Density and Egg Parasitism of Stink Bugs (Hemiptera: Pentatomidae) in Elderberry and Dispersal Into Crops

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Abstract

Chinavia hilaris (Say), Euschistus servus (Say), Euschistus tristigmus (Say), and Thyanta custator custator (F.) (Hemiptera: Pentatomidae) are serious pests of crops in the southeastern United States but little is known concerning their dispersal from noncrop hosts in woodlands into crops. This 2-yr study was conducted to investigate whether elderberry [Sambucus nigra subsp. canadensis (L.) R. Bolli] in woodlands serves as a source of stink bugs dispersing into adjacent crops and to examine parasitism of C. hilaris and E. servus eggs on this plant. Elderberry was a reproductive host for each of the four stink bug species; females oviposited on plants with subsequent nymphs feeding on elderberry and developing into adults. Anastatus mirabilis (Walsh & Riley) (Hymenoptera: Eupelmidae), Anastatus reduvii (Howard), and Trissolcus edessae Fouts (Hymenoptera: Scelionidae) were prevalent egg parasitoids of C. hilaris but A. reduvii was the prevalent parasitoid of E. servus. Newly developed stink bug adults were first detected on elderberry around mid-July. Then in late July and early August, as elderberry fruit senesced and cotton bolls became available, stink bugs began dispersing from elderberry into cotton based on recapture of stink bugs on cotton that had previously been marked on elderberry. In addition, in 2015, density of C. hilaris, E. servus, and E. tristigmus was higher in cotton with elderberry than in cotton without it. Over the study, economic threshold was reached for four of seven cotton fields. Elimination of elderberry in woodlands adjacent to cotton may be a viable management tactic for control of stink bugs in cotton.

Key words: Noncrop host plant, dispersal, cotton, Anastatus, Trissolcus
bug species in the field (Aldrich et al. 1991, Mizell and Tedders 1995, Cottrell et al. 2000, Leskey and Hogmire 2005, Tillman et al. 2010).

The density of stink bug species can be higher in crops that are closely associated with woodland habitats than in crops further from these habitats (Mundinger and Chapman 1932, Reay-Jones 2010, Reeves et al. 2012, Olson et al. 2012, Tillman et al. 2014, Venugopal et al. 2014). Elderberry [Adoxaceae, Sambucus nigra subsp. canadensis (L.) R. Bolli] was a noncrop host plant of *C. hila-
ris*, *E. servus*, and *E. tristigmus* in woodland habitats in South Carolina (Jones and Sullivan 1982). The shrub was an early-to-mid-season host of *C. hilaris*; adults began colonizing plants in late-May and nymphs were observed from mid-June through July. The first generation of *C. hilaris* was completed on elderberry and black cherry (*Prunus serotina* Ehrh.) while the second generation developed on soybean indicating that those two noncrop hosts were sources of this stink bug dispersing into soybean (Jones and Sullivan 1982). Two recent studies further indicated that elderberry growing near field edges in corn and cotton farmscapes was a source of *C. hilaris* dispersing into cotton (Cottrell and Tillman 2015, Tillman and Cottrell 2015). This study was conducted to investigate whether elderberry in woodland habitats serves as a source of stink bugs into crops alongside these habitats and to examine parasitism of naturally occurring *C. hilaris* and *E. servus* eggs on this plant.

**Materials and Methods**

**Study Sites**

The study was conducted at six sites during 2014 and at six sites during 2015 near Ocilla, GA. Sites, years, locations (i.e., Global Positioning System, GPS, coordinates; Yuma 2, Trimble Navigation Limited, Sunnyvale, CA), planting dates, and field plot sizes for crops are listed in Table 1. In 2015, there were three cotton sites with elderberry, Bark, Bee, and Snake, and three cotton sites without elderberry, Pear, Deer, and Powerline. During 2014, cotton was grown at four sites and peanut at two sites. During 2015, cotton was grown at the two peanut sites from the year before and at four additional sites not used during 2014; no peanut sites were used during 2015. Delta Pine 1252 B2RF cotton was used both years and Georgia-06G peanut was used in 2014. Crop rows were planted 0.91 m apart. For each site, elderberry plants occurred in the woodland habitat 3–15 m from the edge of the crop field. This shrub was the only known early-to-mid-season host plant of stink bugs in woodlands. The first 12 cotton rows were planted around the field (i.e., parallel to the woodland habitat). Interior cotton rows were planted across the field (i.e., perpendicular to the woodland habitat).

When a grower decided that an insecticide application was necessary for control of stink bugs in cotton, dicrotophos (Bidrin 8, Amvac, www.amvac-chemical.com) was applied to the crop at a rate of 420 g a.i./ha. The treatment threshold for cotton is set at 20% internal boll injury the second week of bloom, 10–15% boll injury the third to fifth weeks of bloom, 20% boll injury the sixth week of bloom, and 30% internal boll injury from the seventh week of bloom onward (Collins 2014). In 2015, location (i.e., GPS coordinates) of each elderberry plant, crop sampling site, and pheromone-baited stink bug trap was recorded.

**Pheromone-Baited Stink Bug Traps**

Yellow pyramid traps (Mizell and Tedders 1995 [photo in Tillman et al. 2013]) baited with stink bug pheromone were used as an additional way to recapture marked stink bugs as well as sample stink bugs. The insect-collecting device was made from a 2.8-liters clear plastic PET jar (United States Plastic Corp., Lima, OH) with a screw-cap lid (10.2 mm in dia.) and seated atop the 1.22-m-tall yellow pyramid base (Cottrell et al. 2000). An insecticidal ear tag (10% λ-cyhalothrin and 13% piperonyl butoxide) (Saber Extra insecticides ear tags, Schering-Plough Animal Health Corp., Union, NJ) was placed in the collecting device to kill stink bugs (Cottrell 2001). The *Euschistus* spp. pheromone (MDD) was purchased from Degussa AG Fine Chemicals (Marl, Germany). Lures were produced by pipetting 40 μl of the *Euschistus* spp. pheromone into the opening of rubber septa (11 mm natural, rubber sleeve stoppers, Wheaton, Millville, NJ), holding septa upright in a laboratory rack, and allowing septa to absorb the pheromone at room temperature (Cottrell and Horton 2011). Lures with the *C. hilaris* attractant (methyl [E,E,Z]-2,4,6-decatrienoate) were purchased from Agbio, Inc. (Westminster, CO). At the time of the study, *N. viridula* pheromone was not commercially available. Pheromone lures were replaced weekly. Stink bugs were collected weekly in Ziploc bags and taken to the laboratory for identification.

**Table 1. Site, year, location (GPS coordinates), planting date (PD), and field plot size (ha) for crops in 2014 and 2015**

| Site       | Year | Location       | Peanut PD  | Peanut ha | Cotton PD | Cotton ha |
|------------|------|----------------|------------|-----------|-----------|-----------|
| House      | 2014 | 31° 32'57.99" N 83° 19'16.86" W | .          | .         | 5/8       | 3.34      |
| Pond       | 2014 | 31° 33'17.62" N 83° 19'54.31" W | .          | .         | 5/8       | 0.74      |
| Little Lake| 2014 | 31° 31'17.63" N 83° 20'01.14" W | .          | .         | 5/6       | 0.74      |
| Grain Bin  | 2014 | 31° 33'07.23" N 83° 18'01.90" W | .          | .         | 5/14      | 1.11      |
| Bee        | 2014 | 31° 33'38.99" N 83° 18'00.09" W | 5/23       | 0.1       | 5/26      | 0.84      |
|            | 2015 | .              | .          | .         | 5/26      | 1.39      |
| Snake      | 2014 | 31° 33'35.99" N 83° 18'00.86" W | 5/23       | 0.17      | .         | .         |
|            | 2015 | .              | .          | .         | 5/26      | 0.84      |
| Bark       | 2015 | 31° 35'20.03" N 83° 18'21.95" W | .          | .         | 5/5       | 1.11      |
| Deer       | 2015 | 31° 36'12.06" N 83° 27'10.93" W | .          | .         | 5/29      | 1.11      |
| Powerline  | 2015 | 31° 56'18.90" N 83° 30'39.73" W | .          | .         | 5/26      | 0.84      |
| Pear       | 2015 | 31° 60'36.61" N 83° 27'56.09" W | .          | .         | 5/29      | 1.11      |
For both years, traps were placed at least 15.2 m from a crop field edge (sampling scheme in Insect Sampling Procedures). Traps positioned this distance from a crop field edge likely were not attractive to stink bug adults in elderberry in woodlands. In support, Leskey and Hogmire (2007) found significantly greater numbers E. servus adults on mullein plants located 1 m from a Euschistus spp., baited trap compared with plants at 5 and 10 m (Leskey and Hogmire 2007). Additionally, significantly more Halyomorpha halys (Stål), Euschistus spp., C. hilaris, and N. viridula adults were captured in a centrally baited (lure with aggregation pheromone of H. halys and synergist) trap compared with those captured in nonbaited traps 2.5 m from the baited one; trap capture was not significantly different for nonbaited traps 2.5, 5, and 10 m from the baited trap (Morrison et al. 2016).

Insect Sampling Procedures
In both years, elderberry was sampled weekly for stink bugs. In 2014, elderberry was sampled from early June through mid-September. The number of plants sampled was 10 at House, nine at Grain Bin, eight at Snake, seven at Bee, five at Pond, and four at Little Lake. In 2015, elderberry was sampled from early June through mid-August. The number of plants sampled was 56 at Snake, 33 at Bee, and 23 at Bark. Plant height was recorded for 40 plants at Bark in 2015 and at each of the other sites in 2014. The whole plant was visually examined for stink bugs. When necessary, a 1.5 m hooked cattle show stick (Valley Vet Supply, Marysville, KS) was used to gently pull a branch within reach to check for stink bugs. Species and developmental stages of stink bugs were identified and recorded in the field using a HP iPAQ pocket personal computer (Hewlett-Packard Co., Palo Alto, CA). Stink bug behavior (i.e., sitting, walking, or feeding), plant part (i.e., leaf, stem, or fruit) and phenology of fruit (i.e., green immature or purple ripened fruit) fed on by stink bugs, and the location of any egg mass on leaves were recorded for each sampling date.

In 2014, peanut was examined weekly for the presence of stink bugs during the weeks of 6, 13, and 20 August. Samples were obtained 14.6 m from the crop field edge adjacent to elderberry. Six samples were taken at the Snake site and four at Bee. Sweep net (38 cm diameter) samples were taken along 7.31 m of a peanut row and examined in the laboratory for stink bugs.

For both years, for each cotton sample, all plants within a 1.83-m length of row were shaken over a drop cloth and visually examined for stink bug egg masses. Species and developmental stages of stink bugs were identified and recorded in the field using a HP iPAQ pocket personal computer. Boll injury was assessed by examining one cotton boll (~2.5 cm in dia.) for each cotton sample (1.83-m length of row) for internal injury (i.e., warts and stained lint) caused by stink bugs as described by Bundy et al. (2000).

In 2014, cotton was sampled weekly for stink bugs from the first (week of 23 July) through ninth week of bloom (week of 17 September). Cotton at each site was divided into 15.5 m sections along the field edge adjacent and parallel to elderberry. We sampled 18 sections at House, 10 at Grain Bin, six at Pond, and four at Little Lake. For the center row in a section, cotton samples were obtained along a transect at 0.5 (row 1), 4.6 (row 5), 8.2 (row 9), 15.2, 30.5, 61.0, and 121.9 m from the field edge into the field. Traps were placed 15.2 m from each edge of a cotton and peanut field and were adjacent to the woodlands harboring elderberry. Traps were spaced 7–11 m from crop samples, depending on the location of tractor tire paths in the field. Traps were monitored weekly from the week of 6 August to the week of 17 September. We used 27 traps in cotton at House, 10 at Grain Bin, six at Pond, and four at Little Lake. There were six traps in peanut at Snake and four at Bee.

In 2015, cotton was sampled weekly for stink bugs from the first (July 1 at Bark and 22 July at the other two sites) through the fourth week of bloom (22 July) at Bark and through the eighth week of bloom (9 September) at the other five sites. Cotton at each site was divided into 23.8 m sections along the field edge adjacent and parallel to elderberry. Three sections were sampled at Bee and Powerline, four at Bark, Pearl, and Deer, and five at Snake. Pheromone-baited traps were positioned along a transect at 15.2, 30.5, 61.0, and 121.9 m from the field edge into cotton at the three field sites with additional traps placed at 182.9, 243.8, and 274.3 m from the field edge at the Bee site. At the center of each 23.8 m section, a trap transect was placed in a furrow with 13 parallel cotton rows on each side. Cotton was sampled from the two adjacent rows on each side of a trap. In addition, cotton was sampled along a transect at 0.5 (row 1), 1.8 (row 2), 4.6 (row 5), 8.2 (row 9), 15.2, 30.5, 61.0, and 121.9 m from the field edge into the field along two cotton rows (one row on each side of a trap transect) that were seven rows away from the traps (~5.5 m from traps).

Additionally, in 2015, six pheromone-baited traps were established in peanut at the Grain Bin location. Trap capture was examined for 3 wk from 10 through 24 September. Traps were aligned along a peanut row, and the distance between each trap was 6.1 m. Traps were positioned 15.2 m from the peanut field edge.

While sampling in elderberry and cotton, visibly parasitized (darkened) egg masses were collected and held in the laboratory for emergence of adult parasitoids. Otherwise, egg masses were left in the field but thereafter checked daily for parasitism or emergence of first instars. This was done to maximize exposure of parasitoids to eggs.

Identification of stink bug species and developmental stages were based on years of rearing these insects in our laboratory. Trissolcus species (Hymenoptera: Scelionidae) were identified using a key to Nearctic species of Trissolcus (Talamas et al. 2015a). The species of Anastatus (Hymenoptera: Eupelmidae) females were identified using a key to North American species of this genus (Burks 1967). Currently, a key to identify species of Anastatus males is not available. Telenomus podisi Ashmead (Hymenoptera: Scelionidae) was identified using a key to species of Telenomus (Ashmead 1893). Voucher specimens of stink bug and parasitoid species are stored in the USDA, ARS, Crop Protection and Management Research Laboratory, Tifton, GA.

Marking Stink Bugs
All adults observed on elderberry plants while sampling were marked; the number of bugs that were marked are included in the results. In 2014 and 2015, an opaque, medium line, oil-based paint marker (Uni Paint, Sanford, L. P., Oak Brook, IL) was used to paint a mark on the insect's prothorax. This is an effective method for marking stink bugs that can remain on an adult for at least 54 d in the field (Tillman 2006). Using a combination of color(s) and design(s), the mark designated elderberry as the host plant and the date the insect was found on elderberry. Generally, stink bugs exhibit negative geotaxis, moving upward on objects (Millar et al. 2009).
So after an insect was marked with paint, it was gently allowed to walk onto the underside of an elderberry leaf near the location where it had been found. Thus, the stink bug remained on the plant once it was released. In 2014, green fluorescent ink (The Hitt Companies, Santa Ana, CA) also was sprayed onto some individual stink bugs using a 100 ml Nalgene pump-spray aerosol spray bottle (United States Plastic Corp.) to mark them in elderberry. The insect was not removed from the plant but marked in place; application of the light mist fluorescent ink onto the stink bug did not elicit flight from the plant. In 2014, stink bugs on elderberry were marked with fluorescent ink from the week of June 11 through the week of 16 July. For the 2 wk of 23 and 30 July, some individuals were marked with fluorescent ink, and the rest were marked with paint; no insects were marked using both techniques. All stink bugs observed in elderberry from the week of 6 August through the week of 20 August were marked with paint. In 2015, an ultra fine-point permanent marker (Sharpie, Sanford, L. P., Oak Brook, IL) was used to write the number, hence the location within the woodland habitat, of the sampled elderberry plant on the dried paint mark. Stink bugs on elderberry were marked from the weeks of 15 July through 12 August. In 2014, stink bugs found while sampling cotton were collected in 37 ml plastic cups (Solo Cup Co., Urbana, IL) and brought into the laboratory. Stink bugs collected from cotton and pheromone-baited traps were examined for a paint mark and a fluorescent mark using ultraviolet light. In 2015, only those stink bugs with a paint mark were collected while sampling. Sampling for marked insects continued at the Bark location even after application of dicrotophos (see later). For both years, marking information and recapture site were recorded for each marked insect recaptured.

Data Analysis

All data were analyzed using SAS statistical software (SAS Institute, Inc. 2010, Cary, NC). Weekly means for \textit{C. hilaris}, \textit{E. servus}, \textit{E. tristigmus}, and \textit{T. c. custator} were calculated for the number of stink bugs per sample in elderberry, cotton, and peanut (only \textit{E. servus} in 2014), and the number of stink bugs per pheromone-baited trap in peanut (only 2014) and cotton (PROC MEANS). Mean percentage parasitism of \textit{E. servus} and \textit{C. hilaris} egg masses over both years also was calculated (PROC MEANS). For seasonal graphs of stink bug abundance, date refers to the mid-week date for the week in which samples were collected in elderberry for both years and in cotton in 2014. However, in 2015, only the week of bloom was used for sample collection time because cotton at the Bark site matured earlier than cotton at the other two sites. For elderberry for both years, chi-square analyses were used to compare frequencies of stink bug behaviors (i.e., sitting, walking, or feeding), plant parts (i.e., leaf, stem, or fruit) and phenology of fruit (i.e., green immature or purple ripened fruit) fed on by stink bugs, locations of egg masses on leaves, and parasitoid species parasitizing \textit{E. servus} and \textit{C. hilaris} egg masses (PROC FREQ). Mean plant height for elderberry at each site was calculated (PROC MEANS). Plant height among sites was compared using analysis of variance (PROC ANOVA).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig1.png}
\caption{For \textit{C. hilaris} in 2014, mean density of eggs, nymphs, and adults in elderberry and nymphs and adults in cotton (drop sample), mean number of adults in pheromone-baited traps in peanut and cotton, and number of marked adults recaptured in peanut and cotton. Date refers to mid-week date for the week in which samples were collected. Fl, flowering; Fr, fruiting; G Fr, green fruit; R Fr, ripened fruit; N Fr, fruit gone. Arrows, dates dicrotophos applied to cotton for stink bug control.}
\end{figure}
For the 2 wk that adults began colonizing cotton adjacent to elderberry in 2014 and 2015, stink bug count data (all species combined) were modeled using a Poisson distribution. Stink bug count data for row 2 (i.e., 1.8 m from the field edge) and the two rows on each side of a trap in 2015 were not included in the analysis because these data were not collected in 2014. The analysis was done using PROC GLIMMIX. The KENWARD-ROGER option and the LINK = LOGIT function were used in the model statement. Model fit was evaluated by use of the chi-square and df statistic provided by PROC GLIMMIX (Littell et al. 2006). Fixed effects were week, row, and week by row. Random effects were replicate and residual error. Because the row by week interaction was insignificant, it was dropped from the model, which was then rerun. Means were back transformed using the ILINK option in the LSMEANS statement and compared using Tukey’s honestly significant difference (HSD).

For cotton fields with and without elderberry, stink bug count data (by species) for cotton and pheromone-baited traps during 2015 were not included in the analysis because these data were not collected at the other cotton sites. The analysis was done using PROC GLIMMIX. The KENWARD-ROGER option and the LINK = LOGIT function were used in the model statement. Model fit was evaluated by use of the chi-square and df statistic provided by PROC GLIMMIX (Littell et al. 2006). The fixed effect was treatment (i.e., site with or without elderberry). Except for C. hilaris in pheromone-baited traps, random effects were replicate, section, and residual error. For C. hilaris in pheromone-baited traps, random effects were replicate and residual error due to the high frequency of zero counts. Means were back transformed using the ILINK option in the LSMEANS statement and compared using Tukey’s HSD. Means and SEs were calculated for distance from the field edge but these data were not included in the analysis due to the high frequency of zero counts.

**Results**

Over both years of the study, populations of C. hilaris, E. servus, E. tristigmus, and T. c. custator occurred on elderberry (Figs. 1–8). One N. viridula, a nymph, was detected in elderberry during the 2014 growing season but none were detected the following season. Oviposition by C. hilaris, E. servus, and T. c. custator occurred as often on the bottom of an elderberry leaf (41.5%) as on the top...
were observed feeding (74.5%) more often than walking (14.0%) or not parasitized. Three C. hilaris egg masses in elderberry was 49.4% (\( \chi^2 = 6.3, P < 0.0839 \)) and 47.9% (\( \pm 6.3, P < 0.0001 \)) percentage parasitism of E. servus and C. hilaris egg masses in elderberry (Table 2). The Anastatus species, A. reduvii and Anastatus mirabilis (Walsh & Riley), and Trissolcus edessae Fouts were the prevalent egg parasitoids of A. reduvii species, A. servus naturally occurring including four scelionids, one eupelmid, and an encyrtid, parasitized and an encyrtid, parasitized Four species of parasitoids, comprising two eupelmids, a scelionid, (Howard) was the prevalent egg parasitoid of this stink bug. In 2014, 6.3% of the adults marked with fluorescent ink in cotton were recaptured in cotton (\( P < 0.0001 \)). Six species of parasitoids, including four scelionids, one eupelmid, and an encyrtid, parasitized naturally occurring E. servus eggs in elderberry (Table 2). Anastatus reduvii (Howard) was the prevalent egg parasitoid of this stink bug. Four species of parasitoids, comprising two eupelmids, a scelionid, and an encyrtid, parasitized C. hilaris eggs (Table 2). The Anastatus species, A. reduvii and Anastatus mirabilis (Walsh & Riley), and Anastatus mirabilis C. hilaris egg masses in elderberry was 49.4% (\( \pm 9.4 \)) and 47.9% (\( \pm 6.3 \)), respectively. Three C. hilaris egg masses collected from cotton were not parasitized. All four stink bug species fed on elderberry. Adults and nymphs were observed feeding (74.5%) more often than walking (14.0%) or sitting (11.5%) on elderberry (\( \chi^2 = 704.2; df = 2; P < 0.0001 \)). Adults and nymphs fed more often on elderberry fruit (96.4%) than on leaves (1.8%) or stems (1.8%) of the plant (\( \chi^2 = 704.2; df = 2; P < 0.0001 \)). In general, stink bugs fed more often on green immature fruit (70.6%) than on purple ripened fruit (29.4%) (\( \chi^2 = 100.9; df = 1; P < 0.0001 \)) perhaps due more to timing of fruit availability than fruit phenology. Plant height for elderberry was not significantly different among sites (\( F_S = 1.88, P > 0.0839 \)). Mean plant height for these sites ranged from 3.2 to 3.4 m.

In 2014, C. hilaris was present on elderberry on 11 June to 20 August (Fig. 1). Late-instars and adults peaked on ripening elderberry fruit on 23 and 30 July and then decreased as the fruit senesced. C. hilaris, including fifth instars on row 1 and adults, initially was present on cotton the second week of bloom. Adult density peaked in the crop the following week (6 August). Dicrotophos was applied after sampling to cotton at House (13.3% boll damage), Pond (8.3% boll damage), and Little Lake (10.8% boll damage) the third week of bloom and at Grain Bin (11.3% boll damage) the fourth week of bloom. An egg mass was found on cotton at the House location the fifth week of bloom.

The next year, C. hilaris occurred on elderberry on 3 June to 12 August (Fig. 2). Early instars peaked in mid-June and subsequent adults were present from mid-July to early August. Adults began colonizing cotton the second week of bloom and continued as elderberry fruit senesced. The following week, adults were captured in pheromone-baited traps and capture increased as adults peaked in cotton. Two egg masses were detected on cotton at the Bark location during the fourth week of bloom. Dicrotophos was applied after sampling cotton at this location (13.2% boll damage) the fourth week of bloom (21 July). At the Snake site, the highest boll damage was 8.9% the sixth week of bloom (26 August) and at the Bee site, the highest boll damage was 3.9% during both the sixth and seventh weeks of bloom.

In 2014 and 2015, C. hilaris males and females dispersed 3.7–280.2 m from elderberry into adjacent cotton and peanut (Tables 3 and 4). In 2014, 6.3% of the adults marked with fluorescent ink in elderberry (\( n = 112 \)) and 9.4% of the adults painted in elderberry (\( n = 32 \)) were captured in crops. Nine C. hilaris were recaptured in
three of four cotton sites the third and fourth weeks of bloom (Fig. 1, Table 3). Even though *C. hilaris* was not detected in peanut sweep samples, two marked adults were captured in pheromone-baited traps in this crop. In 2015, 18.8% of the adults marked in elderberry (n = 32) were recaptured in cotton. Seven marked *C. hilaris* were recaptured in two of three cotton fields the third through sixth weeks of bloom (Fig. 2, Table 4). One cotton field was at the same site where a marked *C. hilaris* was captured in a trap in peanut the previous year.

In 2014, *E. servus* was present on elderberry on 11 June to 20 August 2014 (Fig. 3). Late-instars peaked on maturing elderberry fruit on 6 August while adults peaked on 6 and 13 August. This stink bug species began colonizing cotton the second and third weeks of bloom; fourth and fifth instars were present on rows 1 and 5. Even after dicrotophos was applied to cotton, adults continued to enter the crop. In 2015, *E. servus* occurred on elderberry on 3 June to 5 August (Fig. 4). Egg masses and early instars were detected on this host plant early in the season. Adults peaked on maturing elderberry fruit on 5 August. Adults began colonizing cotton the second week of bloom, peaking the third and fourth weeks of bloom. Trap capture followed a similar pattern. Late-instars were present on rows 1 and 2 during the second and third weeks of bloom.

Over both years, *E. servus* males and females dispersed 9.1–75.4 m from elderberry into adjacent peanut and cotton (Tables 3 and 4). In 2014, 4.8% of the adults marked with fluorescent ink in elderberry (n = 64) and 13.2% of the adults painted in elderberry (n = 121) were captured in crops. Two marked *E. servus* adults were recaptured in two of four cotton fields the ninth week of bloom, and 17 marked adults were recaptured in both peanut fields the fourth, fifth, and seventh weeks of bloom (Fig. 3, Table 3). In 2015, 27.3% of the adults marked in elderberry (n = 187) were captured in cotton. Six marked *E. servus* adults were recaptured in cotton fields the third, fourth, and seventh weeks of bloom (Fig. 4, Table 4). Two cotton fields were at the same site where marked *E. servus* were captured in peanut the previous year. One adult traveled 71.4 m from elderberry at the Snake location through woodlands to the edge of the Bee cotton field and then dispersed another 4 m into cotton. A marked *E. servus* female dispersed 873 m from the Snake location across a paved road into a pheromone-baited trap in peanut at the Grain Bin site.

In 2014, *E. tristigmus* was detected on elderberry on 18 June to 20 August (Fig. 5). Adults peaked on maturing elderberry on 13 August and then decreased as elderberry fruit senesced. Adults entered cotton the second and third weeks of bloom, peaking the
sixth week of bloom when elderberry fruit were no longer available. Trap capture followed a similar pattern. The following year, *E. tristigmus* was present on elderberry on 3 June to 12 August (Fig. 6). Early instars peaked in elderberry in late June and early July with subsequent adults peaking on this host plant on 12 August. Adults entered cotton and were captured in traps the first week of bloom and increased in number on cotton and in traps the third and fourth weeks of bloom as elderberry fruit senesced. Late-instars were present on row 1 the third and fourth weeks of bloom.

In 2014 and 2015, *E. tristigmus* males and females dispersed 4.2–354.6 m from elderberry into adjacent cotton (Tables 3 and 4). In 2014, 4.0% of the adults marked with fluorescent ink in elderberry (*n* = 25) and 1.0% of the adults painted in elderberry (*n* = 98) were captured in cotton. Two marked *E. tristigmus* females were recaptured in two of four cotton fields the fourth and seventh weeks of bloom (Fig. 5, Table 3). Even though marked *E. tristigmus* were not captured in peanut samples at the Snake and Bee sites in 2014, they were captured in cotton at these sites the following year. In 2015, 30.0% of the adults marked in elderberry (*n* = 90) were captured in cotton. Thirty-seven marked *E. tristigmus* males and females were recaptured in the three cotton fields the third through eighth weeks of bloom (Fig. 6, Table 4). Two adults traveled 71.1 and 73.6 m each from elderberry at the Snake location through woodlands to the Bee cotton field edge and then dispersed another 5.9 (77 m total distance) and 281 m (354.6 m total distance), respectively, into cotton.

In 2014, *T. c. custator* was detected on elderberry on 9 July to 13 August when adults and nymphs peaked on the host plant (Fig. 7). This stink bug species entered cotton the third week of bloom and peaked in the crop the fifth week of bloom when it was no longer present on elderberry. For this year, 10.0% of the adults marked with fluorescent ink in elderberry (*n* = 10) and none of the adults marked in the shrub (*n* = 11) were captured in crops. A marked female was recaptured in a trap in cotton the fifth week of bloom (Fig. 7, Table 3). In 2015, *T. c. custator* was present on elderberry on 17 June to 29 July (Fig. 8). Nymphs peaked on elderberry on 1 July, and adults peaked 2 wk later on 15 July. Then the next week (22 July), adults were captured in pheromone-baited traps located in cotton. Afterwards, adults occurred on cotton from the fourth through the seventh weeks of bloom. For this year, 4.2% of the adults marked in elderberry (*n* = 24) were captured in crops. A marked *T. c. custator* adult was recaptured on cotton the fourth week of bloom (Fig. 8, Table 4). *N. viridula* (three adults and a nymph) were detected in cotton over the 2014 growing season but none were detected the following season.

For cotton adjacent to elderberry, stink bug adults began colonizing cotton the second and third weeks of bloom both years (Figs. 1–8). For these 2 wk, adult density (number per 1.83-m length of
Discussion

Elderberry in woodland habitats was a source of *C. hilaris*, *E. servus*, *E. tristigmus*, and *T. c. custator* dispersing into adjacent cotton and peanut. Elderberry was a reproductive host plant for these four stink bug species. Females oviposited on plants and subsequent nymphs fed on the berries and completed development. Jones and Sullivan (1982) previously reported elderberry as a reproductive host for *C. hilaris*, *E. servus*, and *E. tristigmus* in South Carolina but this is the first report of this shrub as a reproductive host of *T. c. custator*. In this study, as elderberry fruit senesced and cotton bolls became available, adults dispersed from elderberry into cotton as strongly suggested by the sequence of occurrence of stink bugs on elderberry, then on both elderberry and cotton, and finally solely on cotton. Even though an edge effect concerning dispersal of stink bugs into crops is known to occur (Tillman et al. 2009, 2014; Reay-Jones 2010; Reeves et al. 2010; Venugopal et al. 2014), the location, as well as the timing, of colonization of cotton adjacent to elderberry indicated that stink bugs dispersed from elderberry into cotton. Indeed, direct evidence of dispersal of *C. hilaris*, *E. servus*, and *E. tristigmus*, and *T. c. custator* into crops is provided by the recapture of these stink bug species on cotton and peanut that had previously been marked on elderberry. In addition, in 2015, density of *C. hilaris*, *E. servus*, and *E. tristigmus* was higher in cotton with elderberry than in cotton without it. Over the 2-yr study, the economic

![Graph showing the presence and development of stink bugs in elderberry and cotton](image)

*Fig. 6. For *E. tristigmus* in 2015, mean density of eggs, nymphs, and adults in elderberry and nymphs and adults in cotton (drop sample), mean number of adults in pheromone-baited traps in cotton, and number of marked adults recaptured in cotton. Date refers to mid-week date for the week in which samples were collected in elderberry. For Bark cotton, mid-week date for the first week of bloom was 7/1. For the other two cotton sites, mid-week date for the first week of bloom was 7/22. Fl, flowering; Fr, fruiting; G Fr, green fruit; R Fr, ripened fruit; N Fr, fruit gone. Arrow, week of bloom dicrotophos applied to cotton for stink bug control.*
threshold (based on percent internal boll damage) was reached for four of the seven cotton fields. Thus, dispersal of stink bugs from elderberry into cotton can result in economic damage to this crop.

Previous studies have indicated that stink bug adults move from noncrop host plants into closely associated crops in response to deteriorating suitability of noncrop hosts. Ehler (2000) determined that the first generation of *E. conspersus* Uhler developed on roadside weeds such as wild radish, *Raphanus sativus* L., and black mustard, *Brassica nigra* (L.) Koch, and the second generation developed in an adjacent tomato crop suggesting that after developing on roadside weeds, this stink bug dispersed into tomato. The timing of completion of development of *C. hilaris* in elderberry and the initial appearance of this stink bug species in soybean suggested that this noncrop host was a source of this pest dispersing into this crop (Jones and Sullivan 1982). Miner (1966) reported that the first generation of *C. hilaris* developed almost entirely on noncrop hosts, in particular elderberry, and that later infestations in soybean were greater wherever these hosts were nearby. Two recent studies indicated that elderberry growing in woodlands were sources of *C. hilaris* dispersing into nearby cotton (Cottrell and Tillman 2015, Tillman and Cottrell 2015). This current study is the first to demonstrate via mark-recapture studies that a noncrop host plant in woodlands is a source of the stink bugs in nearby crops.

In addition, this study provided information on distances of dispersal of *C. hilaris*, *E. servus*, and *E. tristigmus* in these agricultural landscapes. For cotton adjacent to elderberry, each stink bug species was detected at sampling locations from 0.5 to 274.3 m into cotton over the season. Even during the first 2 wk of colonization, some stink bug adults occurred in cotton 121.9 m from the field edge along elderberry. Dispersal of elderberry-marked stink bugs into cotton ranged from 3.7 to 354.3 m. Three elderberry-marked adults actually dispersed from elderberry through the woodlands to a cotton field. One *E. servus* female dispersed 873 m from a cotton location into a peanut field. In 2016, a marked *E. servus* female dispersed ~900 m from elderberry at the Snake location across a paved road, a cotton field, and into elderberry at the Grain Bin location (P.G.T., unpublished data). Even though very little research has been reported in the literature on distance of dispersal of stink bugs in agricultural landscapes, the distances that stink bugs dispersed from elderberry may not be unusual. Kiritani and Sasaba (1969) reported that within 24 h, *N. viridula* females migrated at least 1 km from the place where they developed into the closest available suitable host plant habitat, a rice paddy field at a susceptible stage for feeding and reproduction. In another study, a *E. servus* female dispersed ~400 m across a newly planted peanut field into the edge of a nearby corn field with maturing fruit (Tillman et al. 2009). Because stink bugs have the ability to disperse at least up to ~350 m from elderberry into cotton and potentially may disperse across and beyond a field, studies examining dispersal of stink bugs from woodland sources should be conducted by field, not field edges. Furthermore, spatial

**Fig. 7.** For *T. c. custator* in 2014, mean density of eggs, nymphs, and adults in elderberry and adults in cotton (drop sample), mean number of adults in pheromone-baited traps in peanut and cotton, and number of marked adults recaptured in cotton. Date refers to mid-week date for the week in which samples were collected. Fl, flowering; Fr, fruiting; G Fr, green fruit; R Fr, ripened fruit; N Fr, fruit gone. Arrows, dates dicrotophos applied to cotton for stink bug control.
distribution and dispersal of stink bugs in agricultural landscapes with woodland sources should be studied at a landscape level.

Very little is known on parasitism of naturally occurring stink bug eggs in woodland habitats. This is especially important in light of the fact that various noncrop hosts harbor stink bugs in agricultural landscapes, and the diversity of parasitoids in woodland habitats may differ from that of crop habitats. Notably, *Anastatus* species parasitizing naturally occurring *C. hilaris* and *E. servus* eggs

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**Table 2.** Occurrence of stink bug egg parasitoid species attacking *E. servus* and *C. hilaris* in elderberry in 2014 and 2015

| Parasitoid                  | Frequency (%) | $\chi^2$ | df | P     |
|-----------------------------|---------------|----------|----|-------|
| *E. servus*                 |               |          |    |       |
| *Anastatus* spp.            | (27/212)$^a$  | (51/697)$^a$ |    |       |
| *T. podisi*                 | 60.85$^c$     | 46.34$^c$ |    |       |
| *T. thyantae*               | 15.57         |          |    |       |
| *Tr. edessae*               | 8.02          | 45.63$^c$ |    |       |
| *T. brochymenae*            | 7.08          |          |    |       |
| *Ooencyrtus* sp.            | 5.19          |          |    |       |
| *E. servus: all species*    | 309.2         | 5        | 0.0001 |
| *E. servus: *Anastatus* spp vs *T. podisi* | 7.08 | 1 | 0.0001 |
| *C. hilaris: all species*   | 200.8         | 2        | 0.0001 |
| *C. hilaris: *Anastatus* spp vs *Tr. edessae* | 0.04 | 1 | 0.8434 |

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$^a$ Total number of parasitized egg masses/total number of parasitized eggs from which adult parasitoids emerged.

$^b$ Only *A. reduvii*.

$^c$ *A. reduvii* and *A. mirabilis*. 

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**Fig. 8.** For *T. c. custator* in 2015, mean density of nymphs and adults in elderberry and adults in cotton (drop sample), mean number of adults in pheromone-baited traps in cotton, and number of marked adults recaptured in cotton. Date refers to mid-week date for the week in which samples were collected in elderberry. For Bark cotton, mid-week date for the first week of bloom was 7/1. For the other two cotton sites, mid-week date for the first week of bloom was 7/22. Fl, flowering; Fr, fruiting; G Fr, green fruit; R Fr, ripened fruit; N Fr, fruit gone. Arrow, week of bloom dicrotophos applied to cotton for stink bug control.
Table 3 Stink bugs marked in elderberry and subsequently recaptured in cotton or peanut associated with woodland habitats at five locations in Irwin County, GA in 2014

| Marked insects | Date | Recaptured insects |
|----------------|------|--------------------|
| Site           | Crop | Type mark<sup>a</sup> | Species | Date | Sample type<sup>b</sup> | No./sex | Distance (m)<sup>c</sup> |
| House cotton   | paint | 8/4                 | C. hilaris | 8/8 | drop | 1 ♀ | 12 |
| House cotton   | FI    | 7/21; 7/28         | C. hilaris | 8/8 | drop | 2 ♂, 2 ♀; 1 ♀ | 14–21 |
| Grain Bin      | cotton | FI | 7/22                 | C. hilaris | 8/15 | drop | 1 ♀ | 18 |
| Snake peanut   | paint | 7/30                 | E. servus | 8/7; 9/5; 9/15 | PT | 1 ♂; 1 ♀ | 18 |
| Pond cotton    | FI    | 7/23                 | E. servus | 7/5; 8/15 | PT | 1 ♂; 1 ♀ | 24 |
| Bee peanut     | paint | 7/23; 7/30; 8/13    | E. servus | 8/22; 9/5; 15 | PT | 1 ♂; 1 ♂; 5 ♀; 3 ♀ | 18 |
| Snake peanut   | FI    | 7/23                 | E. servus | 8/1; 9/5 | PT | 1 ♂; 1 ♀ | 18 |
| Snake peanut   | paint | 7/30; 8/13; 8/13    | E. servus | 8/18; 8/18; 9/5 | PT | 1 ♂; 1 ♂; 1 ♀; 1 ♀ | 18 |
| House cotton   | paint | 8/4                 | E. tristigmus | 9/15 | PT | 1 ♂ | 24 |
| Pond cotton    | paint | 8/6                 | T. c. custator | 9/16 | PT | 1 ♂ | 24 |
| Grain Bin      | cotton | FI | 7/22                 | T. c. custator | 8/18 | PT | 1 ♂ | 18 |

<sup>a</sup> FI (fluorescent ink).
<sup>b</sup> Drop (drop sample in cotton), PT (Euschistus spp. pheromone-baited trap).
<sup>c</sup> Straight line distance from elderberry habitat into cotton or pheromone-baited trap.

Table 4 Stink bugs marked in elderberry and subsequently recaptured in cotton associated with woodland habitats at three locations in Irwin County, GA in 2015

| Marked insects | Date | Recaptured insects |
|----------------|------|--------------------|
| Site           | Date | Species | Site | Date | Sample type<sup>a</sup> | No./sex | Distance (m)<sup>b</sup> |
| Bee            | 8/25 | C. hilaris | Bee | 8/27 | drop | 1 ♂ | 3.7 |
| Snake          | 8/3  | Snake | 8/5 | drop | 1 ♂ | 12.4 |
| Snake          | 8/10 | Snake | 8/12; 8/26 | drop | 1 ♂; 1 ♂ | 25–30 |
| Snake          | 8/3  | Snake | 8/12; 8/19 | PT | 1 ♂; 1 ♂ | 117 |
| Bee            | 7/28 | Bee | 8/20 | PT | 1 ♂ | 280.2 |
| Bee            | 8/4  | E. servus | Bee | 8/7 | drop | 1 ♂ | 9.1 |
| Bark           | 7/14 | Bark | 7/21 | drop | 1 ♂ | 21.1 |
| Snake          | 8/3  | Snake | 8/12 | PT | 1 ♂ | 29.9 |
| Snake          | 8/4  | Snake | 8/10 | drop | 1 ♂ | 34.9 |
| Bark           | 7/28 | Bark | 8/11 | PT | 1 ♂ | 59.3 |
| Snake          | 8/3  | Snake | 8/12 | PT | 1 ♂ | 75.4<sup>c</sup> |
| Bee            | 8/11 | E. tristigmus | Bee | 8/27 | drop | 1 ♂; 1 ♂ | 4.2 |
| Snake          | 8/10; 8/11; 8/11 | E. tristigmus | Bee | 8/20 | PT | 1 ♂; 1 ♂; 1 ♂ | 6.0–7.3 |
| Snake          | 8/10 | Snake | 9/3; 8/31 | PT | 1 ♂; 1 ♂ | 15.2 |
| Snake          | 8/18 | Snake | 8/12; 8/31 | drop | 1 ♂; 1 ♀ | 7.8 |
| Snake          | 8/10 | Snake | 8/11; 8/27 | PT; drop | 1 ♂; 1 ♀ | 9.5 |
| Snake          | 8/10; 8/17 | Snake | 9/3; 8/31 | PT | 1 ♂; 1 ♀ | 12 |
| Bark           | 7/28 | Bark | 8/11 | PT | 1 ♂ | 14.1 |
| Bee            | 8/11 | Bee | 8/20 | drop | 1 ♂ | 14.6 |
| Bee            | 8/17 | Bee | 8/31 | PT | 1 ♂ | 16.2–18.6 |
| Bee            | 8/4  | Bee | 8/6; 8/15 | PT | 1 ♂; 1 ♀; 1 ♀ | 18.4–27 |
| Snake          | 8/3; 8/3; 8/10 | Snake | 8/5; 8/12; 8/19 | PT | 1 ♂; 1 ♂; 1 ♀; 1 ♀ | 19.9–27.6 |
| Bark           | 7/14; 7/28 | Bark | 7/15; 8/4 | PT | 1 ♂; 1 ♀ | 37.1 |
| Snake          | 8/17 | Snake | 8/31 | PT | 1 ♂ | 50.7–56 |
| Bee            | 8/4; 8/11 | Snake | 8/13; 8/20 | PT | 1 ♂; 1 ♀ | 53.0–66.2 |
| Snake          | 8/10 | Snake | 8/26; 9/10 | drop; PT | 1 ♂; 1 ♀ | 70 |
| Snake          | 8/10 | Snake | 8/26 | drop | 1 ♂ | 77.0<sup>c</sup> |
| Snake          | 8/10 | Snake | 9/24 | PT | 1 ♂ | 119.6 |
| Snake          | 8/3; 8/10 | Snake | 8/12; 9/3 | drop; PT | 1 ♂; 1 ♀ | 127.3 |
| Bee            | 8/4  | Bee | 8/20 | PT | 1 ♂ | 182.4 |
| Snake          | 8/4  | Bee | 8/27 | PT | 1 ♂ | 304.7 |
| Snake          | 8/10 | Bee | 8/26 | PT | 1 ♂ | 354.6<sup>c</sup> |
| Bark           | 7/14 | T. c. custator | Bark | 7/21 | drop | 1 ♂ | 47.5 |

<sup>a</sup> Drop (drop sample in cotton), PT (Euschistus spp. pheromone-baited trap).
<sup>b</sup> Refers to straight line distance from specific elderberry plant to cotton or in pheromone-baited trap.
<sup>c</sup> Distance from Snake elderberry through the woodlands to Bee field ranged from 71 to 74 m.
Table 5 Means (± SE) for number of stink bugs in cotton and pheromone-baited traps in cotton adjacent to elderberry at various distances from the field edge in 2015

| Sample type       | Distance (m) | C. hilaris | E. servus | E. tristigmus |
|-------------------|-------------|------------|-----------|---------------|
| Cotton            | 0.5         | 0.329 ± 0.082 | 0.184 ± 0.052 | 2.250 ± 0.334 |
|                   | 1.8         | 0.026 ± 0.018 | 0.039 ± 0.022 | 0.132 ± 0.043 |
|                   | 4.6         | 0.105 ± 0.040 | 0.013 ± 0.013 | 0.118 ± 0.037 |
|                   | 8.2         | 0.039 ± 0.029 | 0.026 ± 0.018 | 0.039 ± 0.022 |
|                   | 15.2        | 0.184 ± 0.052 | 0.145 ± 0.041 | 0.289 ± 0.064 |
|                   | 30.5        | 0.118 ± 0.042 | 0.079 ± 0.031 | 0.197 ± 0.065 |
|                   | 61.0        | 0.079 ± 0.045 | 0.092 ± 0.050 | 0.158 ± 0.056 |
|                   | 121.9       | 0.105 ± 0.061 | 0.105 ± 0.035 | 0.197 ± 0.073 |
| Pheromone traps   | 15.2        | 0.105 ± 0.063 | 1.000 ± 0.185 | 0.763 ± 0.198 |
|                   | 30.5        | 0.289 ± 0.099 | 1.632 ± 0.270 | 0.921 ± 0.178 |
|                   | 61.0        | 0.368 ± 0.170 | 2.711 ± 0.274 | 1.263 ± 0.235 |
|                   | 121.9       | 0.289 ± 0.146 | 3.211 ± 0.338 | 1.605 ± 0.395 |
|                   | 182.9       | 0.750 ± 0.171 | 2.275 ± 0.539 | 3.563 ± 0.992 |
|                   | 243.8       | 0.188 ± 0.101 | 2.188 ± 0.306 | 2.125 ± 0.584 |
|                   | 274.3       | 0.188 ± 0.101 | 2.250 ± 0.433 | 2.438 ± 0.508 |

Table 6 Means (± SE) for number of stink bugs in cotton and pheromone-baited traps in cotton with and without elderberry in 2015

| Sample type       | Treatment  | C. hilaris | E. servus | E. tristigmus |
|-------------------|------------|------------|-----------|---------------|
| Cotton            | Elderberry | 0.858 ± 0.307a | 0.764 ± 0.184a | 3.143 ± 0.707a |
|                   | Control    | 0.039 ± 0.027b | 0.265 ± 0.086b | 0.899 ± 0.227b |
| Pheromone traps   | Elderberry | 0.614 ± 0.260a | 9.464 ± 1.818a | 9.109 ± 1.798a |
|                   | Control    | 0.122 ± 0.063b | 8.401 ± 1.629b | 6.659 ± 1.338b |

For each sample type, means followed by the same letter in the same column are not significantly different (Tukey’s HSD, P > 0.05).

exist primarily in woodland habitats (Tillman 2016). Interestingly, the Asian egg parasitoid Trissolcus japonicus (Ashmead) was discovered parasitizing H. halys eggs in a woodland habitat in Beltsville, MD (Talamas et al. 2015b).

Both techniques for marking stink bug adults in elderberry were successful in terms of recapturing them in crops. However, the paint mark was more useful for studying dispersal than the fluorescent ink mark because the location where the insect was found could be designated on an individual stink bug. Marked stink bugs at the Snake and Bee sites in 2015 likely dispersed at longer distances than those at the other cotton sites because these two sites were not treated with an insecticide.

In conclusion, elderberry in woodland habitats was a source of C. hilaris, E. servus, and E. tristigmus dispersing into adjacent cotton and peanut. Elimination of this noncrop host plant in woodlands adjacent to cotton field edges may be a viable biologically based management tactic for control of stink bug populations attacking cotton in this region.

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