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# ARTHROPOD MANAGEMENT & APPLIED ECOLOGY

## An Evaluation of Plant-Based Scouting Practices for Tarnished Plant Bug Damage in Traditional and Novel Traited Cotton Technologies Prior to Bloom

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### ABSTRACT

**In the Mid-South region of the U.S., the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is the most economically damaging insect pest of cotton, *Gossypium hirsutum* (L.). During 2022 and 2023, tarnished plant bugs accounted for more than 70,000 lost bales of cotton and caused almost $50 million of economic losses in Mississippi per annum. Scouting for tarnished plant bug in cotton prior to bloom is accomplished by direct insect counts using a sweep net and indirect sampling through evaluation of cotton square (flower bud) retention. Cotton consultants across the Mid-South use a variety of square retention monitoring methods. Research was conducted in the Mississippi Delta to evaluate six different square retention monitoring methods compared to whole plant assessment in non-ThryvOn and ThryvOn cotton. Cotton was planted as a split-plot arrangement within a randomized complete block design with insecticide program as the main-plot factor and technology as the subplot factor. Cotton technology and tarnished plant bug control did not affect method of square retention monitoring, whereas sampling interval and the growth stage of cotton did. These data suggest that assessing damage to the third node during the first 2.5 weeks of squaring best reflects whole plant damage. After this time, accessing square damage in the top three nodes or third node only from the terminal until bloom offers the best sampling efficiency while reflecting whole plant damage prior to bloom. These practices could enable scouts to make management decisions in a timely manner to reduce input costs.**

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Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), damage can occur as early as the seedling stage in cotton, *Gossypium hirsutum* (L.). Damage at this stage of growth causes a reduction in plant height and a delay in fruiting up to several days (Bariola, 1969). Tarnished plant bug feeding at the seedling stage also can result in loss of apical dominance leading to secondary terminal formation and further delays in cotton maturity (Hanney et al., 1977; Scales and Furr, 1968). Oviposition is unlikely to occur at the seedling stage of growth (Bariola, 1969), so monitoring for nymphs is unwarranted. Scouting for tarnished plant bug during the seedling stage of cotton should be focused primarily on detecting migrating adults (Layton, 1995).

Tarnished plant bugs can damage cotton from first square (flower bud) formation until the last harvestable boll (fruit) accumulates at least 300 heat units (Russell, 1999), with most economic damage occurring from first square through the first few weeks of flowering (Black, 1973; Tugwell et al., 1976). Numerous studies have documented the occurrence of tarnished plant bug damage to fruiting cotton prior to bloom (Black, 1973; Layton, 2000; Phelps et al., 1997; Scott and Snodgrass, 2000; Teague et al., 2001). Reproductive growth occurs approximately four or five weeks after planting (formation of first square) and lasts approximately four weeks. During this time, new fruiting branches are formed every three days (vertical growth). New squares are formed horizontally on existing branches at six-day intervals (Eaton, 1955; Oosterhuis, 1990; Tharp, 1960). Small squares, < 3.2 mm in diameter, are the preferred feeding site for tarnished plant bug in cotton and a single adult can feed on 0.6 to 2.1 squares per day (Gutierrez et al., 1977; Tugwell et al., 1976). Yield losses prior to bloom might not always occur, even with high tarnished plant bug populations, due to compensatory growth (Black, 1973; Layton, 2000). High levels of sustained feeding can cause cotton to exhibit abnormal fruiting and branching patterns, excessive vegetative growth, and delays in maturity (Black, 1973; Layton, 2000).

Two methods for scouting pre-bloom cotton for tarnished plant bug are recommended (Crow et al., 2023). The first is a direct sampling method using a sweep net to estimate insect density. The second method of scouting is an indirect, plant-based approach to estimate square retention (Crow et al., 2023). The current economic threshold for tarnished plant bugs prior to bloom is eight adults per 100 sweeps during weeks one and two of squaring and 15 adults per 100 sweeps during weeks three and four of squaring and when square retention falls below 80% on the three uppermost nodes (Crow et al., 2023). Sweep net sampling prior to bloom should always be supplemented with square retention monitoring, because evidence of tarnished plant bug infestation observed with one scouting method might not be observed with another scouting method (Musser et al., 2007). This is especially true following an insecticide application when new adults migrate into the field but might not cause significant injury or reduce square retention (Gore et al., 2012; Musser et al., 2009).

Methods used to estimate square retention prior to bloom varies among cotton consultants and extension services in the Mid-South. Mississippi State University Extension's recommendation is to maintain at least 80% square retention on first and second position fruit in the five upper-most nodes (Crow et al., 2023). However, other Mid-South entomologists recommend slight variations to their scouting guidelines. Prior to bloom, the University of Tennessee recommends maintaining 80% of first position squares on the top five nodes (Kelly and Brown, 2024). Louisiana State University Agricultural Center advocates for maintaining 70 to 85% of first position fruit from all nodes (Ashbrook et al., 2023); whereas the University of Arkansas Extension recommends maintaining greater than 75% of first position fruit on the third node during first few weeks of squaring (Thrash and Bateman, 2024). The variability among different scouting methods could lead to untimely or unwarranted insecticide applications due to underestimating or overestimating damage (Catchot and Gore, 2014). Management decisions for tarnished plant bug control in cotton can become more complicated with the commercialization of ThryvOn cotton (Bayer Crop Science, St. Louis, MO). ThryvOn cotton expresses the Cry51Aa2.834_16 protein, a novel Bt, *Bacillus thuringiensis* (Berliner), trait with activity against tarnished plant bug (Baum et al., 2012; Gowda et al., 2016). It has been estimated that insecticide efficacy could be increased and have an extended duration with the introduction of this technology (Corbin et al., 2020; Huoni et al., 2024). However, little current information exists about how this trait will impact pre-bloom scouting and management of tarnished plant bug. Therefore, the purpose of this study was to evaluate several tarnished plant bug square retention monitoring methods to determine the most accurate and to determine if scouting practices need adjustments in ThryvOn cotton.

## MATERIALS AND METHODS

Experiments were conducted in 2022 and 2023 at the Delta Research and Extension Center in Stoneville, MS to assess different square retention monitoring methods during the pre-flowering period in ThryvOn and non-ThryvOn cotton. A randomized complete block design with a split-plot arrangement of treatments with four replications was implemented. Whole-plot factor consisted of two levels of insecticide management, treated or untreated. Treated plots were sprayed with acephate (Orthene 90S, Amvac Chemical Corporation, Newport Beach, CA) at 0.84 kg ai ha$^{-1}$ tank mixed with bifenthrin (Brigade 2EC, FMC Ag US, Philadelphia, PA) at 0.11 kg ai ha$^{-1}$ applied during the middle of the second week of squaring. The first application was followed by sulfoxaflor (Transform WG, Corteva Agriscience, Indianapolis, IN) at 0.053 kg ai ha$^{-1}$ 7 d later. All plots were sprayed with a MUDMASTER$^{TM}$, 4WD Multi-Purpose Sprayer (Bowman Manufacturing, Newport, AR), equipped with a compressed air high clearance mounted multi-boom spray system calibrated to deliver 93.5 L ha$^{-1}$ at 480 kPa through TX-6 ConeJet Visiflow Hollow Cone spray tip nozzles (TeeJet Technologies, Glendale, IL). Whole plots were eight rows wide on 102-cm centers and 12.2 m in length. Each whole plot contained two subplots that were four rows wide. Plots were separated by 3-m fallow alleys.

The subplot factor was cotton genotype. Deltapine 2131 BT3XF (Bayer Crop Science, St. Louis, MO), modified to express the Cry51Aa2.834_16 Bt protein, was planted as the ThryvOn variety, and Deltapine 2055 B3XF was planted as the non-ThryvOn variety. Both varieties express three Bt proteins for control of lepidopteran pests to minimize their impact on square retention. Field experiments were planted 10 May 2022 and 16 May 2023, at a seeding rate of 113,000 seed $ha^{-1}$ at 2 cm in depth. All crop management practices were conducted based on the recommendations of Mississippi State University Extension.

Seven different plant scouting methods were evaluated. These included quantifying square retention on the third node only, third node first position only, top three nodes, top three nodes first position only, top five nodes, top five nodes first position only, and whole plant. Starting at the first week of square, 25 whole plants per plot were mapped twice per week for the first 3 wk of square and again 7 and 10 to 14 d after the last insecticide application. During plant mapping, each fruiting site (square) on all reproductive nodes were scouted for tarnished plant bug damage, which was indicated if a square was necrotic or missing entirely. Tarnished plant bug densities were determined weekly by taking 100 sweeps with a 38.1-cm diameter sweep net on adjacent unsprayed blocks of ThryvOn and non-ThryvOn cotton. These samples were collected only to provide information on tarnished plant bug densities in the field. Samples were collected from separate blocks of cotton to prevent damage to plants in the plots where square retention was being monitored. Yields were taken on the center two rows of each plot using a modified two-row cotton picker. Lint turnout was estimated to be 40%.

**Data Analysis**. A general linear mixed model analysis of variance was used to analyze square retention and yield data (Proc Glimmix, SAS version 9.4, SAS institute, Cary, NC). A separate analysis was conducted for comparison of square retention methods and yield. Square retention methods were analyzed as a repeated measure due to the partitioning of whole plant square retention data into each individual method for analysis. The fixed effects were insecticide program, cotton technology, and method of square retention monitoring. The random effects were site year, replication nested within site year, and replication by cotton technology nested within insecticide program. The random error effect, for split plot repeated measures in Proc Glimmix, was site year by cotton technology nested within insecticide program. In the yield analysis, the fixed effects were insecticide program and cotton technology. The random effects were site year, replication nested within site year, and replication by insecticide spray nested within site year. Degrees of freedom were calculated using the Kenward-Roger method (Kenward and Roger, 2009). Means of all data were calculated using LSMEANS and separated according to Fisher's Protected LSD at $\alpha = 0.05$.

## RESULTS

Based on samples just outside the test area, tarnished plant bug densities were below the current action threshold in both non-ThryvOn and ThryvOn cotton (Fig. 1). At all sample dates, tarnished plant bugs were slightly below the threshold in non-ThryvOn cotton. Conversely, tarnished plant bug numbers in ThryvOn cotton were at approximately half of the current action threshold. As a result, square retention remained greater than 80% throughout the squaring period. There were no three-way or two-way interactions among factors for square retention at any sample date (Table 1). However, each sample date had at least one significant main effect (Table 1).

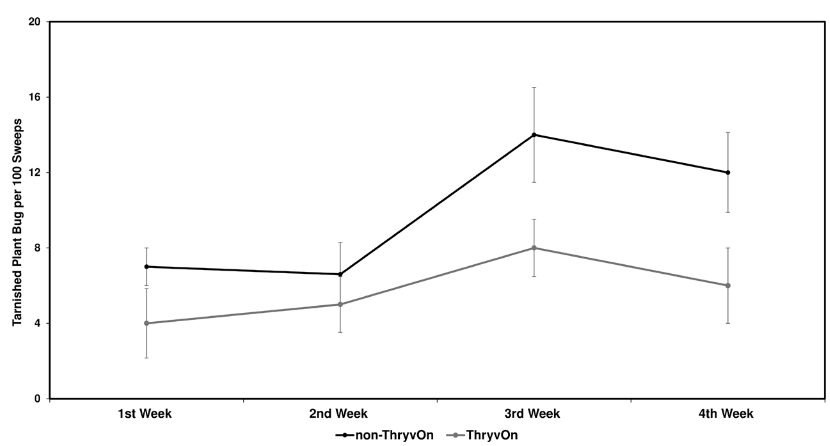


**Figure 1. Tarnished plant bug densities per 100 sweeps in adjacent cotton.**

**Table 1. Type III test of fixed effects for each sample date and lint yield turnout according to Fishers protected LSD ($\alpha < 0.05$)**

| Effect | Num. DF[z] | Den. DF[y] | *F*-Value | *Pr >F* |
|---|---|---|---|---|
| **First Week of Square Second Sample Date** | | | | |
| Spray | 1 | 2.828 | 1.1 | 0.3750 |
| Technology | 1 | 9.324 | 3.09 | 0.1114 |
| Spray*Technology | 1 | 2.828 | 0.04 | 0.8632 |
| Method | 4 | 87.89 | 7.69 | <0.001 |
| Spray*Method | 4 | 87.89 | 0.05 | 0.9958 |
| Technology*Method | 4 | 87.89 | 0.41 | 0.7990 |
| Spray*Technology*Method | 4 | 87.89 | 0.58 | 0.6776 |
| **Second week of Square First Sample Date** | | | | |
| Spray | 1 | 2.783 | 3.84 | 0.2442 |
| Technology | 1 | 4.128 | 2.15 | 0.1858 |
| Spray*Technology | 1 | 2.783 | 4.52 | 0.2228 |
| Method | 4 | 124.5 | 4.58 | 0.0017 |
| Spray*Method | 4 | 124.5 | 0.50 | 0.7392 |
| Technology*Method | 4 | 124.5 | 0.44 | 0.7812 |
| Spray*Technology*Method | 4 | 124.5 | 0.97 | 0.4287 |
| **Second Week of Square Second Sample Date** | | | | |
| Spray | 1 | 2.889 | 5.60 | 0.1282 |
| Technology | 1 | 3.866 | 11.28 | 0.0157 |
| Spray*Technology | 1 | 2.889 | 0.22 | 0.6782 |
| Method | 6 | 180.0 | 5.12 | <0.001 |
| Spray*Method | 6 | 180.0 | 0.13 | 0.9926 |
| Technology*Method | 6 | 180.0 | 0.57 | 0.7511 |
| Spray*Technology*Method | 6 | 180.0 | 0.61 | 0.7227 |
| **Third Week of Square First Sample Date** | | | | |
| Spray | 1 | 2.889 | 18.06 | 0.0256 |
| Technology | 1 | 3.866 | 16.00 | 0.0172 |
| Spray*Technology | 1 | 2.889 | 0.71 | 0.4620 |
| Method | 6 | 180.1 | 11.78 | <0.001 |
| Spray*Method | 6 | 180.1 | 0.29 | 0.9419 |
| Technology*Method | 6 | 180.1 | 0.52 | 0.7959 |
| Spray*Technology*Method | 6 | 180.1 | 0.06 | 0.9990 |
| **Third Week of Square Second Sample Date** | | | | |
| Spray | 1 | 2.889 | 11.26 | 0.0349 |
| Technology | 1 | 3.866 | 13.27 | 0.0276 |
| Spray*Technology | 1 | 2.889 | 0.60 | 0.4870 |
| Method | 6 | 180.1 | 13.29 | <0.001 |
| Spray*Method | 6 | 180.1 | 0.21 | 0.9744 |
| Technology*Method | 6 | 180.1 | 0.75 | 0.6130 |
| Spray*Technology*Method | 6 | 180.1 | 0.28 | 0.9438 |

**Table 1. continued**

| Effect | Num. DF[z] | Den. DF[y] | *F*-Value | *Pr >F* |
|---|---|---|---|---|
| **Fourth Week of Square First Sample Date (7 Days after Sulfoxaflor)** | | | | |
| Spray | 1 | 2.843 | 7.17 | 0.0796 |
| Technology | 1 | 3.889 | 3.09 | 0.1558 |
| Spray*Technology | 1 | 2.843 | 0.86 | 0.4256 |
| Method | 6 | 180.0 | 9.1 | <0.001 |
| Spray*Method | 6 | 180.0 | 0.44 | 0.8483 |
| Technology*Method | 6 | 180.0 | 0.36 | 0.9005 |
| Spray*Technology*Method | 6 | 180.0 | 0.08 | 0.9982 |
| **First Bloom (10-12 Days after Sulfoxaflor)** | | | | |
| Spray | 1 | 2.843 | 7.23 | 0.0758 |
| Technology | 1 | 3.889 | 7.87 | 0.0437 |
| Spray*Technology | 1 | 2.843 | 0.37 | 0.5854 |
| Method | 6 | 180.0 | 16.91 | <0.001 |
| Spray*Method | 6 | 180.0 | 0.86 | 0.5239 |
| Technology*Method | 6 | 180.0 | 1.14 | 0.3411 |
| Spray*Technology*Method | 6 | 180.0 | 0.63 | 0.7092 |
| **Lint Yield** | | | | |
| Spray | 1 | 6.632 | 0.44 | 0.5312 |
| Technology | 1 | 13.73 | 9.51 | <0.001 |
| Spray*Technology | 1 | 13.73 | 0.11 | 0.7418 |

[z]Numerator Degrees of Freedom

[y]Denominator Degrees of Freedom

**Square Retention Monitoring Methods**. The first sample date during the first week of squaring was excluded from analysis because not enough squares were present on plants at that time. For the second sample date during the first week of squaring and the first sample date during the second week of squaring, only the top three nodes had enough squares to evaluate square retention. Therefore, the square retention monitoring methods consisting of the top five nodes first position only; the top five nodes were excluded. Differences among square retention monitoring methods were observed at every sampling date (Table 1). On the second sample date during the first week of squaring, the top three nodes, top three nodes first position only, and whole plant square retention monitoring methods resulted in higher square retention than the third node only and third node first position only approaches (Fig. 2A, $p < 0.0001$). The third node only method resulted in greater square retention than the third node first position only method. For the first sample date during the second week of squaring, the top three nodes and top three nodes first position only monitoring methods recorded higher square retention than the third node first position only and whole plant square retention approach. The top three node monitoring method also recorded higher square retention than the third node only monitoring method (Fig. 2B, $p = 0.001$).

All plots consistently had five or more nodes with squares at every sample interval after the first sample date during the second week of square, therefore all seven square retention monitoring methods were analyzed. For the second sample date during the second week of squaring, the plant scouting methods observing the top three nodes first position only and top three nodes resulted in higher square retention than all other monitoring methods (Fig. 2C, $p < 0.001$). All other square retention monitoring methods from this sample date resulted in similar rates of square retention. For the first sample date during the third week of squaring, the top three nodes and top three nodes first position only monitoring methods recorded the highest rates of square retention (Fig. 2D, $p < 0.001$). The top five nodes, top five nodes first position only, third node and third node first position only monitoring methods resulted in higher square retention than the whole plant square reten-

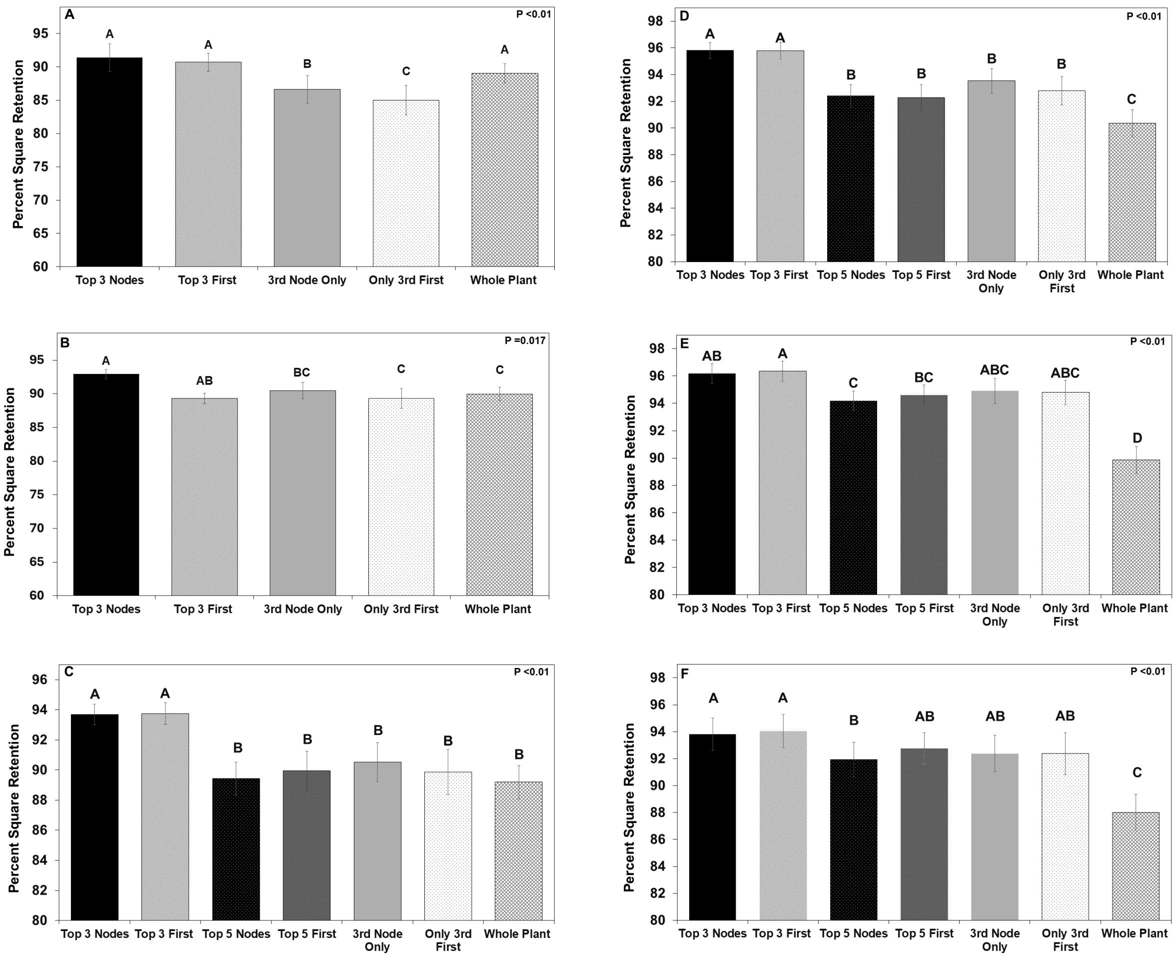


**Figure 2. Main effect of square retention monitoring for: the second sample during first week of squaring (A), the first sample during the second week of squaring (B), the second sample during the second week of squaring (C), the first sample during the third week of squaring (D), the second sample during the third week of squaring (E), and the first sample during the fourth week of squaring (F). Means within the graphs with the same letter are not significantly different according to Fishers protected LSD (α = 0.05).**

tion approach. For the second sample date during the third week of squaring, the top three nodes first position only monitoring method resulted in higher square retention than whole plant square retention, the top five nodes, and top five nodes first position only monitoring methods (Fig. 2E, $p < 0.001$). Square retention determined using the top three nodes was higher than that for the whole plant and the top five nodes monitoring methods. The whole plant square retention approach resulted in the lowest square retention of all monitoring methods (Fig. 2E, $p <$ 0.001). For the first sample date at the beginning of the fourth week of squaring, the top three nodes and top three nodes first position only square retention monitoring methods resulted in higher square retention than the whole plant square retention and top five nodes monitoring methods (Fig. 2F, $p <$ 0.001). Square retention determined by the top five nodes, top five nodes first position only, third node, or third node first position only monitoring methods was higher than the whole plant square retention approach (Fig. 2F). For the sample date at first bloom, the top three nodes, top three nodes first position only and top five nodes first position only monitoring methods resulted in higher square retention than the whole plant square retention, third node, and third node first position only monitoring methods (Fig. 3). The top three nodes first position only and top five nodes first position only monitoring methods resulted in higher square retention than the top five nodes monitoring method. The lowest square retention was calculated using the whole plant square retention approach (Fig. 3).

**Influence of Technology**. Cotton technology had an effect on square retention on the second sample date during the second week of squaring ( $p$

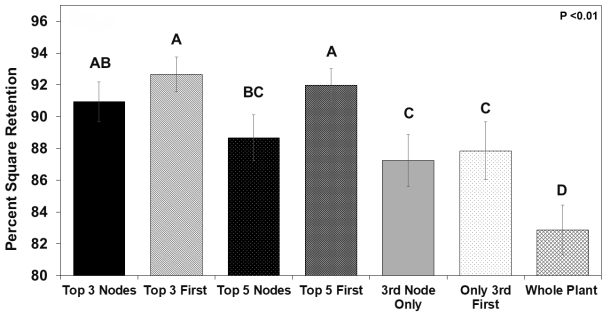


**Figure 3. Main effect of square retention monitoring methods during first week of bloom. Means within the graphs with the same letter are not significantly different according to Fishers protected LSD (α = 0.05).**

= 0.0160), for both sample dates during the third week of squaring ( $p = 0.017$; $p = 0.028$) and for the sample date during the first week of bloom (Table 1, $p = 0.044$). During these sample dates, square retention in ThryvOn cotton was 92.85 (±0.57 SEM), 95.16 (±0.037), 96.36 (±0.30), and 92.92% (±0.57), respectively. Square retention in non-ThryvOn was 88.46 (±0.67), 91.4 (±0.54), 92.44 (±0.53), and 84.83% (±0.84), respectively. Tarnished plant bug densities were lower in ThryvOn at all sample dates (Fig. 3).

**Influence of Insecticides**. Insecticide applications had a significant effect on square retention during the first sample date of the third week of squaring (six days after the first application) ( $p = 0.028$) and second date during the third week of squaring (two days after the second application) (Table 1, $p = 0.035$). Tarnished plant bug management with insecticides resulted in higher square retention, 95.13 (±0.39) and 96.21% (±0.34) compared to where plant bugs were not managed 91.42 (±0.53) and 92.60% (±0.51) on these sample dates, respectively.

**Yield**. There was no interaction between cotton technology and insect management, and insect management did not affect cotton yield (Table 1). However, cotton technology had a significant effect on yield. ThryvOn cotton averaged 1,193.3 (±69.90) kg $ha^{-1}$ compared to the non-ThryvOn average, 994.5 (±62.14) kg $ha^{-1}$ ( $p < 0.001$).

## DISCUSSION

Neither cotton technology nor insect management interacted with methods of square retention monitoring over the course of this study. Therefore, the same plant-based scouting methods can be used in both ThryvOn and non-ThryvOn cotton. However, there was variability in the square retention monitoring among sample dates (Fig. 2). In general, monitoring square retention on the top three nodes and first position sites on the top three nodes overestimated square retention relative to the whole plant, except at the first sample date. By the third week of squaring, all methods focusing on specific nodes underestimated square retention relative to the whole plant. Cotton forms new fruiting branches in the terminal portion of the plant every three days (Eaton, 1955; Oosterhuis, 1990; Tharp, 1960), therefore by the third week of squaring a large portion of previously observed damage was below the top five nodes, while new damage continued to accumulate within the top five nodes. The overestimation of square retention observed beginning at the third week of square, from all methods of square retention compared to the whole plant, is likely due to older damage accumulating over time.

Over the course of the pre-bloom period, the third node and third node first position square retention monitoring methods most accurately reflected whole plant square retention until the third week of squaring. Once five fruiting nodes were present, observing square retention with the top five nodes and top five nodes first position was similar to whole plant square retention. However, this is to be expected, because the two monitoring methods based around the fifth node make up the majority of total fruiting sites during this time. When observing abscission scarring, square retention based on the top five nodes could increase the likelihood of observing old damage. In contrast, using only the third node or top three nodes while observing more plants can increase scouting efficiency, allow for greater sample sizes across more plants (in one sample area) and more total area across a field, and decrease the likelihood of observing older damage. The differences observed across all methods (excluding whole plant monitoring) during this time never exceeded more than 3.5% higher than the top five node method, and the top five nodes method never had greater than 4% square retention compared to the whole plant approach.

Tarnished plant bugs have been observed to prefer small squares ≤ 3 mm compared to larger fruit (Bariola, 1969; Pack and Tugwell, 1976; Tugwell et al., 1976). This preference aligns with fruit sizes observed on the third node and three uppermost nodes throughout squaring. Using a square retention methodology centered around these feeding preferences would ideally be based on the third node or top three nodes, which can allow for greater sampling efficiency compared to a method based on the top five nodes. Moving forward, greater efficiency would follow the best sampling practices of using a more rapid and simple scouting approach that allows for a larger sample size in a smaller amount of time on a field scale, when variance among insect counts is high and justifies more sample (Stern et al., 1959; Wilson et al., 1989). However, more research is needed in larger plots on grower fields and in situations where plant bug densities are greater resulting in lower levels of square retention.

Overall, plant-based, pre-bloom scouting practices can vary as the season progresses and among scouts. However, plant development appears to be the only factor causing variability among methods of square retention monitoring. These data indicate that tarnished plant bug control, or lack thereof, achieved with the two-pass insecticide program did not alter the results obtained with different methods of square retention monitoring. Also, plant-based scouting approaches did not differ greatly when comparing ThryvOn cotton to non-ThryvOn cotton prior to bloom. Not having to alter scouting methods when observing ThryvOn cotton is meaningful with more hectares of ThryvOn cotton expected to be planted in the years to come. Observing square retention on either method based on the top three nodes or on only the third node could underestimate plant damage at different time periods throughout squaring, but this is due to whole plant damage accumulating over time. With representative sample sizes and scouting efficiency in mind, it is paramount that plant-based scouting strategies observe new damage accurately to trigger insecticide applications and to evaluate the efficacy of previous insecticide applications. In conclusion, observing square retention on the third node from the beginning of square formation until first bloom allows for higher scouting efficiency and a greater sample size across a field setting. These practices coupled with sweep net counts should ensure that tarnished plant bug management is as efficient as possible.

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