An Examination of the External and Internal Signs of Cotton Boll Damage by Stink Bugs (Heteroptera: Pentatomidae)[1]

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Abstract Small- and medium-sized bolls were exposed to stink bugs, primarily *Nezara viridula* (L.), *Acrosternum hilare* (Say), and *Euschistus servus* (Say), for a 48-h feeding period. Bolls were then examined for external and internal evidence of feeding 2, 4, 6, 8, and 10 d later. No relationship was documented between numbers of external feeding marks and internal warts that form when the interior of the boll is pierced. In fact, approximately 20% of damaged bolls with internal warts lacked external marks. Therefore, external marks cannot be used to accurately estimate the occurrence or amount of internal boll damage by stink bugs. Neither size nor number of external marks or warts increased significantly among the five post-feeding sampling dates. All visual signs of damage were present by the second day. There were significantly more damaged bolls with the combination of external marks, stylet sheaths, and warts (approximately 70%) than any other combination of feeding signs. There was a significant increase in lint and seed damage through time. Finally, a strong relationship existed between the presence of a feeding stylet sheath and wart number. A regression equation was generated to predict the presence of internal wart damage (warts) based on the number of stylet sheaths observed. A sampling program based on the incidence of stylet sheaths could potentially be used in a cotton pest management program to effectively assess stink bug injury to cotton bolls without destroying the developing bolls.

Key Words *Acrosternum hilare, Euschistus servus, Nezara viridula,* Pentatomidae, Bt cotton, boll damage

Stink bugs (Heteroptera: Pentatomidae) have been reported as pests of cotton since the beginning of the 20th Century (Morrill 1910). However, the heavy use of organophosphate and pyrethroid insecticides to combat the boll weevil, *Anthonomus grandis grandis* Boheman, and lepidopterous pests have kept pentatomid numbers below economic levels (Turnipseed and Greene 1996). Recently, stink bugs have reemerged as major pests of cotton in the Southeast. Reasons for the emergence of stink bugs as economic pests include the eradication of the boll weevil from North Carolina, South Carolina, Georgia, and Alabama (Grefenstette 1999), the utilization of highly specific insecticides, such as those containing the endotoxin from *Bacillus thuringiensis* Berliner (Bt), and the rapid adoption of the recently introduced transgenic Bt cultivars. These factors have effectively reduced the number of applications of broad-spectrum insecticides to cotton (Barbour et al. 1988), resulting in increased

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populations of pests such as stink bugs that were once secondarily controlled by these insecticides (Bachelor and Mott 1996, Turnipseed and Greene 1996).

The most important pentatomid species on cotton in the Southeast are the southern green stink bug, *Nezara viridula* (L.), the green stink bug, *Acrosternum hilare* (Say), and the brown stink bug, *Euschistus servus* (Say) (Roach 1988, Bundy et al. 1998). These pests feed on developing seeds and lint (Barbour et al. 1988) causing shedding of young bolls, yellowing of lint, and reduction in harvestable locks (Wene and Sheets 1964, Roach 1988). Internal evidence of stink bug damage has been reported to be a "yellowish to brownish discoloration . . . beneath insertion area" (Barbour et al. 1988) and "a watery or blisterlike, bright green area" (Morrill 1910). Feeding lesions, generally known as "warts," form on the inside carpel wall of the cotton boll as a result of penetration by the pentatomid stylet sheaths during feeding. External evidence of stink bug feeding needs clarification. One report indicates that damage is visible as small, purple spots on a green boll (Barbour et al. 1988). Another source reported that damage was not visible externally (Greene and Turnipseed 1996). Morrill (1910) found no indication of a connection between external spots and internal damage.

Another sign of feeding produced by these insects is the presence of the stylet sheath, although this structure is not usually mentioned. This sheath, composed primarily of lipoproteins, is a rapidly solidifying oral secretion that forms during feeding. It surrounds the mouthparts of the insect and creates a canal through which digestive enzymes are passed from the insect to the plant tissue (Miles 1972). The tissue is then liquified and imbibed by the insect. The stylet sheath remains at the feeding site after feeding has ceased and the stylets are removed. This structure may be found as an artifact of feeding in many of the true bugs, including the Pentatomomorpha (Schuh and Slater 1995). The presence of the stylet sheath has been used as an indicator for feeding of the rice stink bug, *Oebalus pugnax* (F.), on rice (Bowling 1979), but has not been utilized for cotton.

Limited research has been reported on the relationship of boll age with damage caused by stink bug feeding. One recent study (Greene et al. 1998) found that during a 5-d feeding period, young bolls (4 to 15 d from white bloom) were significantly damaged by pentatomids; whereas, mature bolls (18 d from white bloom) were not. The current work was initiated to examine both external and internal signs of boll damage caused by stink bug feeding, and to determine if there is a relationship between the observed external feeding injury and internal boll damage.

Materials and Methods

Plots of Bt cotton (NuCotn 33b) were grown using standard production practices. Two planting dates were utilized (13 May and 20 August 1998) in order to have access to the appropriate boll sizes for an extended period. Large field cages (1.82 m × 1.82 m × 3.65 m) were placed in the plots just prior to beginning of white bloom (1-d-old flower). Plants within the cages were sprayed with pesticides to kill the arthropods present. The earlier planting of cotton was sprayed with Baythroid 2 EC (cyfluthrin) on 16 July and Capture 2 EC (bifenthrin) plus Provado 1.6 F (imidacloprid) on 20 July. The later planting of cotton was sprayed with Capture 2 EC on 1 October. The application rate for all pesticides was 0.06 kg (AI)/ha. Insecticides were applied with a CO^2-powered backpack sprayer with 4 TX-12 nozzles on a 1.8 m boom (3 nozzles per row) at 40 psi (276 Kpa). White blooms were tagged with flagging tape to accurately age the developing bolls. Two sizes of bolls were used in this study: small (aged 7 to 8 d from white bloom) which were approximately 1.5 cm diam and medium (aged 11 to 12 d from white bloom) which were approximately 2.5 cm diam. Single bolls of the appropriate age were isolated using small cages modified from Greene et al. (1998). Each cage was composed of a 355 ml styrofoam cup with its bottom removed. A nylon stocking, with the toe removed, was placed over the cup and secured to the stem of the cotton plant with paddle wire. The nylon on the bottom was closed with a round cardboard disk (4 cm diam) that was slit to hold the nylon firmly in place and to provide easy cage entry when necessary. Field-collected adults of *N. viridula, A. hilare,* and *E. servus* were used in this experiment depending upon availability. Lab-reared individuals were occasionally supplemented when necessary. Preliminary data showed no significant differences in visual signs of feeding among the 3 species. Therefore, stink bug damage was assumed to be the same for the purpose of this experiment.

Bugs were starved for a 24-h period prior to being placed into the cages to better facilitate feeding responses. Two stink bugs were isolated on each caged boll and removed after a 48-h feeding period. The bolls were removed at 2, 4, 6, 8, and 10 d after the initial feeding exposure. Cages remained in place until the bolls were removed in order to prevent the possibility of additional feeding damage by other insects. Controls consisted of bolls surrounded by small cages without stink bugs and were maintained for the same periods. After removal, all bolls were examined externally for the number of stylet sheaths and external markings, and internally for the number of warts, and amount of damaged lint and seed. A rating system (1-5) for lint damage was used, where each lock within a boll was examined. Yellowing of a single lock equal to or greater than the size of one seed was given a rating of 1. The ratings for all locks within a boll were combined, thus giving a maximum boll damage rating of 5. A cotton seed was considered damaged if puncture marks were present. Total number of damaged seeds was recorded for each boll. Using a dissecting microscope, the diameters of the warts and external markings also were measured on each of the post-feeding dates.

The experiment was duplicated on 21 August and 9 September, in the cotton planted in May, and on 9 and 21 October in the cotton planted in August. Each exposure period after the stink bug feeding was replicated a total of 16 (small bolls) or 17 (medium bolls) times. A total of 215 bolls was examined for stink bug feeding damage.

The experimental design was a RCB, and the treatments were assigned in a 2 (bolls) × 5 (exposure period) factorial arrangement. Tukey's pairwise comparisons were performed on the percentages of the two bolls sizes that exhibited the various combinations of boll damage (external marks, stylet sheaths, warts, etc.). Regression analyses were performed to compare the relationship numbers of external marks and stylet sheaths with numbers of warts. An ANOVA was performed on sizes of both external marks and warts for the post-feeding dates. All statistical analyses used the Statistical Analysis System (SAS Institute 1989).

Results and Discussion

External marks. No significant relationship was found between numbers of external marks and internal warts (Table 1). Exposed bolls having no marks often had

Table 1. Percentages of cotton bolls (two sizes) with various combinations of external marks (EM), stylet sheaths (SS), and warts for 5 time periods following a 48-h stink bug feeding exposure

Boll size	Days after exposure	EM + SS + wart	SS + wart (no EM)	EM + wart (no SS)	EM + SS (no wart)	SS only (no wart or EM)	Bolls with no damage
Small	2	61.6	6.2	0.0	3.6	3.6	25.0
	4	40.6	29.9	0.0	0.0	0.0	29.0
	6	52.9	32.5	0.0	0.0	0.0	14.6
	8	83.9	16.1	0.0	0.0	0.0	0.0
	10	79.4	13.4	0.0	0.0	3.6	3.6
Medium	2	84.9	0.0	0.0	0.0	9.5	5.6
	4	66.2	29.0	0.0	0.0	0.0	4.8
	6	75.4	19.8	0.0	0.0	0.0	4.8
	8	62.3	28.2	0.0	0.0	4.8	4.8
	10	76.6	23.4	0.0	0.0	0.0	0.0

No significant differences ($P > 0.05$) in the numbers of external marks, stylet sheaths, or warts among the 5 time periods and two boll sizes. A total of 215 bolls was examined.

high levels of internal injury. In fact, over 20% of the bolls with warts had no external marks.

There were no significant differences in the numbers of external marks among the 5 post-feeding dates. Also, none of the control bolls had any signs of external damage. This indicates that external marks from within 48 h after the bolls are injured by stink bug feeding. Also, there were no significant differences in number of external marks among the two bolls sizes sampled.

Warts. Wart numbers did not increase significantly among the 5 post-feeding dates. Warts were present in some form within 48 h after stink bug injury. Medium-sized bolls did have a significantly greater number of warts than small bolls ($P = 0.0312$). This may be due to a size preference or possibly the result of a greater surface area exposed to the bugs for feeding in larger bolls. No warts were found in any of the control bolls.

Lint and seed damage. There were significant increases in both lint and seed damage through time ($P = 0.0453$ and $P = 0.0025$, respectively) (Figs. 1, 2). The slight decrease in damage at days 8 and 10 was probably due to sampling error. A larger sample size would probably yield an increase in damage for these days or at least not a decrease. No controls had any lint or seed damage.

Stylet sheaths. There was a significant linear relationship between stylet sheath numbers and numbers of warts ($P \leq 0.0001$) (Fig. 3). All of the bolls with wart damage also had stylet sheaths (Tables 1 and 2). A mean of 68.4% (±23.46) of the damaged bolls had stylet sheaths, external marks, and warts. A mean of 19.9% (±17.36) of the bolls had stylet sheaths and warts, but no external marks, and only 0.3% had external

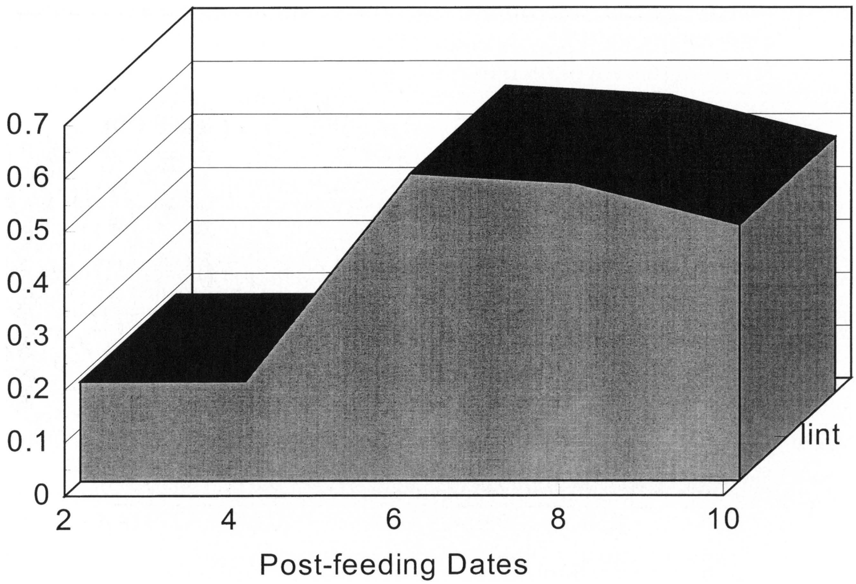

Fig. 1. Lint damage (number of yellowed locks) caused by stink bugs on five time periods following a 48-h feeding exposure.

marks and stylet sheaths, but no warts. A mean of 2.1% (±6.21) had stylet sheaths without external marks or warts, and 9.2% of the bolls examined were not fed upon by the stink bugs. No bolls had external marks and warts without stylet sheaths. The stylet sheath was always present when warts were observed. None of the control bolls had stylet sheaths.

A regression equation was formulated ($y = .915 + .655x$, where y = wart number and x = stylet sheath number) in order to predict the amount of internal damage (warts) from the number of stylet sheaths observed (Fig. 3). The correlation coefficient was not high ($r^2 = 0.611$), probably due in part to sampling error. Stylet sheaths were occasionally knocked off when handled and thus overlooked. However, a small remnant of the sheath was generally present even when this occurred. Therefore, closer scrutiny would most likely lower the sampling error and increase the r^2 value.

Overall comparisons. In both small- and medium-sized bolls there were no differences in the combinations of external (external marks and stylet sheaths) and internal (warts) signs of stink bug-damaged bolls observed among the 5 post-feeding dates. However, when results were pooled for all post-feeding durations, comparisons of the various combinations of these symptoms did produce significant differences within boll size. There were significantly more bolls ($P \leq 0.0001$) that bore all three symptoms (external marks, stylet sheaths, and warts) than any other combination. Bolls with stylet sheaths and warts, but no external marks, were significantly more numerous ($P \leq 0.0001$) than bolls with external marks and stylet sheaths (no warts). There were more bolls with stylet sheaths and warts (no external marks) than those without damage, but the difference was not statistically significant. No bolls

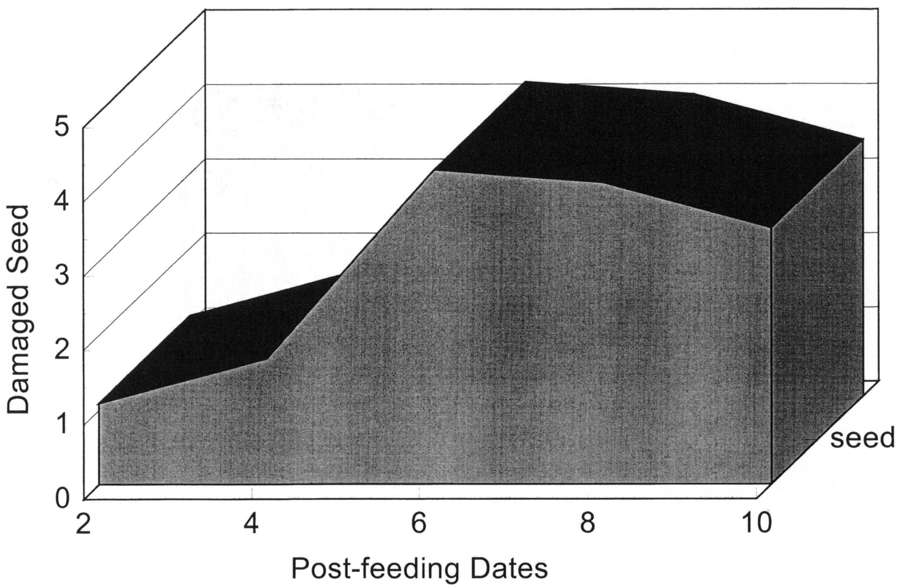

Fig. 2. Cotton seed damage (seeds with feeding punctures) caused by stink bugs on five time periods following a 48-h feeding exposure.

during this study produced external marks or warts in the absence of stylet sheaths. Again, this supports the stylet sheath as a necessary artifact of feeding.

Measurements. Wart size increased slightly, but not significantly, as the days progressed after feeding (Table 3). External mark sizes appeared to be random. Statistical analyses over the 5 post-feeding dates showed no significant difference in the diameter of warts for either boll size over time. Also, there was no significant difference in the diameters of external marks for either boll size over time. The sizes of the external marks appeared to be much more random. This indicates that the relative sizes of warts or external marks are not good indicators of the length of time since stink bug feeding occurred.

This study examined the external and internal signs of boll damage caused by stink bugs. More particularly, the relationships between external signs of damage and the manifestation of internal damage over time were compared. These results demonstrate that damage caused by stink bug feeding appears relatively quickly. All evidence of feeding damage is present in some form within 48 h of initial contact with stink bugs. None of the control bolls in this experiment had any external or internal signs of stink bug feeding. No significant relationship was found between the numbers of external marks and internal warts. These observations indicate that the external mark is not a reliable indicator of the presence or amount of internal damage (numbers of warts) caused by stink bug feeding. This supports the findings of Morrill (1910). The cotton field scout, examining a field for stink bug damage, might greatly underestimate potential loss if he or she relies heavily on the presence of external feeding marks. Stink bug feeding can produce these external marks, but not consis-

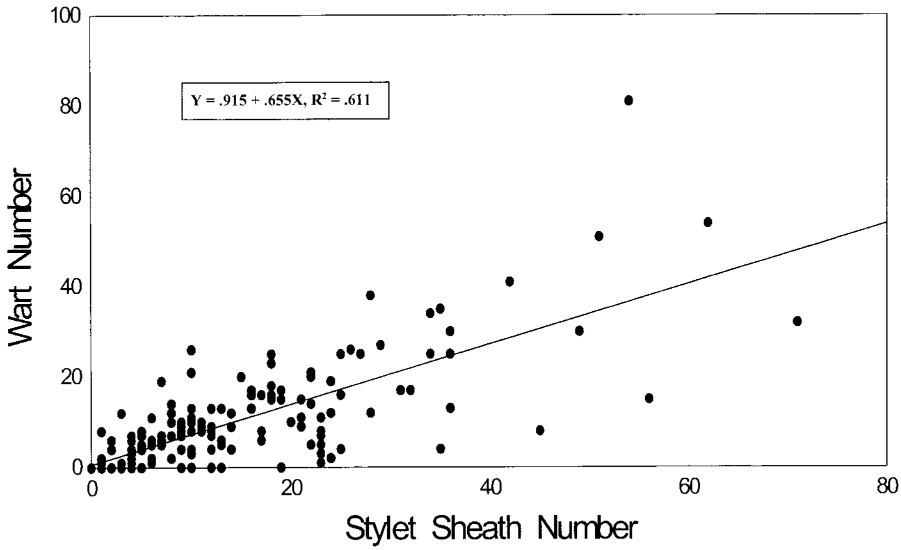

Fig. 3. Regression equation ($Y = .915 + .655X$, $R^2 = .611$) for the correlation between numbers of stink bug stylet sheaths and internal warts on cotton bolls.

Table 2. Total combined percentages of cotton bolls with various combinations of external marks (EM), stylet sheaths (SS), and warts for 5 time periods following a 48-h stink bug feeding exposure

Boll size	EM + SS + wart	SS + wart (no EM)	EM + wart (no SS)	EM + SS (no wart)	SS only (no wart or EM)	Bolls without damage
Small	63.7 a	19.6 b	0.0 c	0.7 c	1.5 c	14.5 bc
Medium	73.1 a	20.1 b	0.0 c	0.0 c	2.8 c	4.0 bc
Mean	68.4	19.9	0.0	0.3	2.1	9.2

Data transformed [arcsine(square root/100)] and analyzed using Tukey's pairwise comparisons. Row means followed by the same letter are not significantly different, $P > 0.05$.

tently. Approximately 20% of all non-control bolls examined in this study had internal warts, but did not have external marks. However, all of the bolls with warts had feeding stylet sheaths. Stylet sheaths are readily visible with the use of a hand lens and are a much more reliable external indicator of internal damage, and may be utilized to estimate the number of internal warts. An exceptionally high correlation was found between these two signs of damage, and a regression equation was formulated to predict the numbers of internal warts based on the numbers of external stylet sheaths. This equation will need to be tested in the field, but could offer a reliable means of predicting internal damage without destroying bolls.

Table 3. Mean (±SEM) diameter (mm) of warts and external marks on cotton bolls for 5 time periods following a 48-h stink bug feeding exposure

Boll size	Days after exposure	Wart size	n	External mark size	n
small	2	1.49 (±1.07)	44	0.95 (±0.20)	24
	4	1.37 (±0.58)	89	0.76 (±0.07)	21
	6	1.47 (±0.37)	147	1.00 (±0.10)	15
	8	1.67 (±0.57)	90	1.03 (±0.19)	34
	10	2.31 (±0.47)	55	1.04 (±0.15)	28
medium	2	1.28 (±0.12)	147	1.12 (±0.12)	39
	4	1.43 (±0.38)	216	0.94 (±0.18)	19
	6	1.82 (±0.45)	193	1.00 (±0.14)	29
	8	1.78 (±0.50)	76	0.92 (±0.08)	24
	10	1.64 (±0.27)	173	1.19 (±0.20)	31

No significant differences ($P > 0.05$) in the sizes of warts or external marks between small- and medium-sized bolls or among the 5 post-feeding dates for either boll size.

Both warts and external marks were present in some form within 48 h of feeding by stink bugs, and no significant increase in numbers of warts or external feeding marks was found for the 5 post-feeding time periods. A significant increase in lint and seed damage was seen over time, with damage reaching its highest level 6 d after exposure. The sizes of warts and external marks were not statistically different between the second to the tenth day after feeding. Thus, the sizes of these structures cannot be accurately used to determine when feeding has occurred.

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