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DEFINING A PREFERRED BOLL COHORT FOR BROWN STINK BUG, *EUSCHISTUS SERVUS* (SAY)

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Abstract

Whole-plant infestation studies were conducted during 2002 and 2003 to define cotton boll cohorts (based on heat unit accumulation beyond anthesis) that are preferred by brown stink bug, *Euschistus servus* (Say), for feeding during each of the five initial weeks of flowering. Bolls that accumulated 165.2 through 672 heat units beyond anthesis (ca. 7 to 27 d old) were more commonly injured by brown stink bug when a range of boll ages were available. The susceptible boll ages in our studies corresponded to diameters of 1.161 to 3.586 cm. These data will refine sampling protocols that estimate the level of stink bug-injured bolls within a field.

Introduction

Brown stink bug, *Euschistus servus* (Say), southern green stink bug, *Nezara viridula* (L.), and green stink bug, *Acrosternum hilare* (Say), have become more common pests of cotton in the mid-southern United States. Stink bugs have become a more significant problem because of the reduction in broad-spectrum insecticide applications against key cotton pests, the use of target-selective insecticides, adoption of Bollgard cotton, and producer participation in boll weevil, *Anthonomous grandis grandis* Boheman, eradication programs (Greene and Herzog 1999, Leonard et al. 1999, Roberts 1999). Stink bug pests infested 5,604,138 acres across the seventeen states of the cotton belt in 2002, ranking fifth among all insect pests (Williams 2003).

Stink bugs are observed in cotton fields from seedling emergence until harvest. During pre-flowering growth stages they do not significantly injure cotton seedlings or flower buds (squares) (Willrich et al. 2003). Stink bug infestations that occur during flowering can result in significant boll injury. Feeding by stink bugs can cause small bolls to abscise, and reduce lint quality, seed germination, and overall yield (Wene and Sheets 1964, Barbour et al. 1990, Greene et al. 1999, Turnipseed et al. 2003, Willrich et al. 2003). In no-choice feeding studies, brown stink bug adults and southern green stink bug nymphs have reduced seedcotton yield in bolls that have accumulated ≤550 heat units (HU) (ca. 22 d) and ≤472 HU (ca. 21 d) beyond anthesis, respectively (Greene et al. 2001, Willrich et al. 2003).

Currently, limited data describes stink bug feeding preferences for bolls in various age classes. Therefore, these studies were conducted to more clearly define those boll cohorts that are more likely to be injured by brown stink bug during each of the initial five weeks of flowering.

Materials and Methods

These studies were done at the Macon Ridge Research Station near Winnsboro, Louisiana (Franklin Parish) during 2002 and 2003. The soil at the site was a Gigger-Gilbert silt loam complex. Field plots used for whole-plant infestations were planted to ‘DP458BR’ on May 23, 2002 and April 30, 2003. General agronomic practices for optimum fertilization and pest control were followed as recommended by the LSU AgCenter. Supplemental irrigation was applied to all plots on an as-needed basis. All non-target pests were suppressed with weekly applications of insecticides at recommended rates.

Plot size was two rows (101.6 cm row centers) x 3.3 m in length. Plant densities were thinned to nine plants per meter at three weeks after plant emergence. Treatments were placed in a randomized complete block design with a 5 x 2 factorial treatment arrangement in four replications. The first factor consisted of stink bug-infested (30 adults/cage) and non-infested plots. Brown stink bug adults were collected from soybean, *Glycine max* (L.), ca. 24-h prior to infestation during each week, using a standard 15.0 cm diameter sweep net. Stink bugs were held in a polypropylene cage (30.0 x 30.0 x 30.0 cm, Bug-Dorm, Megaview Science Education Services CO. Ltd., Taichung, Taiwan) to reduce mortality from physical injury and were fed a small quantity of washed green beans, *Phaseolus vulgaris* (L.).

The second factor consisted of flowering interval and included each of the first five weeks of flowering. The first week of flowering was determined to be when 50% of plants in each plot across the test area had at least one flower or boll. Subsequent infestations occurred every 7 d following the first week of flowering. The growth stage of the plants within the study site was recorded as number of main stem nodes above a sympodial branch with a flower on the first node (NAWF). In 2002, the NAWF growth stage during weeks one, two, three, four, and five was 7-9, 6-8, 5-6, 3-4, and <4, respectively, and in 2003 was 8-10, 7-9, 5-8, 4-6, and <3, respectively.

All flowers on one row of each plot were marked with a yellow "snap-on-tag" (A.M. Leonard, Inc. Piqua, OH), placed on the peduncle (stem) between the flower and the fruiting branch. The date of anthesis was recorded on the tag to ascertain the boll's age upon removal from the plant. Boll age was calculated using heat unit (HU) accumulation beginning at anthesis, as described by Bagwell and Tugwell (1992). HU were calculated as: {[(Maximum daily temperature – Minimum daily temperature)/2]-60}. Flowers in the experimental plots were tagged prior to the initiation of week one until cages were removed at the completion of week five. Translucent cages (32 nylon mesh/linear cm, Synthetic Industries, Greenville, Georgia) were placed over each plot. All tagged, green bolls from each infested and non-infested plot were removed at the completion of each week of flowering. Bolls were grouped according to their date of anthesis, transported to the laboratory, and stored in chilled coolers and refrigerators until inspected for injury.

An individual green boll was classified as injured if at least one wart (callous tissue) or puncture (water-soaked lesion) was present on the internal carpel wall (Bundy et al. 2000). Boll diameter was determined using a dial caliper (Forestry Suppliers, Inc., Jackson, MS). Individual boll measurements were taken at the widest diameter using two diametrically opposite points. Bolls were then placed into six discrete cohorts (Table 1). The intervals for each of the first five cohorts were calculated as: average number of heat units accumulated per day during the study x seven d. In 2002, an average of 23.6 HU were accumulated per day for a cohort interval of 165.2, whereas, in 2003, an average of 24.0 HU were accumulated per day for a boll cohort interval of 168 HU. A shorter interval in the sixth cohort during 2002 as compared to other cohorts may be due to natural boll abscission that occurred during the first week of flowering.

Within each week, the percentage of bolls representing each cohort of the total bolls tagged was determined. The percentage of bolls within each cohort that were injured of the total bolls representing that cohort during each week and within infested and non-infested treatments was also determined. Injury data for infested plots was corrected for boll injury within non-infested plots based upon Abbott's formula (Abbott 1925): {[(% injury in infested treatment)–(% injury in non-infested treatment)]/[100-% injury in non-infested treatment]} x 100. Corrected injury data were then analyzed using analysis of variance (ANOVA). A general linear model was used for each week, with cohort as the independent variable (PROC GLM, SAS 1998). The Fisher protected least significant difference (LSD) test was used for mean separation (α=0.05).

Results and Discussion

There were significant differences between years describing the preference of stink bugs for boll cohorts ($P<0.05$); therefore, data for 2002 and 2003 are presented separately. During 2002, mean number of bolls in all cohorts during week one through five ranged from 40.2 to 200.4 (Figure 1a). In week one, mean number of bolls in cohorts 1 (<165.2 HU) and 2 (165.3-330.5 HU) were 30.9 and 9.3, respectively. Boll densities in week two for cohorts 1, 2, and 3 (330.6-495.8 HU) were 50.6, 21.5, and 9.3, respectively. In week three, the initial four cohorts included 78.1, 51.0, 41.8, and 12.4 bolls. Cohorts 1, 2, 3, 4 (495.9-661.1 HU) and 5 (661.2-826.4 HU) were present during week four with densities of 12.0, 47.5, 60.3, 34.3, and 11.3, respectively. In week 5, cohorts 1, 2, 3, 4, 5, and 6 (826.5-850.5 HU) were present with means of 8.0, 32.6, 61.9, 52.5, 36.8 and 8.6 bolls, respectively.

In 2002, during week one there was no significant difference in injury between boll cohorts 1 and 2 with means of 13.7 and 40.4%, respectively (Figure 1b). In weeks two and three, significantly less boll injury was observed for cohort 1 compared to other cohorts. Boll injury for the oldest two cohorts (3 and 4) in week three was significantly greater than cohort 1. Bolls in cohort 1 comprised 62.2 and 42.6% of the total bolls present during weeks two and three (Figure 1a). In week four, there was no significant difference in the amount of injury observed among cohorts. Mean injury across cohorts ranged from 9.6 to 17.4%. Injury during week five ranged from 0 to 32.8% among cohorts. Boll injury significantly greater in cohorts 2 and 3 compared to all other cohorts. Stink bug preference for these bolls was apparent because cohorts 2 and 3 represented 47.1% of the total bolls present (Figure 1a).

During 2003, boll densities during week one through five ranged from 65.5 to 314.2 for all cohorts (Figure 2a). In week one, number of bolls in cohorts 1 (<168 HU), 2 (169-336 HU), and 3 (337-504 HU) were 37.0, 23.0, and 5.5, respectively. Boll densities in week two for cohorts 1, 2, 3, and 4 (505-672 HU) were 56.3, 49.8, 26.5, 2.6, respectively. In week three, the initial five cohorts were present with respective means of 84.6, 84.0, 44.8, 20.4, and 2.2. Boll cohorts 1, 2, 3, 4, 5 (673-840 HU), and 6 (841-1014 HU) were present during week four with mean densities of 32.1, 44.9, 86.9, 47.3, 16.6, and 2.5. In week 5, boll cohorts 1, 2, 3, 4, 5, and 6 were present with respective means of 40.8, 21.5, 83.0, 92.3, 53.5, and 23.1.

During week one, boll injury for cohort 3 was significantly greater than cohort 1, with respective means of 44.2% and 0% (Figure 2b). In week two, boll injury was significantly greater within cohort 4 compared to cohort 1 and 2, with respective means of 66.5, 0.3, and 24.7%, respectively. In week three, injury within cohort 2 and 3 was significantly greater than cohorts 1 and 5. Boll injury among cohorts 1, 4, and 5 was not significantly different, but was significantly less than cohort 2. Cohorts 1, 4, and 5 comprised 45.3% of the total bolls within week three; whereas, cohort 2 comprised 35.6% of the total bolls (Figure 2a). Stink bugs preferred bolls from cohort 2. In week four, there was no significant difference in the amount of injury observed among all cohorts. Boll injury across cohorts ranged from 0 to 19.8%. Injury during week five was not significantly different among cohorts with means ranging from 3.6 to 14.2%. Similar to 2002, bolls in cohort 1 comprised 56.5, 41.7, and 35.8% of the total sampled during weeks one, two, and three (Figure 2a). For significant differences in injury to be demonstrated among cohorts, a greater number of bolls would have to exhibit injury within cohort 1.

Brown stink bug will injure bolls of different ages and in different proportions during the initial five weeks of flowering. Generally, bolls from cohort 2 (165.3-661.1 HU in 2002 and 169-672 HU in 2003) were most injured by brown stink bug during the initial three weeks of flowering. When injury among cohorts was significantly different within any week, bolls representing cohort 1 (≤168 HU) were least preferred. During the initial three weeks, bolls from cohort 1 were present in greater densities compared to other cohorts; therefore, more bolls would have to be injured to demonstrate preference. During week four in both years and during week five in 2003, brown stink bug did not demonstrate a preference for any boll cohort. Compared to previous weeks (1, 2, and 3), this could be explained by a plant canopy that was much larger, presence of boll cohorts on the plant in more equal proportions, and presence of bolls on sympodial positions beyond the first position that are younger. These conditions may have impaired the ability of brown stink bug to find preferred bolls. However, during week five in 2002, bolls in cohorts 2 and 3 (165.3-495.8 HU) were preferred by stink bugs.

Based on these data, bolls that accumulated 165.2 through 672 HU beyond anthesis (ca. 7 to 27 d old) were most preferred by brown stink bug for feeding when a range of boll ages were available. However, this preference was less distinct during weeks four and five when bolls were available in greater quantities and more equal proportions. Although injury was observed in cohorts that included bolls accumulating through 672 HU, previous studies have indicated that significant yield losses from brown stink only occur in bolls that have accumulated ≤550 heat units beyond anthesis (Willrich et al 2003). The susceptible boll ages in our studies corresponded to a boll diameter of 1.161-3.586 cm with a mid-range of 2.375 cm. The preferred boll ages and sizes, as determined in these studies, are broader than the currently recommended sampling protocol [(12 to 16 d old boll and quarter-size (2.426 cm diameter)]. However, the boll recommended for sampling occurs within our defined range.

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Table 1. Boll cohorts and their relationship to age (heat unit accumulation beyond anthesis) and diameter (cm)[1].

	2002		2003	
Boll Cohort	**Age (HU)**	**Diameter**	**Age (HU)**	**Diameter**
1	<165.2	0.607-2.014	<168	0.607-2.068
2	165.3-330.5	1.334-2.979	169-336	1.161-3.134
3	330.6-495.8	2.184-3.322	337-504	1.613-3.388
4	495.9-661.1	2.182-3.434	505-672	1.935-3.586
5	661.2-826.4	2.100-3.421	673-840	2.446-3.444
6	826.5-850.5	2.624-3.424	841-1014	1.976-3.493

[1]Range of boll diameters during the five weeks of flowering.

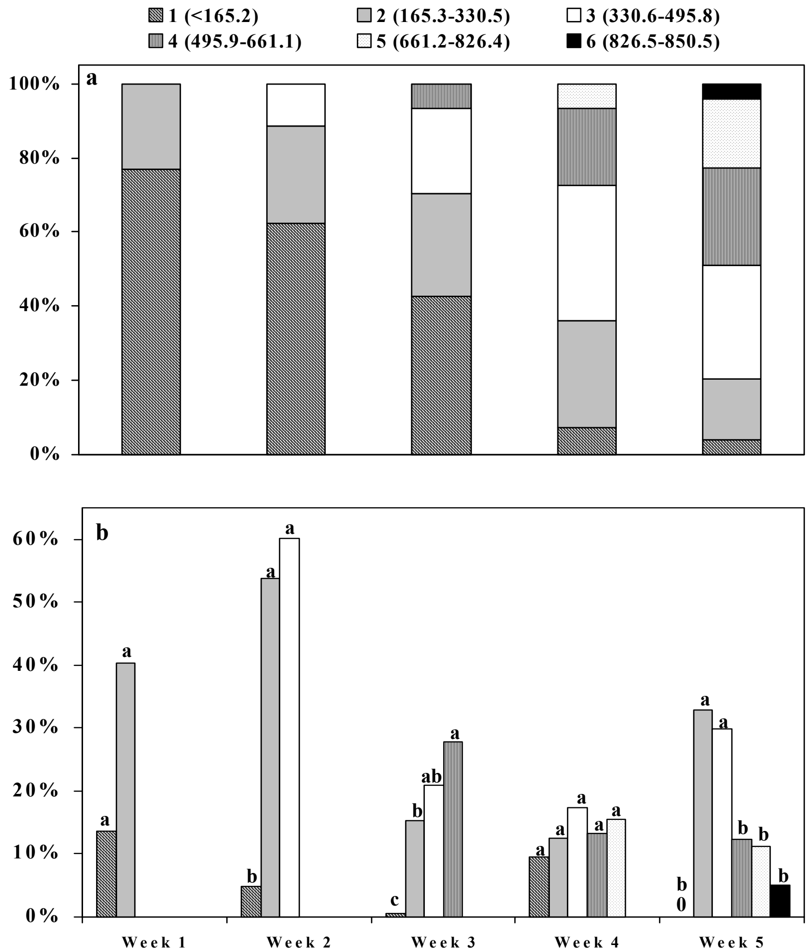

Figure 1. Distribution of each boll cohort (heat unit accumulated) of the total bolls (a) and percent of bolls within eath cohort with injury [bolls with ≥ carpel with a wart or puncture] by brown stink bug (b), 2002. Injury data are

corrected for injury in non-infested plots and injury for cohorts within weeks followed by the same letter are not significantly different (P<0.05, LSD).

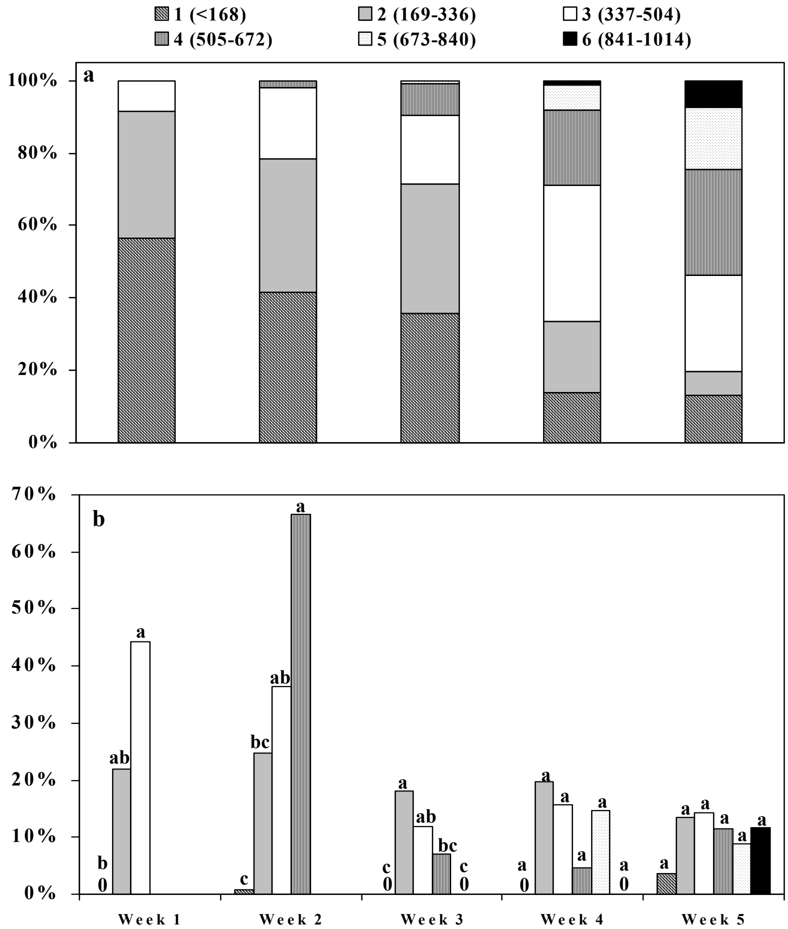

Figure 2. Distribution of each boll cohort (heat units accumulated) of total bolls (a) and percent of bolls within each cohort with injury [bolls with ≥1 carpel with a wart or puncture] by brown stink bug (b), 2002. Injury data are corrected for injury in non-infested plots and injury for cohorts within weeks followed by the same letter are not significantly different (P<0.05, LSD).