Determining the Optimum Sampling Interval for the Control of Tarnished Plant Bugs (Hemiptera: Miridae) in ThryvOn® Cotton[1]

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J. Entomol. Sci. 61(1): 000–000 (Month 2025)
DOI: 10.18474/JES24-115

Abstract A new cotton technology expressing the *Bacillus thuringiensis* (Berliner) (*Bt*) protein Mpp51Aa2, ThryvOn® (Bayer CropScience, St. Louis, MO), has been commercialized. An experiment was conducted in Stoneville, MS, Glendora, MS, and Marianna, AR during the 2023 growing season to determine the optimum scouting interval to control the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), in Deltapine 2131 B3TXF and Deltapine 1646 BG2XF cotton. Treatments included ThryvOn and non-ThryvOn cotton scouted at 3- and 7-d intervals, with a threshold treatment for each factor. Untreated control plots and positive control plots (treated with insecticide weekly regardless of plant bug density) also were included. Treatments that received a weekly automatic insecticide application or that reached an established economic threshold were treated with sulfoxaflor at 70.02 grams of active ingredient per hectare (Transform® WG, Corteva Agriscience, Indianapolis, IN). Where insecticide applications were made, results indicated that 3- and 7-d scouting intervals in the non-ThryvOn cotton variety resulted in statistically similar yields in plots that received weekly insecticide applications. In the ThryvOn plots, there were no significant differences between lint yields among any of the treatments. These results indicate that regardless of variety or sampling interval the management of tarnished plant bug densities based on current thresholds minimizes crop losses. The untreated control plot yield was similar to that of the treated ThryvOn plots, suggests that alteration of current thresholds to account for the population suppression effects of the *Bt* technology is possible, but further research is needed to confirm this hypothesis.

Key Words ThryvOn, *Lygus lineolaris*, *Bacillus thuringiensis*, *Bt* cotton

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is a highly polyphagous, yield-limiting pest of cotton, *Gossypium hirsutum* (L.), in the United States. Estimates of losses caused by this insect pest combined with the cost of control make it one of the most economically important arthropod pests of U.S. cotton (Cook et al. 2023). Historically, tarnished plant bugs were a pest of cotton only during the squaring stage (Musser et al. 2009b), which is the first reproductive growth stage where floral bud development has begun but no

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buds have opened into mature flowers. This stage typically lasts 3–4 weeks (Oosterhuis 1990, Tharp 1960). However, the eradication of the boll weevil, *Anthonomus grandis grandis* (Boheman), and control of lepidopteran pests such as the cotton bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Chloridea virescens* (F.), through the usage of transgenic *Bacillus thuringiensis* (Berliner) (*Bt*) cotton varieties have reduced the number of broad-spectrum insecticide applications, contributing to elevating the tarnished plant bug as a primary pest (George et al. 2021, Musser et al. 2007).

Tarnished plant bugs tend to migrate from alternative hosts onto cotton as squaring begins (Layton 1995). The preferential feeding sites of tarnished plant bugs are squares, blooms, small bolls, and terminal growing points (Stewart 2003, Young 1969). Because of its indeterminate growth habit, cotton has various fruiting structures present throughout most of the growing season, allowing tarnished plant bugs to feed from plant emergence through the final stages of physiological maturity (George et al. 2021, Layton 1995, Musser et al. 2009a). This span of susceptibility to feeding, which can extend for over 2 mo, allows tarnished plant bugs to complete multiple generations during the growing season (Musser et al. 2009b). Plant bug feeding on squares can result in abscission or flower deformation, whereas feeding damage to small bolls may result in malformation, reduced lint quality, or abscission (George et al. 2021, Layton 2000, Pack and Tugwell 1976, Russell 1999, Stewart 2003). Feeding on terminal growing points may result in deformed growth or loss of apical dominance (George et al. 2021, Young 1969).

Because of the destructive nature of the tarnished plant bug, effective pest monitoring and management decisions based on economic thresholds is a proven method for reducing insect management costs (Bateman et al. 2023, Crow et al. 2023, Musser et al. 2009a). Because the tarnished plant bug can rapidly cause significant fruit loss, twice per week sampling of cotton for insect pests is often recommended (Crow et al. 2023, Musser et al. 2009a) and involves a combination of direct and indirect sampling methods (Ashbrook et al. 2023, Bateman et al. 2023, Brown et al. 2023, Crow et al. 2023, George et al. 2021, Musser et al. 2009a). Economic thresholds for tarnished plant bugs vary across the Cotton Belt but are similar. These thresholds are based on economic injury levels (EILs) that incorporate pest management costs, market value of the crop, injury by the insect, crop damage due to insect injury, and the reduction in the pest population from the control practice (Musser et al. 2009a, Pedigo et al. 1986). Because of the number of variables, true EILs fluctuate when any of these variables change (Musser et al. 2009a). Musser et al. (2009a) found an EIL of tarnished plant bugs in blooming cotton sampled once per week of 1.6–2.6 plant bugs per drop cloth sample. However, the authors recommended more frequent sampling due to the ability of tarnished plant bug populations to increase quickly. Increased sampling frequency would result in a higher EIL and, therefore, a higher threshold (Musser et al. 2009a). Before first bloom in cotton grown in Mississippi, when plant bug populations are monitoring through a combination of sweep net sampling and visual square retention counts, the threshold is eight plant bugs per 100 sweeps but can be lowered when square retention is $<$80% (Crow et al. 2023, Musser et al. 2009b). After first bloom when sampling is conducted using a drop cloth, the current threshold is $\geq$3 tarnished plant bugs per drop cloth in Mississippi, Tennessee, Arkansas, and Louisiana (Ashbrook et al. 2023, Bateman et al. 2023, Brown et al. 2023, Crow et al. 2023).

Historically, transgenic crops expressing a gene found in the soil bacterium *Bt* have been approved and utilized to suppress populations of selected U.S. insect pest species (George et al. 2021). The U.S. Environmental Protection Agency recently approved the commercialization of a new transgenic *Bt* cotton trait, ThryvOn® (Bayer CropScience, St. Louis, MO). This trait in cotton expresses the *Bt* protein Mpp51Aa2 that was developed and optimized for control of the tarnished plant bug and various thrips species (Bachman et al. 2017). Research has revealed lower densities of tarnished plant bugs on this cotton variety compared with non-ThryvOn varieties managed similarly (Corbin et al. 2020, Graham et al. 2019). Evidence suggests that density reductions may be due to increased insecticide susceptibility in response to the added stress caused by the *Bt* toxin (Graham et al. 2019). Ongoing research conducted at the Delta Research and Extension Center in Stoneville, MS suggests that population suppression and the added stressor of the ThryvOn trait may allow for less costly and less effective insecticide chemistries to be reevaluated as potential options for control of the tarnished plant bug.

This study was conducted to determine whether the population suppression capabilities of ThryvOn technology would allow for a longer sampling interval compared with nontraited cotton. This information could decrease the workload of producers, licensed agricultural consultants, and certified pesticide applicators. There is also potential for a reduction in the amount of foliar insecticides required, reducing the amount of active ingredients in the environment and potentially increasing the economic potential for producers. Although the current EILs and tarnished plant bug thresholds were developed by sampling cotton once per week, extension personnel have suggested sampling cotton twice per week to avoid potential yield loss caused by the previously mentioned variables. Findings from this study may indicate the potential for altering tarnished plant bug thresholds or allowing more forgiving timeframes for management decisions to be made in ThryvOn cotton if sampling intervals were to be extended.

Materials and Methods

To determine the optimum sample scouting interval for tarnished plant bugs in ThryvOn cotton, experiments were conducted at the Lon Mann Cotton Research Station in Marianna, AR (34.733, −90.765), the Delta Research and Extension Center in Stoneville, MS (33.415, −90.898), and a commercial farm in Glendora, MS (33.815, −90.304) during the 2023 growing season. Cotton varieties were Deltapine 2131 B3TXF expressing the *Bt* proteins Cry1Ac, Cry2Ab, Vip3A, and Mpp51Aa2 (ThryvOn), and Deltapine 1646 B2XF expressing the *Bt* proteins Cry1Ac and Cry2Ab (non-ThryvOn). Both cotton varieties were selected on the basis of genetic similarity to minimize the variation between them. Both varieties were treated with imidacloprid (5 lb/gal active ingredient [ai]; Gaucho® 600, Bayer Crop Science) at a rate of 0.375 mg/seed to mitigate damage from early season arthropod pests.

At the Stoneville location, plots 12.19 m long and 4.06 m wide were planted with cotton at a seeding rate of approximately 112,000 seeds/ha into conventionally tilled rows spaced 101.6 cm apart on 16 May 2023. At Glendora, plots 12.19 m long and 3.86 m wide were planted with cotton at a seeding rate of approximately 135,850 seeds/ha into conventionally tilled rows spaced 96.52 cm apart on 24 May 2023. At the Marianna location, plots 12.19 m long and 3.86m wide were planted with cotton at a seeding rate of approximately 102,000 seeds/ha into conventionally tilled rows spaced 96.52 cm apart on 16 May 2023. Furrow irrigation was applied as necessary at the Stoneville and Marianna locations but was unavailable at the Glendora location. At the Glendora location, overhead pivot irrigation was available but restrained according to the grower's needs. Each location received an application of a preemergent herbicide for control of summer annual weeds. Plots at all locations were fertilized and managed according to local university extension service guidelines. An application of methoxyfenozide + spinetoram (2.5 lb ai/gal [300 g/L] methoxyfenozide, 0.5 lb ai/gal [60 g/L] spinetoram; Intrepid Edge®, Corteva Agriscience, Indianapolis, IN) at a rate of 66.10 g ai/ha and 13.56 g ai/ha, respectively, was made to all plots at each location during the seedling stage to suppress thrips. An application of 70.21 g a/ha chlorantraniliprole (Vantacor®, FMC Corp., Philadelphia, PA) was also made at each location across all plots for control of the cotton bollworm when the economic threshold was reached in the Deltapine 1646 variety. All locations reached the economic threshold for bollworm once throughout the growing season. These treatments were successful in preventing economic injury from bollworms.

The tests were randomized complete block designs with a factorial arrangement of treatments. Factor A was cotton technology: the ThryvOn variety Deltapine 2131 BG3TXF versus the non-ThryvOn cotton variety Deltapine 1646 BG2XF. Factor B consisted of scouting interval treatments that initiated at first square: untreated 3-d sample interval, untreated 7-d sample interval, treated 3-d sample interval, treated 7-d sample interval, and treated weekly. Adult and immature tarnished plant bug densities were monitored prebloom by taking 25 sweeps per plot with a 38.1-cm-diameter sweep net. Beginning at bloom, sampling methods were changed to two drops per plot with a black drop cloth. This method involved placing a black cloth (0.76 by 0.91 m) between the center two rows of each plot. Cotton plants along the sides of the drop cloth were shaken vigorously, dislodging tarnished plant bugs onto the cloth, which were then counted. Numbers of tarnished plant bug nymphs and adults were recorded separately. Damage from tarnished plant bug feeding was monitored by calculating percentage of square retention. This method involves examining the first-position fruiting sites on each of the three upper nodes of a cotton plant for the presence or absence of squares and evidence of tarnished plant bug feeding, such as blasted or flared squares. To be considered missing, the presence of an abscission scar could be observed, whereas blasted or flared squares were characterized by being discolored, dried, or having the bracts opened. All squares, missing or damaged, were counted as missing because damaged squares will abscise and not result in harvestable fruit. The number of retained squares was recorded, and the percentage square retention was calculated based on 25 sampled fruiting sites per plot and then averaged across all four replicates. Insecticide applications were made to the designated treated plots when either the tarnished plant bug density or percentage square retention thresholds were reached or exceeded. In Mississippi, the current thresholds used to initiate insecticide applications in the first 2 weeks of square are eight tarnished plant bugs per 100 sweeps or

80% square retention (Crow et al. 2023). From the third week of square through bloom, the threshold with a sweep net increases to 15 tarnished plant bugs per 100 sweeps (Crow et al. 2023). For the drop cloth sampling method, applications to treated plots were made when the threshold of three tarnished plant bugs per 1.5 m was reached (Crow et al. 2023). The untreated controls were not sprayed for tarnished plant bugs throughout the season. When thresholds were met or exceeded in the treated plots, an application of sulfoxaflor (Transform WG®, Corteva Agriscience) at a rate of 70.02 g ai/ha were made. Plot yield weights were quantified by machine harvesting the center two rows of each plot. Plot weights were converted to lint yield in kilograms per hectare based on a lint turnout of 40%. The number of insecticide applications each treatment plot received was also recorded throughout the study.

Data analysis. Because two sampling methods were utilized throughout this study, tarnished plant bug numbers were mathematically converted to a percentage of threshold relative to the sampling method used and the growth stage of the cotton at the time of sampling. Yield, tarnished plant bug density expressed as a percentage of threshold, and number of insecticide applications were analyzed with a generalized linear mixed model analysis of variance (PROC GLIMMIX) (SAS version 9.4, SAS Institute, Cary, NC). Degrees of freedom were calculated using the Kenward-Rogers method. Means were estimated using LSMEANS and separated based on Fisher's protected least significant difference ($\alpha = 0.05$). Because the aim of this study was to quantify the impact of various sampling intervals on yield in ThryvOn and non-ThryvOn cotton rather than to compare the yields of ThryvOn and non-ThryvOn cotton varieties, data for each variety were analyzed independently. Lint yield data were analyzed with treatment as the fixed effect and with location and replication nested in location as random effects. ThryvOn cotton expresses a *Bt* protein with suppression effects on tarnished plant bug populations, but these effects are not found in the non-ThryvOn variety. Thus, the percentage of threshold data for each variety were analyzed separately. In the percentage of threshold analysis, treatment was set as the fixed effect, with location and replication nested within location set as random effects. Because the control plots were included as a measure of pressure at each location, the untreated control plots and the plots receiving weekly insecticide applications were excluded from this analysis. For the analysis of number of insecticide applications, the untreated control and the treatment receiving weekly insecticide applications also were excluded. Because all replications within a treatment received the same number of insecticide applications, location was considered the random effect. Treatment, variety, and the interaction of the two were set as the fixed effects.

Results and Discussion

No interaction between sample interval and variety ($F = 0.00$; df = 1, 6; $P = 1.00$) was observed for the number of insecticide applications required for tarnished plant bug management. No significant differences were found between the 3- or 7-d sampling interval regimes ($F = 4.36$; df = 1, 6; $P = 0.08$). However, there was a significant difference between varieties in the number of insecticide applications made ($F = 27.27$; df = 1, 6; $P < 0.01$). Across all locations, the ThryvOn

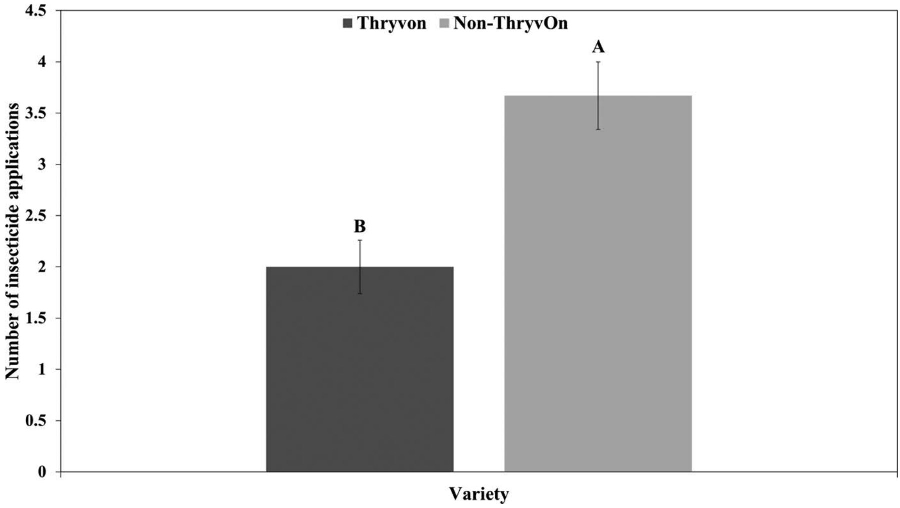

Fig. 1. Mean number of tarnished plant bug insecticide applications by variety. Means for bars with different letters are significantly different according to Fisher's protected least significant difference test (treatment*variety: F = 0.00; df = 1, 6; P = 1.00; treatment: F = 4.36; df = 1, 6; P = 0.08; variety: F = 27.27; df = 1, 6; $P < 0.01$).

variety required a mean (±SEM) of 2.00 (±0.26) insecticide applications, whereas the non-ThryvOn variety required 3.67 (±0.33) insecticide applications for tarnished plant bug management (Fig. 1). These findings are supported by previous research in which fewer insecticide applications targeting tarnished plant bugs were required in ThryvOn cotton than in a non-ThryvOn variety when following the current economic thresholds (Corbin et al. 2020, Graham and Stewart 2018, Thrash et al. 2024, Whitfield et al. 2023).

No significant differences in yield were observed between treatments in the ThryvOn variety (Table 1). These findings are supported by those of Maris et al. (2023), who observed no significant differences in ThryvOn cotton treated with insecticide for tarnished plant bugs compared with an untreated control. However, in the non-ThryvOn variety, lint yield in the untreated control plots was significantly lower than that in the treated plots (Table 1). No other significant differences were observed. Tarnished plant bug infestations in the untreated control plots of the non-ThryvOn variety were 51–229% of threshold at the Glendora location, 124–560% of threshold at the Marianna location, and 66–297% of threshold at the Stoneville location. Tarnished plant bug infestations in the non-ThryvOn variety also exceeded the economic threshold in the untreated control plots in 6 of the 7 weeks of sampling at the Glendora location, 5 of 5 weeks in Marianna, and 4 of 7 weeks in Stoneville.

In the ThryvOn variety, tarnished plant bug infestations in the untreated control plots were 13–263% of threshold at the Glendora location, 78–304% of threshold at the Marianna location, and 41–171% of threshold at the Stoneville location.

Table 1. Lint yield quantifying sample interval effects on yield.

Treatment	Lint Yield Across all Locations (kg/ha)	
	ThryvOn (DP 2131)	Non-ThryvOn (DP 1646)
3-d untreated control	1252.05 (161.23)a*	948.91 (215.47)b
7-d untreated control	1294.04 (174.73)a	923.58 (214.45)b
3-d sprayed	1323.35 (141.41)a	1242.97 (174.13)a
7-d sprayed	1279.88 (135.44)a	1219.09 (198.24)a
Weekly	1414.51 (154.17)a	1288.69 (184.25)a
F	1.50	11.06
df	4, 44	4, 44
$P > F$	0.22	$<$0.01

* Values are mean (standard error of the mean). Means followed by the same letter are not significantly different according to Fisher's protected least significant difference test ($\alpha = 0.05$).

Tarnished plant bug infestations in the ThryvOn variety also exceeded the economic threshold in the untreated control plots in 5 of the 7 weeks of sampling at the Glendora location, 4 of 5 weeks at the Marianna location, and 3 of 7 weeks at the Stoneville location. In the percentage of threshold analysis (data not shown), there were no significant differences in tarnished plant bug density between the 3- and 7-d treated plots when thresholds were met or surpassed regardless of variety (non-ThryvOn: $F = 0.52$; df $= 1, 48.22$; $P = 0.48$; ThryvOn: $F = 0.04$; df $= 1, 17.06$; $P = 0.84$).

Although costs associated with the development of ThryvOn cotton technology result in higher seed costs compared with the non-ThryvOn variety, the decreased number of insecticide applications targeting tarnished plant bugs in the ThryvOn variety offers the potential for producers to reduce input costs later in the season. Cook et al. (2023) estimated the mean price of an insecticide plus application costs targeting tarnished plant bugs in the United States during 2022 was approximately $39.05/ha. In addition to the reduction in monetary insecticide costs, reducing the number of insecticide applications decreases the amount of active ingredients, which can cause off-target effects, entering the environment. Furthermore, reduction in the number of insecticide applications leads to fewer passes across the field, resulting in decreased labor, fuel, and equipment costs. Compounding these benefits, ThryvOn cotton technology also provides excellent protection from thrips in seedling cotton, which is not seen in non-ThryvOn cotton varieties. Some university guidelines currently recommend that no foliar insecticide applications be made targeting thrips in seedling cotton unless warranted by excessive thrips damage (Bateman et al. 2023, Brown et al. 2023, Crow et al. 2023). Given current implications regarding insecticide resistance in thrips populations across the midsouthern United States, this is a significant area where producers may see reduced input costs from utilizing ThryvOn cotton. However, varying levels of insect pest pressure and environmental factors between years may impact the total reduction in insecticide applications required.

Yield and percentage of threshold data indicate that shortening sampling intervals, whether in ThryvOn or non-ThryvOn cotton, provided little benefit in yield when coupled with insecticide intervention when thresholds were met. These results are supported by the findings of Musser et al. (2009a), who developed the economic thresholds for tarnished plant bug control in the midsouthern United States utilized in this trial by conducting sampling efforts weekly. However, Musser et al. (2009a) noted that due to the damage potential and reproductive capabilities of tarnished plant bugs, sampling twice per week is recommended to avoid potential yield-limiting damage caused by subthreshold populations feeding for extended periods or greatly exceeding economic thresholds prior to the cotton being sampled again a week later. Additionally, yield loss may be intensified when a significant weather event such as rain occurs before the scheduled time for a sample in a 7-d regime, causing sampling to be delayed beyond the 7-d period. However, the population suppression capabilities of ThryvOn technology may alleviate some of this damage potential, as observed in the lint yields of the untreated control plots. Although both season-long untreated controls in the ThryvOn variety had yields statistically similar to those of the treated plots in this study, it should not be implied that chemical control for tarnished plant bugs can be omitted from a ThryvOn pest management regime. Research has shown decreased yield in nontreated ThryvOn cotton compared with ThryvOn cotton treated with insecticides for tarnished plant bug control (Corbin et al. 2020, Graham and Stewart 2018, Thrash et al. 2024). Further research is needed to investigate the relationship between ThryvOn cotton technology and the currently established economic thresholds for tarnished plant bugs.

Acknowledgments

The authors express sincere gratitude to all those involved in this research and to Cotton Incorporated for partial financial support of this research.

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