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ARTHROPOD MANAGEMENT

Effect of Leaf Pubescence on Tarnished Plant Bug (Hemiptera: Miridae) Ability to Cause Damage and Yield Loss in Cotton

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ABSTRACT

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is the most important insect pest of cotton in the states of Arkansas, Mississippi, Louisiana, Missouri, and Tennessee. Numerous insecticide applications are required during a growing season to manage this insect, and these associated costs are unsustainably high for growers. Cultural practices could provide an inexpensive means to manage the tarnished plant bug. Experiments were conducted at the Mississippi State University, Delta Research and Extension Center, to determine the impact of leaf pubescence on tarnished plant bug feeding that results in reduced cotton yields. Three separate varieties, each possessing differing levels of pubescence, were utilized to determine if there was any effect on tarnished plant bug density and damage. Varieties possessing high numbers of trichomes retained significantly more squares and yielded significantly higher than varieties with fewer trichomes. The variety with the fewest number of trichomes sustained significantly greater injury and yield loss than the other varieties utilized in this experiment. Variety selection could be an inexpensive cultural practice used to combat rising input costs associated with cotton production.

Considerable research has been conducted on host plant resistance traits for insect pests of cotton, *Gossypium hirsutum* L. In particular, variability in plant chemistry among cotton varieties and plant parts has been shown to have antibiotic activity against tobacco budworm, *Heliothis virescens* (F.), and bollworm, *Helicoverpa zea* (Boddie), in agricultural environments (Hanny, 1980; Hedin et al., 1983; Stipanovic, 1983). Secondary plant chemicals in cotton that negatively impact larvae include tannins, terpenoids, chrysanthemin, and gossypol.

Other traits have been researched in cotton that produce a non-preference response in several non-lepidopteran insect pests. Frego bract cotton was bred as a mechanism to minimize injury from boll weevil, *Anthonomus grandis grandis* Boheman, (Jenkins and Parrot, 1971). Frego bract cotton was characterized as having open bracts that exposed the flower bud (square) and thus reduced oviposition by boll weevil. However, those cotton lines were highly susceptible to injury from tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), due to the exposed flower bud.

Cotton is a unique plant in that it possesses extrafloral nectaries on the leaves which provide a sugar source for several insects. Tarnished plant bug and other insects utilize extrafloral nectaries as a food source prior to flowering. Cotton varieties have been bred and released for commercial production that do not have the extrafloral nectaries. These were referred to as nectariless cotton and showed some resistance to tarnished plant bug (Bailey et al., 1984; Schuster et al., 1976)). However, these varieties have only had limited acceptance commercially and commercial varieties with the nectariless trait currently are not available.

Pubescence is an important trait in cotton that can impact the preference and behavior of several insects (Nawab et al., 2011). Pubescence refers to the presence of trichomes, which are unicellular outgrowths from the epidermis of leaves and Nawab et al. (2011) identified three leaf phenotypes of cotton based upon the density of these trichomes: glabrous (smooth), hirsute (moderate pubescence), and pilose (dense pubescence). Bourland et al. (2003) presented a quantitative rating scale for leaf pubescence based upon abaxial trichome density counts that separates truly pilose (velvety) leaf types from those that are very hairy. The degree of pubescence, or trichome density is related to varying degrees of resistance/susceptibility to various insect pests (Meagher et al., 1997).

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Research has shown that infestation by aphid species can be negatively impacted by leaf trichome density in other crops (Dixon, 1998). This may be due to effects on mobility or the ability to feed. However, some pests, such as the cotton aphid, *Aphis gossypii* (Glover), and silverleaf whitefly, *Bemisia tabaci* (Gennadius), prefer densely pubescent leaves (Butler et al., 1986; Zarpas et al., 2006). There is a direct relationship between population growth of the cotton aphid and trichome density on the abaxial surface of cotton leaves (Zarpas, 2006). Research has also shown that some pests of cotton, such as *Bemisia argentifolii* Bellows and Perring and *Creontiades signatus* Distant, tend to prefer densely pubescent leaves with respect to oviposition (Benedict et al., 1983; Meagher et al., 1997). Additionally, moths of several important lepidopteran species have shown a preference for cotton varieties that are very hairy over ones that are glabrous for oviposition (Chatzigeorgiou et al., 2010; Javed et al., 2009).

Trichome density has been observed to affect the ability of tarnished plant bug to feed on cotton (Bailey et al., 1980; Laster and Meredith ,1974; Meagher et al., 1997; Meredith and Shuster, 1979; Wilson and George, 1986). Laster and Meredith (1974), and Meredith and Shuster (1979) observed that when compared to hirsute varieties, a glabrous variety was more susceptible to tarnished plant bug feeding. Studies have shown glabrous varieties to have significantly more square damage than pubescent varieties with similar plant bug populations (Bailey et al., 1980; Meredith and Schuster, 1979). Bourland et al. (2014) also determined that dense pubescence can confer some level of resistance to tarnished plant bug, yet some glabrous varieties possessed similar levels of resistance which could suggest differing mechanisms of resistance.

With multiple cultivars on the market, it may be economically feasible for a grower to select a cultivar if there is added benefit of protection due to level of pubescence that is expressed. Varieties have previously been selected for nectariless traits that provide some resistance against tarnished plant bug (Platt et al., 1999). If leaf pubescence can provide some resistance to tarnished plant bug damage, then cultivar selection could potentially be employed as a cultural control tactic. Previous research suggests mechanisms of resistance different from trichome density, yet little is known about the impact leaf pubescence on tarnished plant bug. The purpose of this study was to determine if leaf pubescence affects tarnished plant bug populations and damage in cotton.

MATERIALS AND METHODS

An experiment was conducted at the Delta Research and Extension Center, Stoneville, MS, in 2013 and 2014 to determine the impact of leaf pubescence on tarnished plant bug populations and damage to cotton. Treatments were arranged in a randomized complete block design with six replications. Treatments consisted of a glabrous variety (DP 1050 B2RF), hirsute variety (PHY 499 WRF), and a very hairy variety (ST 5288B2F). To determine which varieties to use, a total of eight varieties were planted in a greenhouse on 29 January 2013. Two leaves from the upper three nodes on five plants were collected from each variety. A thread counter lens with 10X magnification was used to count the total number of trichomes per 6.45 cm^2 area of leaf surface. From these trichome counts, the three varieties were chosen to represent a range in trichome densities.

All cotton varieties were planted on 21 May 2013 and 21 May 2014 at 113,668 seeds/ha. Plots consisted of four 1.01 m wide rows that were 15.2 m long. Once flowering began, tarnished plant bug densities were determined by taking two drop cloth samples per plot with a 0.76 m black drop cloth in the center two rows of each plot on a weekly basis. Square retention and nodes above white flower counts were collected weekly using the center two rows of each plot. Mean square retention from 16 plants per plot was determined by dividing the number of first position squares by the total number of first position fruiting sites and multiplying by 100. Square retention can be impacted by multiple factors, but tarnished plant bug feeding is one of the most important contributors to square abscission (Laster and Meredith, 1974). Although other factors may have contributed to square abscission in these trials, they were not quantified and it can be reasonably assumed that square retention from environmental conditions would be relatively similar among varieties. Nodes above white flower data were determined by counting the number of mainstem nodes from the highest first positon white flower to the apical meristem. All plots were irrigated on a regular schedule. No foliar insecticide applications were made to the trial during the growing season. At the end of the season, the center two rows of each plot were harvested using a cotton picker modified for small plot research and seed cotton weights were recorded. Lint yield was determined by taking 38% of the seed cotton weights.

All sampling and yield data were subjected to analysis of variance (PROC MIXED, Littell et al., 1996). Tarnished plant bug densities and square retention were analyzed by year due to differing numbers of samples between years. For tarnished plant bug densities, treatment (variety) was considered a fixed effect in the model and sampling date was used as a repeated measure. Replication was considered random and served as the error term and residual error for treatment. For square retention, treatment and sample date were considered as fixed effects in the model. The replication by sample date interaction was considered random and served as the error term and residual error for treatment. Yield was analyzed across both years. Treatment was considered a fixed effect in the model. Year and replication nested within year were considered random for the analysis of yield. Degrees of freedom were estimated using the Kenward-Roger method. Means were separated using the LSMEANS statement. Differences were considered significant for $\alpha=0.05$.

RESULTS AND DISCUSSION

There were significant differences in trichome densities per 6.45 cm^2 area of leaf surface among the three varieties ($F=457.14$; df=3, 357; $P<0.01$) (Fig. 1). The very hairy variety (307±8) had the greatest density of trichomes, followed by the hirsute variety (140±5) which had significantly fewer trichomes than the very hairy variety. The glabrous variety (56±3) had significantly fewer trichomes than either of the other varieties.

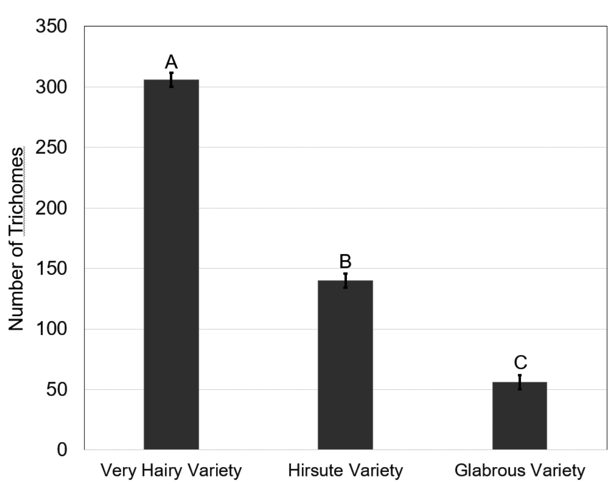

Figure 1. Mean number of trichomes per 6.45cm² by variety averaged for 2013 and 2014. Means separated by common letter are not significantly different at α=0.05.

Mean number of tarnished plant bug nymphs per 3.04-m were analyzed by year because more samples were collected in 2014 than 2013. During 2013, variety had a significant effect on tarnished plant bug numbers ($F=4.55$; df=2, 51; $P<0.01$). The very hairy variety (17.1±1.9) had significantly greater densities of nymphs than the glabrous variety (9.6±1.5) in 2013 (Table 1). The hirsute variety (13.9±1.9) had a similar density of tarnished plant bug nymphs as both the very hairy and glabrous varieties. During 2014, there was no significant effect of variety on number of tarnished plant bugs per 3.04-m of row ($F=1.01$; df=2, 69; $P=0.36$).

Square retention was analyzed by year due to more samples collected in 2013 than 2014. During 2013, there was a significant interaction between variety and sample date ($F=4.52$; df=10, 88.6; $P<0.01$) for mean square retention (Table 2). In general, square retention remained relatively high in the very hairy variety. In contrast, square retention in the glabrous variety started off high early in the year and declined significantly as the season progressed. Similarly, square retention was high in the hirsute variety early in the year, but significantly declined during weeks two and three of flowering. However, the reduction in square retention in the hirsute variety was not as great as that in the glabrous variety late in the flowering period.

During 2014, there was no significant interaction between variety and sample date ($F=0.37$; df=6, 54; $P=0.89$). Variety had a significant effect ($F=18.01$; df=2, 54; $P<0.01$) on square retention (Table 2). The very hairy variety (79.6±3.7) and the hirsute variety (76.1±2.4) had significantly greater square retention than the glabrous variety (59.9±3.7). There also was a significant effect of sample date ($F=10.06$; df=3, 54; $P<0.01$) on square retention. Significantly greater square retention was present at the first week of flowering (83.1±2.4) compared to the second (67.9±3.6) and fourth weeks of flowering (63.1±2.5). Square retention at the third week of flowering (73.3±2.1) was not significantly different than square retention at any week of sampling. These results coincide with the findings of Graham (2016) in which tarnished plant bug populations were not significantly different between a hirsute variety and a glabrous variety, yet the hirsute variety had significantly greater square retention and remained above the 80% threshold. Meredith and Schuster (1979) also found that the average reduction of flower buds in glabrous varieties (74-78%) was significantly greater than reductions in very hairy varieties (15-33%).

Table 1. Mean ± SEM number of tarnished plant bugs per 3.04-m of row for 2013 and 2014 by variety and week in Stoneville, MS. Means in a column followed by a common uppercase letter are not significantly different at α=0.05

2013	Squaring	First Flower	2WOF[a]	3WOF	Mean
Very hairy	nd	12.3±1.4	18.6±3.4	20.3±3.9	17.1±1.9A
Glabrous	nd	5.8±0.9	13±3.1	10±2.6	9.6±1.5B
Hirsute	nd	7.3±0.9	19.3±3.9	15±2.4	13.8±1.9AB
Mean	nd	8.5±1.4	17±3.4	15.1±3.7	
2014	**Squaring**	**First Flower**	**2WOF**	**3WOF**	**Mean**
Very hairy	0.6±0.5	7.6±2.6	16.5±3.6	8.3±1.6	8.2±1.6
Glabrous	0.8±0.5	6.1±1.3	13.5±3.2	5.5±1.2	6.5±1.3
Hirsute	1±0.4	8.3±1.9	20.6±4.4	9±1.8	9.7±1.9
Mean	0.7±0.5	7.6±2.6	16.5±3.6	7.6±1.6	

[a] WOF=Week of Flowering; nd=not determined.

Table 2. Mean (SEM) square retention for 2013 and 2014 by variety and week sample was conducted in Stoneville, MS. Means within a column separated by common uppercase letter or within a row followed by a common lowercase letter are not significantly different at α=0.05

2013	Squaring	First Flower	2WOF[a]	3WOF	4WOF	5WOF	Mean
Very hairy	97.3±1.6a	87.3±1.8a-d	78.6±4.4a-e	91.3±2.3abc	88±0.7a-d	90.6±1.1abc	88.8±1.3
Glabrous	96±1.2ab	77.6±3.5b-e	60.6±4.6efg	56.6±3.7fg	51±8.4g	56±6.3fg	66.3±3.3
Hirsute	96.6±1.8a	85.6±4.1a-d	70±3.5def	76.6±3.7cde	79.3±2.9a-e	81.3±1.7a-d	81.6±1.8
Mean	96.6±1.6	83.5±1.8	69.7±4.4	74.8±2.3	72.7±0.7	76±0.7	
2014	**Squaring**	**First Flower**	**2WOF**	**3WOF**	**4WOF**		**Mean**
Very hairy	nd	91±2.4	72.6±3.6	83.5±2.1	72.3±2.5		79.6±3.7A
Glabrous	nd	72.6±6.9	59.3±5.9	58.5±12.1	49±3.9		59.8±3.7B
Hirsute	nd	85.6±3.9	71.6±4.2	78±5.3	68±2.8		76.1±2.4A
Mean	nd	83.1±2.4a	67.8±3.6b	73.3±2.1ab	63.1±2.5b		

[a] WOF=Week of Flowering; nd=not determined.

During 2013, drop cloth samples were not taken prior to flowering; however, square retention counts were collected. By the time drop cloth samples were initiated, plant bugs had already caused damage as evidenced by square retention during weeks three and four (coincides with when drop cloth samples began). In 2014, drop cloth samples were collected earlier in the season when tarnished plant bug numbers were still low. The differences between sample timing could explain the significant differences in tarnished plant bug densities within each year. The very hairy variety had significantly more tarnished plant bug nymphs per 3.04-m of row in 2013; however, it retained a significantly higher percentage of squares than the other two varieties. In 2014, there were no differences in tarnished plant bug densities among varieties; yet, square retention followed a similar trend as observed in 2013. During 2014, variety had no significant effect on tarnished plant bug numbers, which may suggest there is not a preference of selection in terms of pubescence. Significant differences were observed for variety in 2013 when tarnished plant bug populations shifted out of the glabrous variety plots into the other two varieties. The glabrous variety had been heavily damaged and had minimum fruit left for tarnished plant bugs to feed on.

Variety did not have a significant effect on average node above white flower counts in 2013 ($F=0.19$; $df=2, 45$; $P=0.82$) or 2014 ($F=1.61$; $df=2, 42$; $P=0.21$). These results suggest that the relative maturity of the three varieties did not vary significantly (Data not shown).

There was a significant effect of variety on mean lint yield (F=96.97; df=2, 22; *P*<0.01) (Fig. 2). The very hairy variety (1708.97±82.9 kg/ha) yielded significantly greater than all other varieties. The hirsute variety (1330.42±34.4 kg/ha) yielded significantly less than the very hairy variety but significantly more than the glabrous variety (918.24±48.9 kg/ha). The very hairy variety had over three times the recommended threshold of tarnished plant bugs, yet still yielded significantly greater than the other two varieties. These results are similar to observations by Graham (2016) in which pubescent varieties yielded 26-48% more than glabrous varieties, with similar densities of tarnished plant bug. Meredith and Schuster (1979) also reported that glabrous cotton varieties had significantly higher yield reductions (239 kg/ha) due to tarnished plant bug damage when compared to pubescent varieties (163 kg/ha). It is hypothesized that trichomes interfere with tarnished plant bug feeding and thus tarnished plant bugs do not cause as much injury to a pubescent variety when compared to a glabrous variety. Research has shown that pests, such as *Amrasca devastans* (Distant), are negatively affected by leaf pubescence (Murugesan et al., 2010). Murugesan et al. (2010) showed that oviposition and feeding damage were lower in varieties possessing densely pubescent leaves. However, more research is still needed to determine the mechanism that allows pubescent varieties to sustain high populations of tarnished plant bugs, yet still retain significantly more squares and yield significantly greater than other varieties. Although the very hairy variety yielded the greatest and retained more squares, there could be a trade off at harvest due to leaf trash. Negative impacts on lint quality due to leaf trash contamination commonly occur with very hairy varieties, which results in lower value of the lint. Also, research has shown that lepidopteran pests, such as *Helicoverpa zea* (Boddie), prefer to oviposit onto densely pubescent leaves (Chatzigeorgiou et al., 2010; Javed et al., 2009). The potential need for an insecticide application targeting lepidopteran pests could occur when planting a very hairy variety. However, it could be beneficial for a grower to plant a hirsute variety which still has an impact on tarnished plant bug populations, but could have less negative effects on lint quality and/or lower risk associated with lepidopteran pests. More research is needed to determine if this is the best option available. Additionally, more research is needed to gain a better understanding about how leaf pubescence impacts tarnished plant bug injury in cotton.

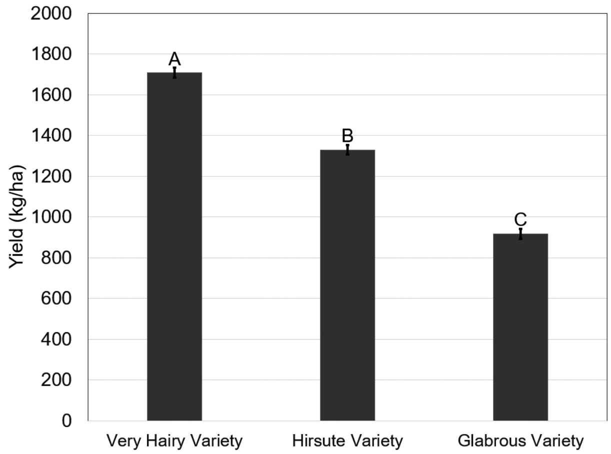

Figure 2. Mean (±SEM) lint yield averaged for very hairy, hirsute and glabrous cotton varieties during 2013 and 2014 in Stoneville, MS. Means separated by common letter are not significantly different at α=0.05.

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