. 5, Supp. Table 7) Micronaire was higher in non-Bt

cotton (4.64 ± 0.04) than in Bt Cry51Aa2 cotton (4.54 ± 0.04). Applying insecticides decreased micronaire. Cotton not treated for tarnished plant bug had higher micronaire (4.76 ± 0.05) than all other treatments. Cotton sprayed aggressively for tarnished plant bug had higher micronaire (4.58 ± 0.04) than cotton sprayed based on threshold (4.43 ± 0.04). Similarly, reflectiveness was also affected by trait and spray regimen (Table 1). A higher reflectance was found in Bt cotton (72.68 ± 0.17) than in the non-Bt cotton (71.78 ± 0.21), and cotton not sprayed for tarnished plant bug had lower reflectance than treatments sprayed with insecticides. Trait did not affect yellowness, but a significant effect of spray regimen and a trait by spray regimen interaction was observed (Table 1). Generally, cotton not sprayed with insecticides was more yellow than cotton treated according to threshold or automatically, but this difference was only significant for the non-Bt cotton (Fig. 5, Supp. Table S7).

Discussion

Based on injury ratings, vigor ratings, and biomass, Bt Cry51Aa2 provided as good or better protection against thrips than non-Bt cotton with an IST plus a foliar application of insecticide made at the first true leaf. When compared to plots without thrips control, Bt Cry51Aa2 cotton with no IST reduced total thrips numbers by 71.3%, while the non-Bt cotton with an IST and a foliar insecticide application reduced thrips numbers by 74.2%. Although the

Table 1. Analysis of variance for the impact of treatment effects on fiber quality parameters when averaged across 2 yr and two locations

Treatment effect	F	df	P	F	df	P
	Gin turnout			Fiber length		
IST	2.79	1, 77	0.090	3.70	1, 77	0.058
Trait	0.69	1, 77	0.395	10.74	1, 77	0.002
IST*trait	0.68	1, 77	0.414	0.33	1, 77	0.569
Spray	0.33	2, 77	0.722	1.09	2, 77	0.341
IST*spray	1.56	2, 77	0.217	2.63	2, 77	0.078
Trait*spray	2.12	2, 77	0.127	1.08	2, 77	0.345
IST*trait*spray	1.78	2, 77	0.175	0.00	2, 77	0.997
	Fiber strength			Fiber uniformity		
IST	0.02	1, 77	0.655	0.02	1, 84	0.896
Trait	29.56	1, 77	<0.001	4.39	1, 84	0.041
IST*trait	0.54	1, 77	0.466	0.75	1, 84	0.388
Spray	3.25	2, 77	0.044	1.07	2, 84	0.352
IST*spray	0.52	2, 77	0.599	0.12	2, 84	0.886
Trait*spray	6.15	2, 77	0.003	2.11	2, 84	0.128
IST*trait*spray	0.21	2, 77	0.814	0.16	2, 84	0.849
	Micronaire			Reflectiveness		
IST	0.37	1, 77	0.546	3.39	1, 83	0.069
Trait	8.53	1, 77	0.005	19.29	1, 83	<0.001
IST*trait	0.00	1, 77	1.00	0.86	1, 83	0.356
Spray	30.70	2, 77	<0.001	12.35	2, 83	<0.001
IST*spray	1.73	2, 77	0.185	0.32	2, 83	0.730
Trait*spray	0.70	2, 77	0.502	0.65	2, 83	0.527
IST*trait*spray	0.83	2, 77	0.441	1.85	2, 83	0.163
	Yellowness					
IST	0.01	1, 77	0.912			
Trait	0.24	1, 77	0.623			
IST*trait	0.28	1, 77	0.595			
Spray	4.68	2, 77	0.012			
IST*spray	0.38	2, 77	0.685			
Trait*spray	3.22	2, 77	0.045			
IST*trait*spray	0.60	2, 77	0.551			

Trait = Bt Cry51Aa2 or non-Bt, and spray = foliar insecticide regimen used to control tarnished plant bug.

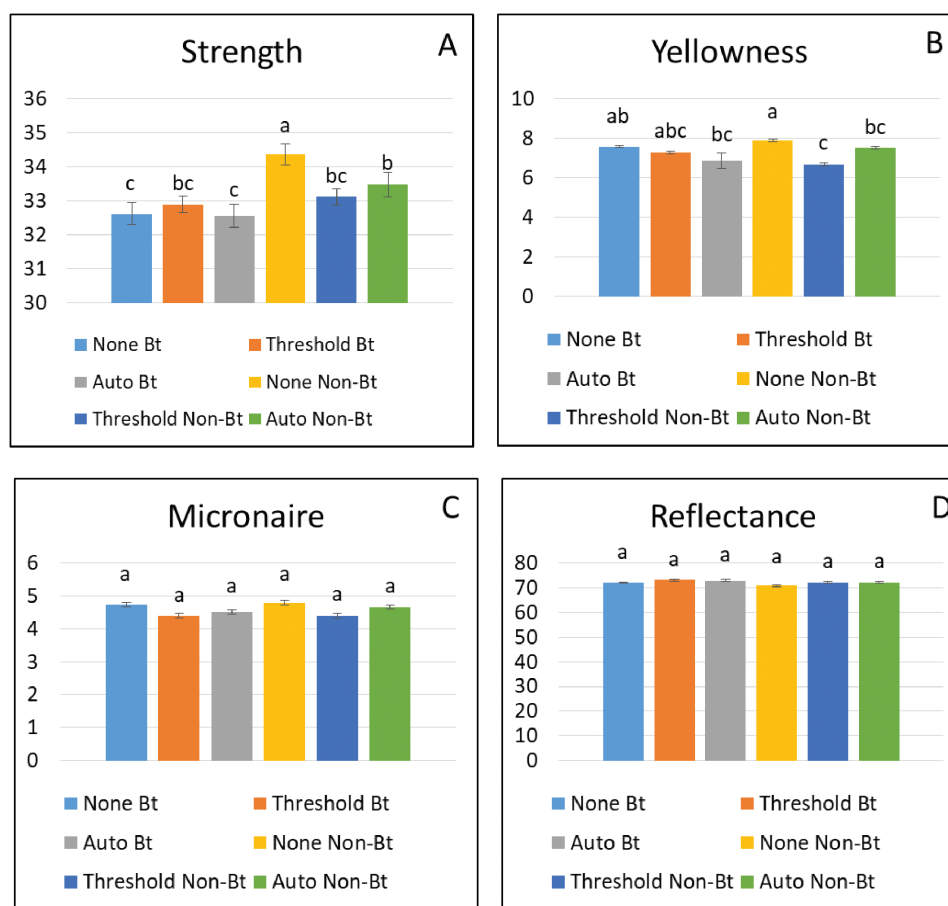


Fig. 5. Influence of Bt Cry51Aa2 and non-Bt cotton managed for tarnished using different spray regimens (automatic [auto], threshold or none) on average cotton fiber quality indices averaged across 2 yr and two locations. Error bars represent 95% confidence intervals. Common letters above bars indicate treatments are not different (Fisher's protected LSD, $\alpha = 0.05$).

reduction in thrips populations was similar between these two treatment combinations, thrips injury ratings between these treatment combinations were significantly different. In terms of both thrips numbers and thrips injury ratings, there was a benefit of using an IST plus the foliar insecticide application in conjunction with the Bt Cry51Aa2. Imidacloprid, a main thrips-control component in the seed treatment used within these experiments (Aeris), is reported to have non-preference qualities for tobacco thrips (Joost and Riley 2005). Our field and laboratory trials have shown Bt Cry51Aa2 is non-preferred (unpublished data). Because at least some of the effect of Bt Cry51Aa2 is due to a non-preference, the effect on thrips might be exaggerated in small plot research where non-Bt cotton is in close proximity. However, the non-preference aspect of imidacloprid is believed to have played a role in the delayed resistance of tobacco thrips to imidacloprid compared to thiamethoxam (Huseth et al. 2017). Having two non-preference modes of action (Bt Cry51Aa2 and imidacloprid) against tobacco thrips might help to delay possible resistance to this technology.

In both aboveground biomass weight and vigor ratings, non-Bt cotton treated with an IST had more biomass and was more vigorous than the Bt Cry51Aa2 cotton without an IST. When comparing these data to thrips numbers and thrips injury, it suggests that something other than thrips caused this difference. Another component of Aeris is thiodicarb, which has activity on root-knot and reniform nematodes (Hall et al. 2017), and it provides some additional thrips control (Cook et al. 2017). Although it is possible that some benefit

was provided from thiodicarb on plant vigor and aboveground biomass, it is unlikely that the increase was associated with control of nematodes, as nematode are present at low levels in the fields where these trials were conducted. Although there appeared to be an added benefit of adding an IST and/or the foliar application of acephate to Bt Cry51Aa2, the increased biomass and vigor did not affect yield. Regardless, it was important to have early-season protection from thrips. Bt Cry51Aa2 cotton had more squares at the first week of bloom than non-Bt cotton, possibly reflecting earliness for the Bt Cry51Aa2 cotton due to thrips protection, which can play a role in tarnished plant bug management later in the season. On average, cotton with only a base fungicide treatment yielded 11.5% less seed-cotton than treatments with some form of thrips control, either Bt Cry51Aa2 or a traditional insecticide approach. In our tests, there was a 181 kg per ha increase of yield when thrips infestations were managed, and this increase is consistent with a meta-analysis by North et al. (2016) who reported a 127 kg per ha increase in yield when a neonicotinoid seed treatment was used in cotton. This yield increase demonstrates the potential importance of Bt Cry51Aa2 for thrips management, particularly when considering the documented occurrence of tobacco thrips resistance to neonicotinoid insecticides (Darnell 2015, 2016; Huseth et al. 2016).

Overall, pre-bloom tarnished plant bug infestations were low to moderate. Averaged across all trials, an average of three bugs per 25 sweeps was observed in plots not treated for tarnished plant bug. Fewer adults were found in the Bt Cry51Aa2 cotton than non-Bt

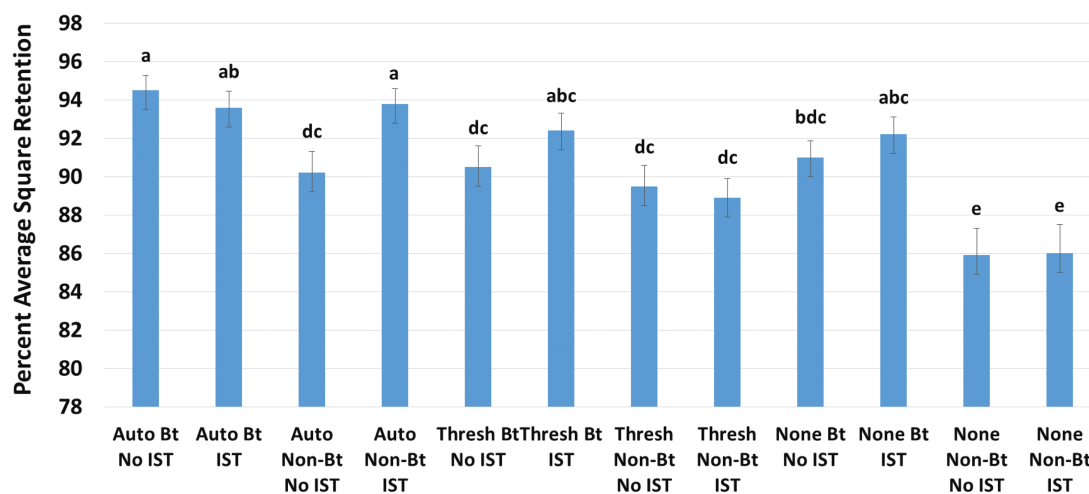


Fig. 6. Average pre-bloom percent square retention graphed by 1) tarnished plant bug spray regimens (automatic [auto], threshold [thresh] or none), 2) trait (Bt Cry51Aa2 or non-Bt) and 3) IST (IST or no IST), averaged across 2 yr and two locations. Error bars represent 95% confidence intervals. Common letters above bars indicate treatments are not different (Fisher's protected LSD, $\alpha = 0.05$).

cotton. Non-Bt cotton required over twice as many total insecticide applications (7) to manage these infestations than Bt Cry51Aa2 cotton (3). Pre-bloom square retention was adequate (>80%) regardless of treatment, but in plots not sprayed with insecticides for tarnished plant bug, square retention was higher in Bt Cry51Aa2 cotton than in non-Bt cotton. In fact, untreated Bt Cry51Aa2 plots had as good or better square retention than non-Bt plots that were managed with the threshold approach. It would be expected to have better retention in sprayed plots than unsprayed, but this was not the case. The difference in square retention suggests that other factors may be contributing to higher square retention in the Bt Cry51Aa2 cotton, perhaps non-preference, as observed with thrips. A non-preference affect may have implications on the efficacy of this technology on tarnished plant bug when implemented in larger fields or for resistance management.

The interaction of IST by spray regimen on tarnished plant bug nymphs found in cotton during bloom is not easily explained. Generally, more bugs were found on cotton treated with an IST. This could be explained by this cotton being more attractive because it had significantly better early season vigor than cotton treated with only a base fungicide seed treatment. However, this seems less likely considering there were no significant differences in total square counts during the first week of squaring for cotton with or without an IST during the first week of squaring, nor was there a difference in overall first position fruit retention at first bloom. No significant difference in the average number of tarnished plant bug nymphs was found between the Bt Cry51Aa2 and non-Bt cotton when they were managed according to threshold. However, across both locations and years, there were fewer total insecticide applications made during this window (11 vs. 8). There were significantly more large nymphs in the non-Bt cotton than the Bt Cry51Aa2 cotton. Large nymphs are especially important because larger nymphs feed more and subsequently cause more injury than small nymphs (Cooper and Spurgeon 2013). Seeing limited effects of Bt Cry51Aa2 on numbers of small nymphs would be expected if Bt Cry51Aa2 slowly killed nymphs and delayed their development. This is consistent with Baum et al. (2012), who reported smaller plant bug nymphs to be more sensitive to the Bt Cry51Aa2 protein than larger nymphs and adults and that mortality at field-relevant rates required 6 d. It is also important to note the Bt Cry51Aa2 protein did not cause a

high level of mortality and that some tarnished plant bug nymphs survived to larger nymphs.

When not treated with insecticides for tarnished plant bug, the Bt Cry51Aa2 cotton yielded more than non-Bt cotton, but there was a substantial yield increase when insecticides were applied to the Bt Cry51Aa2 cotton. Yields were similar between Bt Cry51Aa2 and non-Bt plots managed for tarnished plant bug using the threshold approach but an average of 1.25 fewer insecticide applications (range 0–3) were made to the Bt Cry51Aa2 cotton (Fig. 3). The use of this trait, especially in areas with high tarnished plant bug pressure, may reduce the total number of insecticide application made during the growing season. However, proper scouting and timely applications of insecticides are still needed to manage tarnished plant bug.

Differences in lint quality parameters appeared to be at least partially confounded by inherent differences between the Bt Cry51Aa2 and non-Bt lines, despite the varieties being near-isogenic. While use of an IST did not impact fiber quality parameters, spray regimen impacted micronaire, fiber strength, yellowness, and reflectance. Micronaire and strength were higher in plots that were not sprayed for tarnished plant bug. Higher micronaire and strength likely resulted from a higher percentage of harvestable bolls in these plots coming from early, more mature bolls, as plant bug infestations most likely affected mid- and late-season fruit. Differences in yellowness and reflectance were likely the result of differences in boll injury between the Bt Cry51Aa2 and non-Bt cotton and also between different insecticide regimens. These data indicate tarnished plant bug injury reduced reflectance and increased yellowness. Although it is possible that stink bugs or clouded plant bugs could also affect fiber quality (Pulakkatu-Thodi et al. 2014), no significant difference in the numbers of these pests was observed among treatments, and generally low populations were found. Therefore, these differences were likely caused by tarnished plant bug.

The evaluated Bt Cry51Aa2 trait provided partial control of thrips and tarnished plant bug and thereby could reduce reliance on traditional insecticides to control infestations of these insects. This reduction would help preserve susceptibility to foliar-applied insecticides and alleviate problems with secondary pest outbreaks that are induced by use of broad-spectrum insecticides which disrupt beneficial arthropod populations. Therefore, the Bt Cry51Aa2 trait could be an important component of an integrated pest management plan for both thrips and tarnished plant bug.

Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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