PHOTOPERIOD-SPECIFIC DISTRIBUTION OF THE GREEN STINK BUG (HEMIPTERA: PENTATOMIDAE) ON COTTON

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Abstract

Sampling methods for detecting stink bugs are intensive, time consuming, and yield variable results. In a field study conducted over two years (2016-2017), mark-release-observe experiments were conducted using over five hundred adult green stink bugs, *Chinavia hilaris* (Say) (Hemiptera: Pentatomidae) to identify when and where bugs were observed on cotton. Field collected stink bugs were marked or left unmarked with non-toxic fluorescent sharpie markers, released, and monitored in cotton fields at peak bloom. Stink bugs where monitored visually during day and night, aided by a handheld blacklight for night-time observations. Within-cotton distribution insect observations were categorized by plant section (i.e. bottom, middle, and top branches), by fruiting positions and leaf surface, and by concealed or exposed orientation on floral bracts and leaf surfaces. The green stink bug was primarily distributed in the middle and top branches irrespective of photoperiod, and on bolls of the first position from the main stem. Furthermore, significant difference in stink bug orientation were detected and varied in day and night observations. During the day-time, stink bugs were primarily observed concealed inside the bract or on the lower leaf surface. In contrast, night-time observations indicated stink bugs were primarily exposed outside the bract or on an upper leaf surface. These results are relevant to refining on stink bug detection and monitoring protocols to improve efficiency and reduce variability.

Introduction

Injury to cotton, *Gossypium hirsutum* L. (Malvaceae), by stink bugs (Hemiptera: Pentatomidae) has increased substantially with the widespread adoption of transgenic Bt (*Bacillus thuringiensis)*-cotton cultivars targeting lepidopterans pests (Luttrell et al. 2015). The resulting insecticide use decline has likely released sucking bugs, including stink bugs, that were formerly controlled by broad-spectrum insecticides (Lu et al. 2010). Consistent with higher numbers of these pests, cotton boll injury due to stink bug feeding has increased substantially during the last two decades in the southern United States including Texas, China, and other cotton-producing counties (Lu et al. 2010, Greene et al. 2001, Luttrell et al. 2015, Glover et al. 2019).

The complex of stink bugs on cotton that frequently occur in south Texas include redbanded stink bug, *Piezodorus guildinii* (Westwood), brown stink bug, *Euschistus servus* (Say), southern green stink bug, *Nezara viridula* (Say), and the green stink bug, *Chinivis hilare* (Say) (Hemiptera: Pentatomidae). Historically, the southern green stink bug, brown stink bug, and the green stink bug are known to be economic pests of cotton (McPherson and McPherson 2000), and they have been shown to cause decreased fruit retention, lint staining, lint loss, and seed loss (Greene et al. 2001, Glover et al. 2019). Loss can be further magnified when bacterial boll rot is introduced during probing and feeding activity from stink bugs (Medrano et al. 2015 and 2016).

The management of stink bug infestations on cotton currently relies on use of insecticides when action or economic thresholds are exceeding based on visual sampling methods. Methods available include collecting green bolls (2.5 cm in diameter) for internal injury assessment (Toews et al. 2009) and sampling for stink bugs using a drop cloth (Reay-Jones et al. 2009), sweep net (Outward et al. 2008), or beat bucket (Pyke et al. 1980). The sweep net has been found to be more effective sampling nymphs, while the drop cloth was more effective at sampling adults (Reay-Jones et al. 2009), Conversely, the beat bucket has been found to effective at sampling all life stages but efficiencies in its use are affected by plant growth stage. Sampling time of day and within-plant distribution of stink bugs may also affect the outcomes of monitoring for stink bugs for detection and density estimation. Detection and density estimation of stink bugs require intensive and time-consuming sampling that may be affected by these factors and may introduce bias and variability that negatively affects management.

Information on when (photo-period specific) and where (within-plant distribution) stink bugs are observed on cotton is relevant to these stink bug monitoring and management issues. We used mark-release-observe experiments to acquire this information by using a combination of fluorescent marking techniques applied to a representative stink bug and visual black-light aided observations of the stink bug infesting cotton. The objectives of this study were to assess if stink bugs were observed evenly by plant section (i.e. bottom, middle, and top branches), by fruiting positions and leaf surface, and by concealed or exposed orientation on floral bracts and leaf surfaces across day-time and night-time observations.

Materials and Methods

In a field study conducted over two years (2016-2017), mark-release-observe experiments were conducted using over five hundred adult green stink bugs, *Chinavia hilaris* (Say) (Hemiptera: Pentatomidae) to identify when and where bugs were observed on cotton. Field collected stink bugs were marked or left unmarked with non-toxic fluorescent sharpie markers, released, and monitored in cotton fields at peak bloom. Stink bugs where monitored visually during day and night, aided by a handheld blacklight for night-time observations. Within-cotton distribution insect observations were categorized by plant section (i.e. bottom, middle, and top branches), by fruiting positions and leaf surface, and by concealed or exposed orientation on floral bracts and leaf surfaces. A simple external marking technique was applied to stink bugs which consisted of a quick drying, light weight marker available in several highly visible colors that was resistant to peeling and chipping, durable, nontoxic, non-water soluble, and easy to apply (Wineriter et al. 1981).

Insect Collection and Pre-Infestation Cotton Management

Adult stink bugs were collected on flowering and pod filling stages of soybean in 2016 and 2017. Stink bugs were collected using a modified leaf blower that displaces insects from vegetation and transfers them into an inflatable sock that fits on the opposite end of the blower's fanned nozzle, known as a KISS-sampler (keep it simple sampler) (Beerwinkle et al. 1997). Insects were captured 24 h before being released at the base of the cotton plant in each year. All insects were held individually in plastic portion cups for 24 h fasting period and inspected to confirm that only healthy adults (i.e. bugs with all appendages) were used.

The mark-release-observe field experiments were conducted in 2016, and 2017 at the Texas A&M AgriLife Research and Extension Center farm in Corpus Christi, TX. Cotton used for the within-plant vertical distribution experiments was selected for uniformity from a 1.0 ha field planted to Phytogen 499 WRF (Dow AgroSciences, Indianapolis. IN). Planting occurred in early May on 91-m rows and 96-cm row centers at a rate of 77,800 seeds per ha (31,500 seed per acre). Cotton plots were grown without irrigation under dryland growing conditions. Thiamethoxam insecticide (Centric, Syngenta Crop Protection, Greensboro, NC) at labelled rates was used ca. every 10 days to maintain plots pest free before infestation. Thiamethoxam application was discontinued 2 weeks prior to infesting with insects.

Field Experimental Design, Marking Technique, and Insect Infestation

Marked stink bugs were released into interior experimental plots nested within a larger contiguous and uniform cotton field to characterize the within-plant distribution of the green stink by plant section (i.e. bottom, middle, and top branches), by fruiting positions and leaf surface, and by concealed or exposed orientation on floral bracts and leaf surfaces. Each experimental plot consisted of 10 consecutive rows (96-cm row centers by 15.3 m in length). Adult green stink bugs were marked with non-toxic neon permeant markers (Sharpie neon fine point permanent markers, Sanford L.P., Oak Brook, IL). The adult bugs were chilled at 3 C in a refrigerator for 5 min in preparation for marking the insects. Individual adult bugs were gently held between the thumb and forefinger, cradled by the middle finger, while the dorsal side was broadly covered with a single marking color of blue or orange neon. To minimize adverse effects to the insects, neon marking ink were only applied to the pronotum extending past the scutellum towards the hemelytra including the corium avoiding sensory organs (i.e. antennae, eyes, or wing membranes). The marked and unmarked control bugs were placed in ventilated rectangular plastic and mesh cages (bugdorm cages, BioQuip, Rancho Dominguez, California) to air dry.

In 2016, three experimental plots were infested separately with one of three marking treatments (i.e., blue, orange, and non-marked insects) during the third week of bloom. Third week of bloom was characterized as eight to eleven nodes above first white flower (NAWF) on the first (mainstem) fruiting position (Kerby et al. 2010). In each

experimental plot, 100 stink bugs of an assigned marking treatment were released early morning, before sunrise, at the base of cotton plant main stem approximately at node one (N1, Fig. 1). Stink bugs were released ca. every 1.4 m or row for a total of 10 bugs per row in 10 consecutive rows. A grand total of 300 green stink bugs were used in the experiment (i.e., 100 blue-marked, 100 orange-marked, and 100 non-marked adult stink bugs). In 2017, the same protocol was used in two experimental plots releasing a total of 200 green stink bugs (i.e., 100 orange-marked and 100 non-marked adult stink bugs). Insects were monitored for a period of 3 days and 2 nights in 2016 and 2017.

Photoperiod-Specific within-Cotton Distribution Monitoring and Measurements

Insect monitoring and data collection were taken during day and night photoperiods. Daytime measurements were taken during mid-morning when plants were dry from morning humidity (ca. 10:30am). The data collection period was chosen to reflect a time frame that insect monitoring may occur in a commercial setting. A single observer (JPG) collected all data. The observer walked at a slow pace in-between rows where stink bugs were released. Sampling was initiated at the beginning of each row inspecting all plants within the respective row moving from left to right until all plants within the experimental plot had been observed. The observer walked all experimental plots randomly one after the other over an approximate 45 min time-frame. The observer held the blacklight about 0.5 m from the plants. The sampler visually scanned each individual plant beginning at the terminal moving downward towards the base of the plant to observe the position of stink bugs by plant section (i.e. bottom, middle, and top branches), by fruiting positions and leaf surface, and by concealed or exposed orientation on floral bracts and leaf surfaces. Boll bracts and leaves were gently manipulation to determine the relative concealed or exposed orientation of the stink bug. Caution was used to minimally disturb plants. Nighttime measures were taken during the late-evening (ca. 10:30 pm, US central time zone). Measurements were taken during the moon's first quarter (in perigee), on July 12 and July 13 in 2016, and July 30 and July 31 in 2017 (National Weather Service 2016/17).

Within-plant vertical distribution of the green stink bug was assessed by partitioning the plant into bottom, middle, and top sections. Reproductive or sympodial branches were counted in order from bottom to top of the plant, where the bottom branches (B1-5) corresponded to nodes 6-10 beginning with the first reproductive (sympodial) branch, middle branches (B6-10) corresponded to nodes 11-15, and upper branches (B11-15) corresponding to nodes 16-20 (Fig. 1). Reproductive branches were consistently found at or above the fifth node above the cotyledonary node. Fruiting and leaf surface distribution data were assessed by recording visual observations of stink bugs on first, second, and third position cotton bolls (Fig. 1) or on a leaf surfaces as partitioned among the bottom, middle, and top plant sections. Fruiting positions on reproductive branches were counted in order from the nearest position to the main stem outward. Relative concealment and exposure of the stink bugs were assessed by recording observations of the green stink bug in relation to its position on the boll bract and leaf. Cotton bolls are surrounded by three or sometimes four bracts which are the modified leaves at the base of the fruit. Stink bugs observed inside a bract or on a lower leaf surface were recorded as concealed (Fig. 2A, 2D), and stink bugs observed outside a bract (Fig. 2B, 2E) or on an upper leaf surface were recorded as exposed (Fig. 2C, 2F).

Data Analysis

The number of stink bugs observed were accumluted and aggregated across three daytime observation periods and two nighttime observation periods, as categorized by plant section, by fruiting and leaf surface distribution, and by relative concealment and exposure on bracts and leaves. A contingeny table analysis was used to test three hypotheses related to these distribution categories separately for daytime and nighttime observations (Freund and Walpole 1980). The first hypothesis tested for independence of the porportion of stink bugs observed among plant sections (3: top, middle, and bottom) and marking technique (3 in 2016: unmarked, blue-marked, and orange-marked; and 2 in 2017: unmarked and orange-marked). The second hypothesis tested for independence of the porportion of stink bugs observed among plant sections (3) and fruiting and leaf surface distribution (3: first position boll, second position boll, and leaf). The third hypothesis tested for independence of the porportion of stink bugs observed among fruiting and leaf surface distribution (3) and relative concealment and exposure on bracts and leaves (2: found concealed or exposed on bracts and leaves). The Pearson X^2 statistic was generated and the probability of independence was determined for these three m by n contingency table analyses.

Results and Discussion

This study highlights how differences in photoperiod-specific within-plant distribution of stink bugs may complicates detection of stink bugs using standard know sampling methods (add citations). The field experiments spanned two years and were conducted under dryland conditions that are representative of the south Texas cotton-production region

using a stink bug representative of this and other production regions (Greene et al 2001). Observation rates in 2016 and 2017 during the nighttime were on average similar or higher than similar mark-recapture studies, demonstrating the effectiveness of the marking technique for stink bugs. The discrepancy between day and nighttime observations and the differences in within-plant distribution across plant sections, on bolls and leaves, and relative concealment and exposure of the stink bugs may help to explain the difficulties encountered when using current stink bug detection and monitoring methods.

It can be assumed that marked (fluorescent) stink bugs observed during the nighttime data collection period were experimental insects, not coming from local populations, and detected after insects went undetected during daytime observations. The larger number of nighttime specific observations of stink bugs in both 2016 and 2017 following the limited number of daytime observations support the conclusion that when an infestation occurs there is a portion of the population that in not undetected during the daytime, which complicates insect density assessment which occurs during daytime.

Our data suggest candidate sections of the plant to concentrate sampling efforts when monitoring for the green stink bug. Day-specific observations indicated stink bugs were distributed (56% and 50%) within the upper sections of the plant (i.e. middle) and (35% and 21%) top section of the plant (Fig. 3A, 3B) in 2016 and 2017 field experiments, respectively. Similarly, night-specific observations indicated that bugs were distributed (36% and 60%) within the upper sections of the plant (i.e. middle) and (63% and 30%) in the top section of the plant (Fig. 3C, 3D) during the night in 2016 and 2017 field experiments, respectively, with very few stink bugs were observed on the bottom section of the plant (Fig. 3A, 3B, 3C, 3D).

Results further suggest candidate fruiting sites within-plant sections to concentrate sampling efforts when monitoring for the green stink bug. Data support that as the green stink bug moves up the plant from the base it may transition towards the interior of the plant to first position cotton bolls (34% and 25%) in the middle section of the plant and (32% and 16%) in the top section of the plant (Fig. 4A, 4B) in 2016 and 2017, respectively. The same trend was observed during the night-specific data which indicated stink bugs were distributed (38% and 35%) on first position cotton bolls in the middle plant section, and (32% and 23%) in the top plant section (Fig. 4C, 4D) during the night in 2016 and 2017 field experiments, respectively. Furthermore, significant difference in stink bug-bract distributions were detected between day and night observations (Fig. 5). Data suggest candidate fruiting sites to survey within-plant sections to maximize sampling efficiency when monitoring for the green stink bug. Daytime-specific observations indicated stink bugs were distributed primarily inside the bract (concealed) (Fig. 2A) when observed on first position fruiting sites (53% and 36%, 2016 and 2017, respectively) (Fig. 5A, 4B). Similarly, stink bugs observed on second position fruiting sites were mostly concealed (9% and 12%) inside the bract and observed on lower leaf surfaces (17% and 19%) (Fig. 5A, 4B) in 2016 and 2017, respectively. In contrast, night-specific observations indicated stink bugs were more exposed (Fig. 2D, 2F), primarily observed outside the bract (> 55%) or on the upper leaf surface (Fig. 5C, 4D) in 2016 and 2017, respectively.

The differences in plant section distribution, fruiting and leave position distribution, and relative concealment and exposure during the night in contrast to daytime observation of bugs are important factors to consider when sampling for the green stink bug. Focusing visual observations within the upper portions of the plant and inspecting boll bracts may improve existing detection and monitoring efforts (Reay-Jones et al. 2009, Pyke et al. 1980). Exploration of companion detection techniques may assist in detecting stink bugs in cotton. Xia et al. (2011) reported a fluorescent fingerprint when cotton bolls damaged by stink bugs were exposed to long-wave ultraviolet light. The green bolls emitted a strong blue-green fluorescence in a circular region near the puncture wound. Similarly, stink bugs and their damage have also been detected using an electronic nose (Henderson et al. 2010). Additional research in the applications of fluorescence markers, ultraviolet light, and existing insect detection methods are warranted. Our results here indicate where in the cotton plants, stink bug detection should by focused in isolation or in companion with these developing techniques or the existing technique of opening green bolls to observe visual signs of lint discoloration due to stink bug feeding (Toews et al. 2009). In regard to existing stink bug sampling techniques, further field experimentation using the beat bucket, sweep net, and visual observations to determine is warranted to determine if the methods can be further refined using the distribution data from this experiment.

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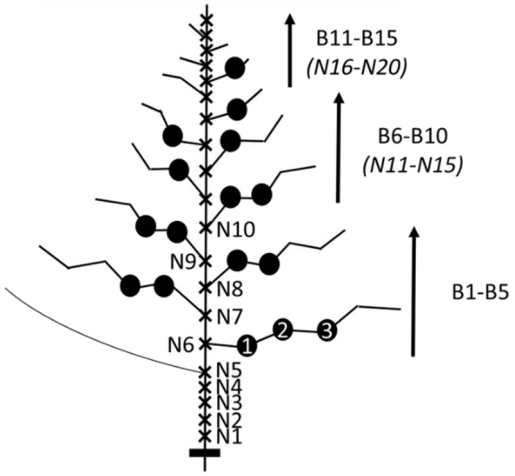

Figure 1. Diagram of a cotton plant illustrating fruiting branches present during time of infestation. In 2016 and 2017, stink bugs were released early morning at the base of the plants main stem (N1) during the third week of bloom. Branches were aggregated in groups of 5 sympodial nodes: bottom branches 1-5 (nodes 6-10), middle branches 6-10 (nodes 11-15), and top branches 11-15 (nodes 16-20). Insects were monitored for a period of 3 days and 2 nights, and mid-season cotton was used, characterized as > 10-12 nodes above first white flower (NAWF).

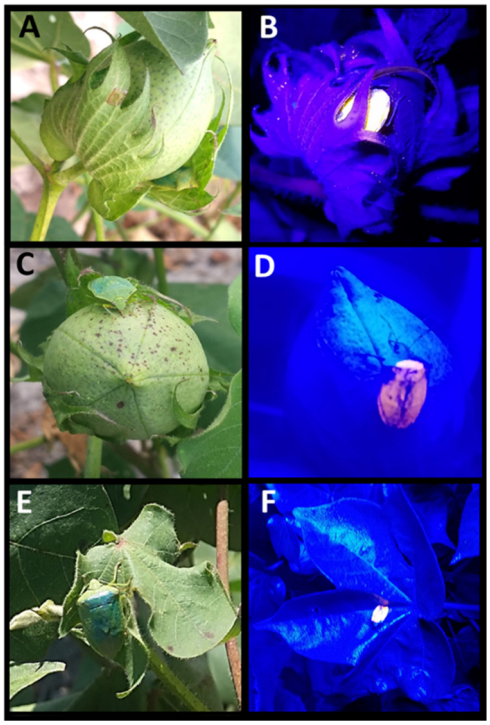

Figure 2. Field measurements taken in 2016 and 2017 photoperiod-specific within-plant distribution experiments. Cotton was infested with marked (fluorescent blue and orange) and non-marked green stink bugs, *Acrosternum hilarum* (S.) (Hemiptera: Pentatomidae). Green stink bug observed on a developing first and second poistion cotton boll concealed (inside) the bract (a: day and d: night), green stink bug observed on a first and second position cotton boll exposed (outside) the bract (b: day and e: night), and green stink bug observed on a leaf surface exposed (upper leaf surface) (c: day and f: night).

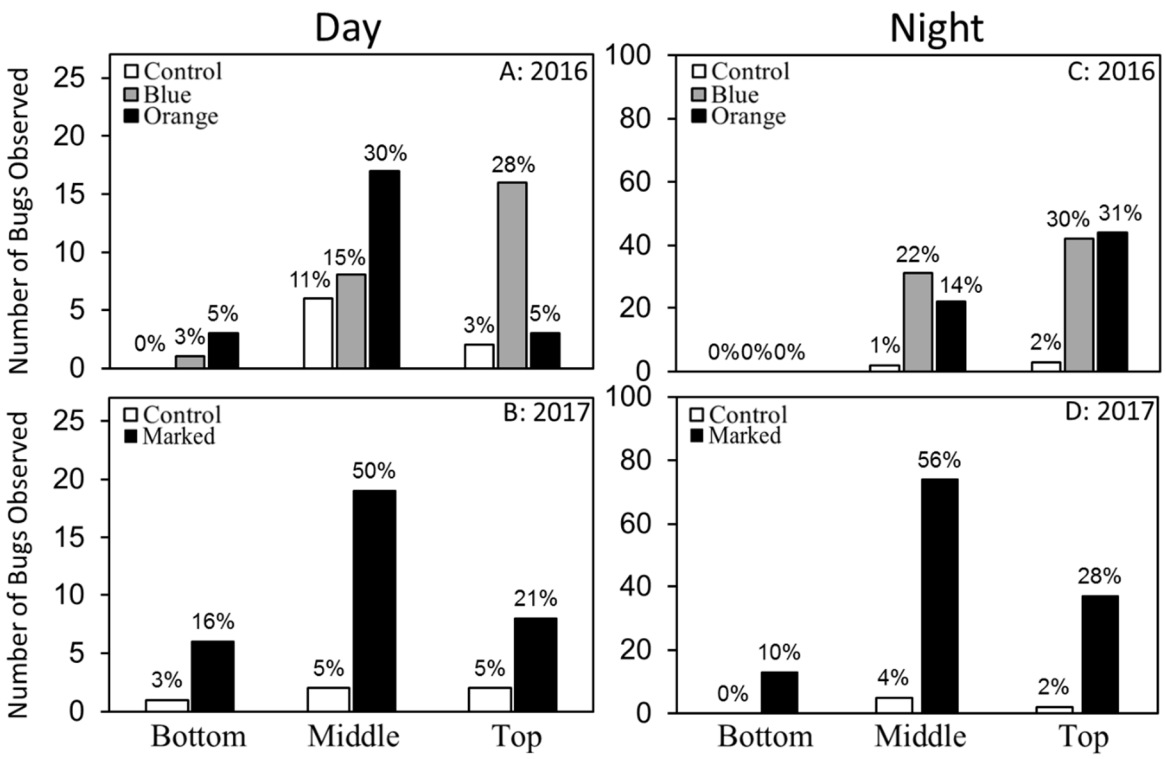

Figure 3. Number of marked and unmarked stink bugs observed and respective probabilities in the daytime (A:2016 and C:2017) and nighttime (B:2016 and D:2017) by branch sections: bottom branches 1-5 (nodes 6-10), middle branches 6-10 (nodes 11-15), and top branches 11-15 (nodes 16-20). Infestation rates of 1insect per plant; infestation duration was 3 days and 2 nights. See legend for marking treatment used each year.

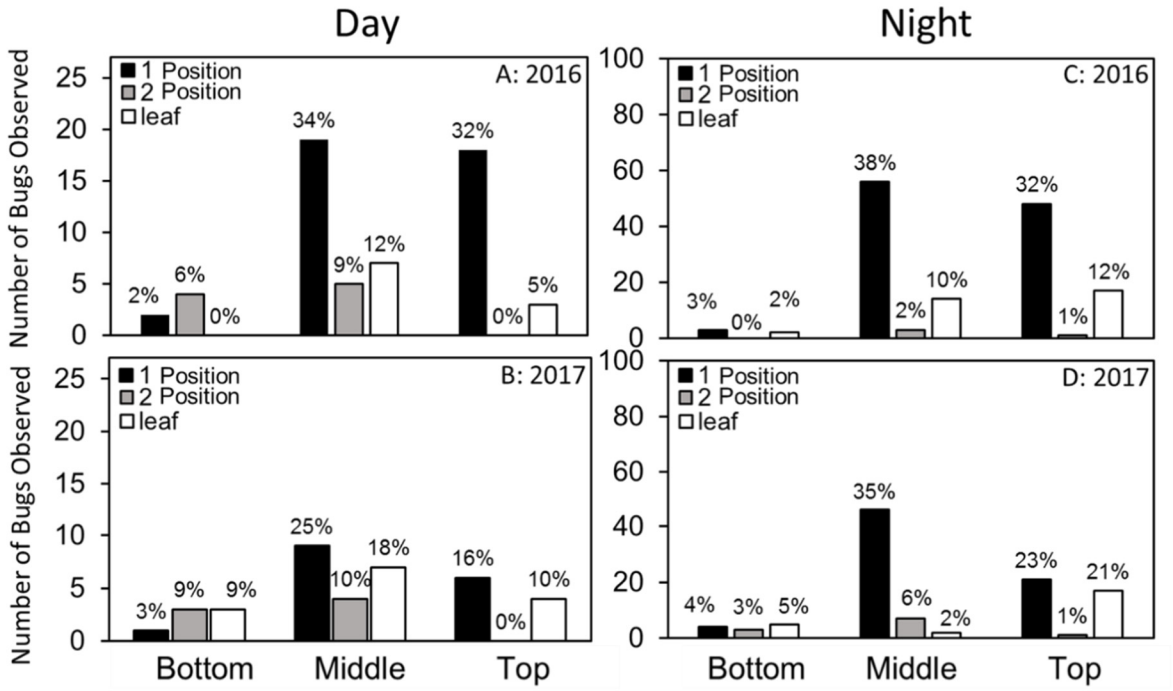

Figure 4. Number of stink bugs observed and respective probabilities in the daytime (A:2016 and C:2017) and nighttime (B:2016 and D:2017) by fruiting position or leaf on bottom branches 1-5 (nodes 6-10), middle branches 6-10 (nodes 11-15), and top branches 11-15 (nodes 16-20). Fruiting positions on reproductive branches were counted in order from the nearest position to the main stem outward. Infestation rates of 1insect per plant; infestation duration was 3 days and 2 nights. See legend for boll position or leaf treatment used each year.

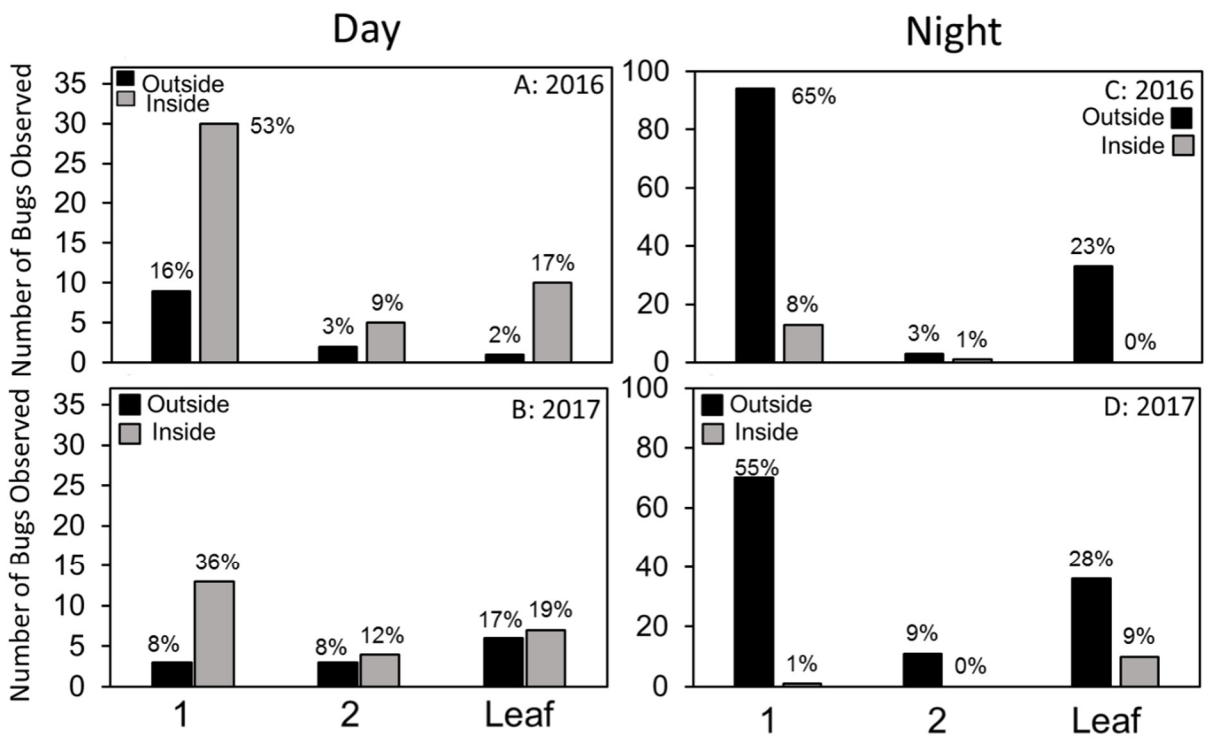

Figure 5. Number of stink bugs observed and respective probabilities in the daytime (A:2016 and C:2017) and nighttime (B:2016 and D:2017) outside or inside the cotton bolls bract by fruiting position or leaf. Fruiting positions on reproductive branches were counted in order from the nearest position to the main stem outward. Infestation rates of 1insect per plant; infestation duration was 3 days and 2 nights. See legend for bract position observed each year.