BOLL WALL THICKNESS IN FOUR COTTON SPECIES AND SUSCEPTIBILITY TO STINK BUG FEEDING

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Abstract

Southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), adults can introduce pathogens into cotton bolls while feeding. Stylet penetration estimates of southern green stink bugs are known, and an understanding of boll wall thickness in cotton species may aid in determining susceptibility of bolls to stink bug feeding. The objective of this study was to determine whether boll wall thickness differed between four cotton species (*Gossypium arboreum, G. barbadense, G. herbaceum* and *G. hirsutum*). Overall, *G. barbadense* exhibited the highest mean wall thickness (mean ± SEM [*n*]; 1.74 mm ± 0.01 [1,102]); lowest mean wall thickness was observed in *G. herbaceum* (0.96 mm ± 0.01 [706]). Mean wall thicknesses for *G. arboreum* and *G. hirsutum* were 1.69 (± 0.01 [1,102]) and 1.56 mm (± 0.01 [1,102]), respectively. At 1-d after bloom, maximum wall thickness was 1.11 mm (*G. barbadense*). For most species, maximum wall thickness was observed at 14-d after bloom, with means ranging from 1.01 (*G. herbaceum*) to 2.14 mm (*G. barbadense*). Results indicate 1-d old bolls of all cotton species are susceptible to stink bug stylets breaching of the internal surface of the carpel wall. Further, despite maximum mean wall thickness at 14-d within most species, these boll walls remained within the range of stylet penetration by adult stink bugs. In addition to potentially contributing to selection of cotton genotypes that may minimize stink bug penetration of carpel walls, these findings should alter the management of stink bugs through proactive monitoring earlier in the cotton-growing season in lieu of reactive control measures after stink bugs are detected later in the season.

Introduction

Southern green stink bug, *Nezara viridula* (L.) (Hemiptera: Pentatomidae), adults feed on cotton bolls and introduce pathogens while feeding. Whether pathogens are introduced because of wounding or complete penetration of the carpel wall is not clearly understood. Comparing potential stylet penetration estimates of southern green stink bug (Esquivel 2011) to the thickness of boll carpel walls would improve our understanding of the mechanics involved in feeding and transmission of pathogens. Stylet penetration values range from a minimum of 900 µm for 1st instars to a maximum of 2,800 µm for female adults (Esquivel 2011). Cauquil (1975) indicated a thickness of 2,000 µm for boll walls; however, thickness of cotton boll carpel walls over time has not been clearly documented. Thus, data relating temporal boll susceptibility relative to carpel wall thickness and stink bug penetration values are lacking. Cotton bolls with thicker walls reportedly mitigated damage by pink bollworm (*Pectinophora gossypiella* Saunders) (Singh et al. 1965). Similar efforts to elucidate boll wall thickness may lead to selection of cotton genotypes that minimize complete penetration of the carpel wall and damage by stink bugs. The objective of this study was to determine the thickness of boll carpel walls in four cotton species to better understand the susceptibility thresholds of developing cotton bolls to stink bug feeding.

Materials and Methods

Cotton Plantings

Sixteen genotypes representing four cotton species (*Gossypium arboreum*, *G. barbadense, G. herbaceum, and G. hirsutum*) were selected for evaluation of carpel wall thickness. There were two *G. arboreum* genotypes, three *G. barbadense* genotypes, two *G. herbaceum* genotypes, and nine *G. hirsutum* genotypes. These genotypes included conventional commercial varieties, accessions from the US Cotton Germplasm Collection, and lines of interest for genetic research. This report will only present data to compare species. Data on individual genotypes will be presented in a subsequent report.

Seeds were planted in peat pellets in a greenhouse on 1 April 2011 and grown until full expansion of cotyledon leaves. Twenty seedlings of each genotype were transplanted on 20 April 2011 to field plots at the Southern Plains

Agricultural Research Center (College Station, TX). Field plots were single rows, 9.1 m long x 1.0 m, wide with 45.7 cm spacing between seedlings. Conventional agronomic practices were used to maintain the crop.

Weekly Boll Cohorts

Cotton plants were monitored daily for the presence of open yellow flowers. Flowers on a given day were tagged and considered as cohorts to monitor boll development. Tags labeled with unique alphanumeric characters were strung around the peduncle and these tags allowed collection of known-age bolls for dissections at later boll age intervals. For each genotype, flowers were initially tagged on three separate dates (i.e., replicates). Bolls were subsequently removed from the plants at 3-, 7-, 14-, 21-, 28-, 35-, and 42-d after tagging (i.e., age after bloom) for each of the replicates. Three bolls were removed from the plants at the designated age intervals for each of the replicates. In total, nine bolls per genotype were examined for each of the age intervals indicated above.

Daily Boll Cohorts

After weekly boll cohorts were tagged, a separate group of bolls was tagged to evaluate boll wall thickness at 1-, 2-, and 3-d after bloom. Establishment of replicates, tagging methodologies, sampling of bolls, and number of bolls examined for each genotype were as previously described for weekly boll cohorts.

Processing of Bolls

On the day of scheduled boll removal, selected bolls were removed from plants before 1000 hours to minimize any effects of water stress on cell turgor that could influence measurements of carpel wall thickness (Anderson and Kerr 1943). Removed bolls were returned to the laboratory for dissection and measurements.

For all boll ages, an initial cut was made at approximately a third of the distance from the distal apex of the boll. A second cut was made at the widest diameter of the boll. The initial cut provided a horizontal surface to stabilize the resulting cross-section for analysis. For bolls ≤3-d-old, a single-edge razor blade was used to obtain cross-sections (≈3-5 mm thick). For bolls ≥7-d-old, a conventional knife was used to obtain cross-sections as described above. Microscope and imaging equipment and software described by Esquivel (2011) were used to document and measure carpel walls near the boll sutures. The sutures on the external surface of the boll wall correspond with the internal longitudinal midline of the locule with developing seeds, and a measurement of wall thickness was recorded on each side of this suture (Fig. 1). Thus, two measurements were recorded for each locule. Because the meeting of the boll carpel wall at the sutures creates a thickened juncture, wall thickness was measured at locations equidistant from the suture at: ≤1 mm for 1- to 3-d old bolls; 1.5-2.5 mm for 7-d old bolls; 2.6-3.0 mm for 14-d old bolls; and 3.1-4.5 mm for bolls ≥21-d old. Additionally, the focus of the measurements was at locations that readily appeared as having the least distance between the exterior surface of the boll and the interior surface of the carpel wall aligning with the developing seed because our interest was determining susceptibility of bolls based on carpel wall thickness.

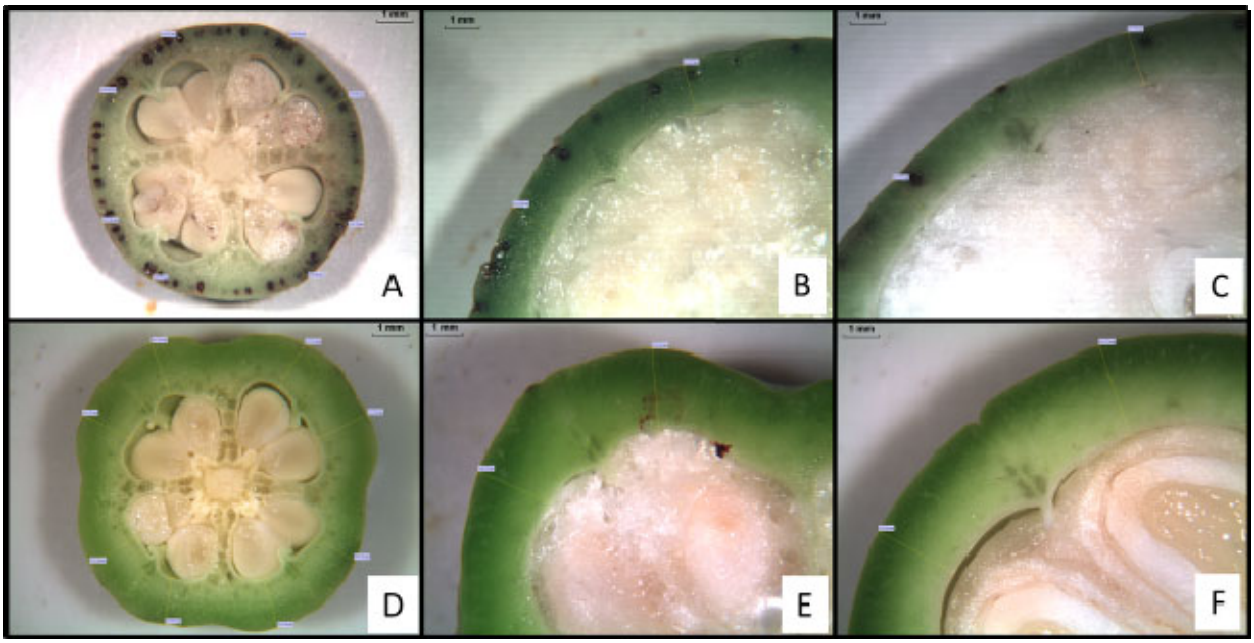

Figure 1. Cross-sections of bolls depicting measurement locations (depicted by gray rectangles) in two *G. hirsutum* genotypes representing thinnest (FM958; A-C) and thickest carpel walls (T582; D-F): 1-d (A & D), 7-d (B & E), and 14-d old bolls (C & F). All frames at 17.6x.

Statistical Analyses

For each group of bolls (i.e., bolls examined at 1-, 2- and 3-d after bloom and bolls examined at 3-, 7-, 14-, 21-, 28-, 35-, and 42-d after bloom), differences in mean carpel wall thickness were compared using the PROC MIXED procedure (SAS Institute 2008). In the MODEL statement, boll wall thickness (mm) was the response variable while cotton species, boll age, and species-by-age interaction were the fixed effects. The model included three RANDOM effects: age nested within replicate (i.e., tagging date); genotype nested within species; and locule nested within bolls nested within genotype. Means comparison was conducted using the PDIFF option of the LSMEANS statement at a conservative significance level ($p = 0.01$). The 35- and 42-d age intervals were excluded from the analyses because of incomplete observations, but means are provided for those cotton species having complete observations at these ages. Although all 16 genotypes were examined, results presented here refer to comparisons between cotton species only. Comparisons of genotypes will be presented in a subsequent report.

Results and Discussion

Weekly Boll Cohorts

Significant differences in carpel wall thickness were observed between cotton species ($F = 6.70$; df = 3, 12; $p < 0.01$) when genotypes were pooled for each species. Overall, *G. barbadense* exhibited the highest mean wall thickness (mean ± SEM [*n*]; 1.74 mm ± 0.01 [1,102]); while the lowest mean wall thickness was observed in *G. herbaceum* (0.96 ± 0.01 [706]). Mean wall thicknesses for *G. arboreum* and *G. hirsutum* were 1.69 (± 0.01 [1,102]) and 1.56 mm (± 0.01 [1,102]), respectively. Carpel wall thickness was significantly affected by age ($F = 209.59$; df = 4, 10; $p < 0.01$) with peak mean wall thickness observed in 14-d old bolls for all species.

The interaction term between cotton species and boll age was significant ($F = 41.28$; df = 12, 2,512; $p < 0.01$). *Gossypium barbadense* and *G. herbaceum* possessed the highest and lowest mean wall thicknesses, respectively, in virtually all ages (Table 1). Within all cotton species, significant differences were observed between ages leading up to the maximum mean wall thickness at 14-d; generally, wall thickness began to decline after 14-d and, in most cases, significant differences were not observed between 21- and 28-d bolls. Observed maximum means for wall thickness in all cotton species are within the stylet penetration range for southern green stink bug adults and late instars (Fig. 2).

Table 1. Mean (± SEM) carpel wall thickness (mm) of *Gossypium* spp. bolls at indicated age (d after bloom) for weekly cohorts (3-d, 7-d and weekly thereafter) of field-grown cotton; number of observations are in parentheses following letters indicative of means separation.

	Species			
Age (d)	*G. arboreum*	*G. barbadense*	*G. herbaceum*	*G. hirsutum*
3	1.11 (0.01) abE (118)	1.26 (0.01) aE (142)	0.72 (0.01) bE (138)	1.20 (0.01) aE (678)
7	1.50 (0.02) aD (108)	1.75 (0.02) aD (166)	0.90 (0.01) bD (140)	1.54 (0.01) aD (672)
14	2.11 (0.04) aA (106)	2.14 (0.02) aA (160)	1.01 (0.01) bC (138)	1.79 (0.01) aA (618)
21	2.04 (0.04) aAB (102)	1.92 (0.03) aB (162)	1.11 (0.01) bA (142)	1.75 (0.01) aB (568)
28	1.78 (0.03) aC (80)	1.84 (0.02) aBC (164)	1.06 (0.01) bAB (142)	1.64 (0.01) aBC (462)
35	---	1.77 (0.02) (170)	0.72 (0.03) (6)	1.44 (0.02) (346)
42	---	1.39 (0.02) (138)	---	1.14 (0.01) (8)

Measurements recorded at two locations per locule (i.e., on each side of external suture). Raw data means are presented but separation of means was based on LSMEANS.
Means within a row and column followed by the same lowercase and uppercase letters, respectively, are not significantly different. Means separation was based on PDIFF option in LSMEANS statement, with significance level at $p \leq 0.01$. Data for 35- and 42-d were excluded from the analyses because of incomplete observations and are provided for informational purposes only.

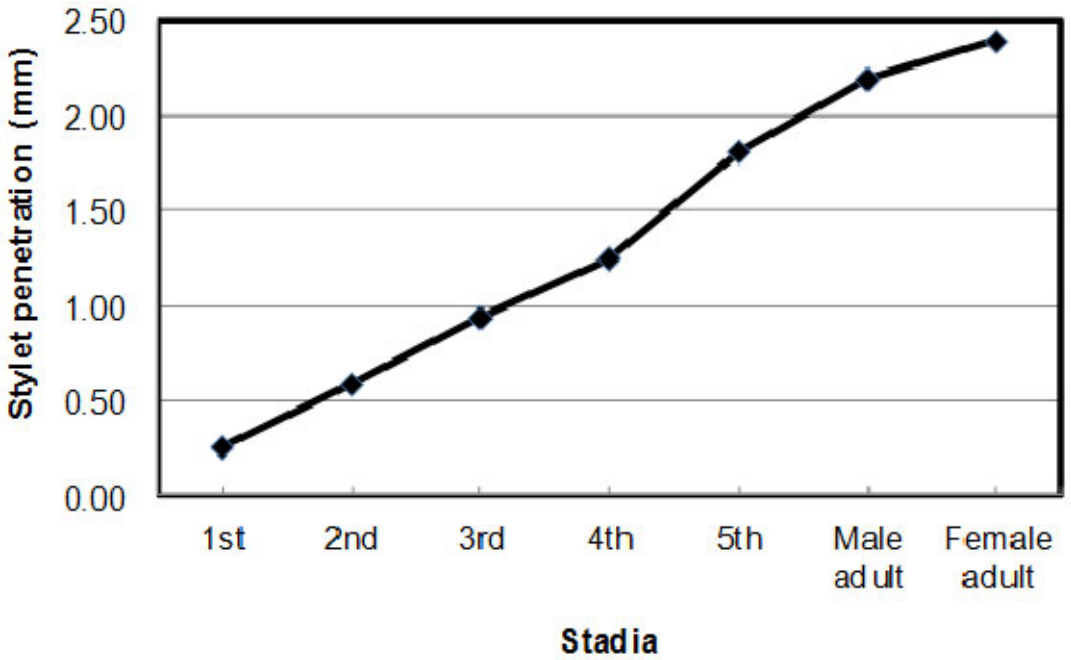

Figure 2. Mean estimates of stylet penetration potential for southern green stink bug nymphs and adults (data from Esquivel 2011).

Daily Boll Cohorts

An identical and significant pattern in overall mean wall thickness among cotton species (F = 3.99; df = 3, 12; p = 0.03) was observed in the 1- to 3-d old bolls as in the weekly boll cohorts. *Gossypium barbadense* and *G. herbaceum* exhibited the highest and lowest mean wall thickness (1.18 mm ± 0.01 [486] and 0.65 mm ± 0.00 [414]). Mean wall thicknesses for *G. arboreum* and *G. hirsutum* were 1.00 (± 0.01 [364]) and 1.04 mm (± 0.01 [2,044]), respectively. Carpel wall thickness was significantly affected by age (F = 7.84; df = 2, 6; p = 0.02) with 3-d old bolls having higher mean wall thickness than 1- or 2-d old bolls. Maximum mean wall thickness at 1-d after bloom was 1.11 mm (± 0.01 [160]) for *G. barbadense* bolls.

No significant interaction was observed between cotton species and age (F = 0.58; df = 6, 1,658; p = 0.75). Older bolls (3-d old) consistently possessed significantly higher mean wall thickness regardless of species, and *G. herbaceum* consistently possessed significantly lower mean wall thickness regardless of age (Table 2). At 1-d after bloom, bolls in all cotton species are susceptible to 2nd through 5th instars and adults because observed wall thickness means are within the stylet penetration range for these southern green stink bug stadia (Fig. 2).

Observed mean boll wall thickness for the four *Gossypium* spp. at 1-d after bloom ranged from 0.60 (*G. herbaceum*) to 1.11 mm (*G. barbadense*) (Table 2). Coupling these findings with known southern green stink bug mean stylet penetration estimates (Fig. 2), 1-d old bolls of all four *Gossypium* spp. are susceptible to penetration of the internal carpel wall by virtually all instars and adult stink bugs. More problematic is that the highest mean boll wall thickness was observed in 14-d old bolls of *G. barbadense* (2.14 mm) yet these boll walls are still thinner than the mean penetration potential of adult stink bugs. *Gossypium hirsutum* cultivars are planted on ≈98% of US cotton acreage (USDA-NASS 2011), and maximum mean wall thickness in this species was 1.79 mm at 14-d of age (Table 1). Thus, *G. hirsutum* bolls are clearly susceptible to stylet penetration of carpel walls at all ages.

Table 2. Mean (± SEM) carpel wall thickness (mm) of *Gossypium* spp. bolls at indicated age (d after bloom) for daily (1- to 3-d) cohorts of field-grown cotton; number of observations are in parentheses following letters indicative of means separation.

	Species			
Age (d)	*G. arboreum*	*G. barbadense*	*G. herbaceum*	*G. hirsutum*
1	0.96 (0.02) abB (120)	1.11 (0.01) aB (160)	0.60 (0.00) bB (140)	0.98 (0.01) aB (680)
2	0.98 (0.01) abB (120)	1.19 (0.01) aB (164)	0.65 (0.01) bAB (138)	1.03 (0.01) aAB (680)
3	1.07 (0.02) abA (124)	1.25 (0.01) aA (162)	0.70 (0.01) bA (136)	1.09 (0.01) aA (684)

Measurements recorded at two locations per locule (i.e., on each side of external suture). Raw data means are presented but separation of means was based on LSMEANS.
Means within a row and column followed by the same lowercase and uppercase letters, respectively, are not significantly different. Means separation was based on PDIFF option in the LSMEANS statement, with significance level at $p \leq 0.01$.

Summary

Boll wall thickness differs between cotton species. At all ages, bolls are susceptible to known stylet penetration potential of late instars and adult southern green stink bugs. Although stylet penetration estimates exceed boll wall thickness, other biotic factors such as firmness of carpel wall and pressure required to penetrate the carpel wall will ultimately affect whether the internal wall is breached. Nonetheless, monitoring efforts for stink bugs may need to be implemented at boll set to mitigate introduction of pathogens and yield losses because bolls for all cotton species are susceptible to breaching of carpel wall at 1-d of age by late instars and adults. These findings provide a foundation for screening of cotton lines to potentially select for increased wall thickness to deter or mitigate penetration of the carpel wall by stink bugs.

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