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Attraction of stink bug (Hemiptera: Pentatomidae) nymphs to *Euschistus* aggregation pheromone in the field

P. Glynn Tillman[1,*] *and Ted E. Cottrell*[2]

Abstract

Phytophagous stink bugs (Hemiptera: Pentatomidae) are primary pests in most fruit, vegetable, grain, and row crops worldwide. Pheromones have been identified and synthesized for several species of economically important stink bug pests. When yellow pyramid traps are baited with lures containing these pheromones, significantly more stink bug adults are captured in the field than without lures. Our specific objective for this study was to examine the attractiveness of the *Euschistus* aggregation pheromone, methyl (*E*,*Z*)-2,4-decadienoate (MDD), to stink bug nymphs by using yellow pyramid traps baited and not baited with MDD lures in peanut fields and alongside pecan and peach orchards. At orchard locations, captured nymphs were predominantly *Euschistus servus* (Say) followed by *E. tristigmus* (Say), *E. ictericus* (L.), and *Thyanta custator custator* (F.). In peanut, *E. servus*, *E. tristigmus*, and *Chinavia hilaris* (Say) nymphs were caught in traps. Nymphal capture of *E. servus*, *E. tristigmus*, and *C. hilaris* was significantly higher in traps with MDD lures than in non-baited traps demonstrating that these nymphs were attracted to the synthetic aggregation pheromone. Pyramid traps baited with aggregation pheromone can be used as monitoring tools to assess the presence and seasonal development of certain stink bug pest species on crop and non-crop host plants and perhaps to predict timing of dispersal into and/or out of a crop.

Key Words: *Chinavia*; peanut; pecan; peach; pheromone-baited trap; monitoring tool

Resumen

Los chinches fitófagos (Hemiptera: Pentatomidae) son plagas principales en la mayoría de frutas, vegetales, granos y cultivos en hileras en todo el mundo. Se han identificado y sintetizado feromonas para varias especies de chinches plaga de importancia económica. Cuando se ceban trampas pirámide amarillas con señuelos que contienen estas feromonas, un número significativamente mayor de las chinches adultos son capturados en el campo que sin señuelos. Nuestro objetivo específico de este estudio fue examinar la capacidad de atracción de la feromona de agregación de *Euschistus*, metilo (*E*,*Z*) -2,4-decadienoato (MDD), a las ninfas de chinches hediondas utilizando trampas pirámide amarillas cebadas con el señuelo MDD y sin cebo en los campos de maní y al lado de pacana y melocotón. En los lugares de la huerta, las ninfas capturadas fueron predominantemente *Euschistus servus* (Say), seguido de *E. tristigmus* (Say), *E. ictericus* (L.) y *Thyanta custator custator* (F.). En cacahuete, ninfas de *E. servus, E. tristigmus* y *Chinavia hilaris* (Say) fueron capturadas en trampas. La captura de las ninfas de *E. servus*, *E. tristigmus* y *C. hilaris* fue significativamente mayor en las trampas con cebos de MDD que en trampas sin cebo lo que demuestra que estas ninfas fueron atraídas por la feromona de agregación sintética. La trampas de pirámide cebadas con la feromona de agregación se pueden utilizar como una herramienta de monitoreo para evaluar la presencia y el desarrollo estacional de ciertas especies de chinches plaga de las plantas de cultivo y no de cultivo y quizás para predecir el momento de la dispersión dentro y/o fuera de un cultivo.

Palabras Clave: *Chinavia*; maní; pacana; melocoton; cebadas con feromonas trampa; herramienta de monitorización

Stink bugs (Hemiptera: Pentatomidae) are primary pests responsible for millions of dollars in losses and cost of control in fruit, vegetable, grain, and row crops (McPherson & McPherson 2000). For example, nearly 131,000 bales of cotton in the U.S. were estimated lost due to pest stink bug species in 2014 (Williams 2015). The 3 main stink bug pests of cotton across the coastal plain of the southeastern United States are *Euschistus servus* (Say), *Chinavia hilaris* (Say), and *Nezara viridula* (L.). Other pest species include *Euschistus quadrator* Rolston, *E. tristigmus* (Say), *E. ictericus* (L.), and *Thyanta custator accerra* McAtee (Bundy & McPherson 2000; McPherson & McPherson 2000).

Stink bugs feed on developing cotton seeds and lint which can cause shedding of young bolls, yellowing of lint, yield reduction, and transmission of a bacterial pathogen, a strain of *Pantoea agglomerans*, which can damage seed and lint (Barbour et al. 1990; Medrano et al. 2009). In contrast, in peanut, stink bugs feed on vegetative parts of plants (Tillman 2008), and thus are not considered economic pests. However, when *E. servus* and *N. viridula* inhabit peanut in peanut–cotton farmscapes, they can lead to a negative impact on cotton because they develop on peanut and then disperse to cotton (Tillman et al. 2009).

Stink bug injury to pecan fruit reduces both kernel quality and yield. Stylet penetration through the shell of a developing fruit causes the kernel to rot, and if the injury occurs before shell hardening, these fruits generally abscise. After the kernel reaches the dough stage and the shell has hardened, stink bug feeding injury is noted by localized black lesions on the mature kernel, but fruits do not abscise (Demaree 1922; Osburn et al. 1966). These pecan fruits are thus harvested, and because the lesions on kernels impart a bitter taste to the affected kernels, they must be separated during processing (Demaree 1922; Turner 1923; Osburn et al. 1966).

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On peach, the type of feeding injury imparted to fruit depends on the phenology of the fruit. Rings (1957) reported that early-season stink bug injury could lead to blossom or fruit drop and catfacing injury. Later in the season, when fruits are larger, injury to fruit can lead to scarred injury, gummosis injury, and water-soaked injury. These types of injury lead to a reduction in fruit yield and quality.

Aggregation pheromones have been identified and synthesized for several species of economically important stink bug pests. Aldrich et al. (1991) identified the major component of the male-specific aggregation pheromone, methyl (*E*,*Z*)-2,4-decadienoate (MDD), of Nearctic *Euschistus* species. Since, this pheromone has been synthesized and is commercially available. Mizell & Tedders (1995) modified a pyramid trap, originally designed to monitor the pecan weevil, *Curculio caryae* (Horn) (Coleoptera: Curculionidae) (Tedders & Wood 1994), to capture stink bugs. This trap combines visual (i.e., yellow base) and chemical (i.e., pheromone) stimuli to capture stink bugs. Several studies have shown that when yellow pyramid traps are baited with MDD lures, they effectively monitor adults of several *Euschistus* species, including *E. servus*, *E. tristigmus*, *E. politus* Uhler, *E. conspersus* (Uhler), *E. ictericus*, and *E. quadrator*, in the field (Aldrich et al. 1991; Mizell & Tedders 1995; Cottrell et al. 2000; Johnson et al. 2002; Leskey & Hogmire 2005; Tillman et al. 2010; Tillman & Cottrell 2012, 2016). Additionally, stink bug species can be cross-attracted to pheromones produced by other stink bug species. Thus, pyramid traps baited with MDD can also capture *C. hilaris* and *N. viridula* adults in the field (Tillman et al. 2010).

Even though pyramid traps baited with *Euschistus* aggregation pheromone have been reported to capture adults of *Euschistus* species, only one study has compared nymphal capture in traps with or without lures containing this pheromone. In that study, greater numbers of early instars of *E. conspersus* were captured in traps baited with MDD lures than in control traps (Aldrich et al. 1991). In that same study, nymphal trap capture also was numerically higher for *E. tristigmus* and *E. politus* in traps baited with MDD than in control traps. In this current study, we examined capture of stink bug nymphs using yellow pyramid traps with or without MDD lures and placed in peanut fields or alongside orchards.

Materials and Methods

To test the ability of the *Euschistus* aggregation pheromone to attract stink bug nymphs in the field, trap capture was examined for yellow pyramid traps (Mizell & Tedders 1995) baited with a lure containing a synthetic aggregation pheromone and traps baited without a pheromone lure. The insect-collecting device of the pyramid trap was modified from that used by Mizell & Tedders (1995) in that it was made from a 2.8 L clear plastic PET® jar (United States Plastic Corp., Lima, Ohio) with a screw-cap lid (10.2 mm in diameter) and seated atop a 1.22-m-tall yellow pyramid base (Cottrell et al. 2000; photo in Tillman et al. 2015). A lure with the *Euschistus* pheromone, methyl [*E*,*Z*]-2,4-decadienoate (Degussa AG Fine Chemicals, Marl, Germany), was placed in the collecting device. Lures were produced by pipetting 40 µL of the *Euschistus* pheromone into the opening of rubber septa (11 mm natural, rubber sleeve stoppers, Wheaton, Millville, New Jersey), holding the septa upright in a laboratory rack, and allowing septa to absorb the pheromone at room temperature (Cottrell & Horton 2011). An insecticidal ear tag (10% λ-cyhalothrin and 13% piperonyl butoxide) (Saber extra insecticide ear tags, Sagebrush Tags, De Smet, South Dakota) was placed in this device to decrease stink bug escape (Cottrell 2001).

The 2 treatments, i.e., traps with or without a pheromone lure, were set up as a randomized complete block design using 6 blocks (i.e., replicates). Lures were changed and insects collected on a weekly basis. The test was conducted in peanut at 3 sites: 1) near a woodland field edge (31.5658889°N, 83.2962500°W), 2) near a peanut–cotton interface (31.5698611°N, 83.3015556°W), and 3) near a shrub field edge (31.5520833°N, 83.3005278°W) in Irwin County, Georgia, USA. In this crop, the test was conducted for 3 wk from 10 through 24 Sep. Traps were lined along a peanut row, and the distance between each trap was 6.1 m. Pheromone-baited stink bug traps were positioned 15.2 m from the peanut field edge. The test was also conducted at the United States Department of Agriculture, Agricultural Research Service, Southeastern Fruit and Tree Nut Research Laboratory in Byron, Georgia. At this location, traps were set up in a randomized complete block design using 6 blocks associated with orchards. Three blocks were alongside edges of peach orchards (32.6557111°N, 83.7392667°W; 32.6522972°N, 83.7341972°W; and 32.6533250°N, 83.7212028°W) with pecan and wooded areas nearby. The remaining 3 blocks were alongside edges of pecan orchards (32.6543500°N, 83.7345583°W; 32.6536778°N, 83.7307972°W; and 32.6527250°N, 83.7184917°W) with peach and wooded areas nearby. Within replicates, spacing between the traps was at least 9 m. Traps were run for 21 wk from 19 May through 9 Oct 2015.

All data were analyzed using SAS statistical software (SAS Institute 2010). Chi-square analyses were used to compare overall frequencies of species and instars of stink bugs in traps in peanut and orchards (PROC FREQ). For traps with lures containing *Euschistus* pheromone, chi-square analyses were used to compare frequencies of traps with both nymphs and adults and those containing nymphs (no adults) or adults (no nymphs) in peanut and orchards (PROC FREQ). For the pheromone lure test, count data (number of stink bugs) were modeled by a Poisson distribution. In peanut, count data for *E. servus* and *E. tristigmus* nymphs and adults were analyzed. Only *C. hilaris* nymphs were analyzed because the number of adults was too low. For orchards, count data for *E. servus* nymphs and adults were analyzed; numbers of *T. c. custator, E. tristigmus*, and *E. ictericus* nymphs were too low to analyze. The analyses were 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). For peanut, fixed effects were treatment, week, and location. For orchards, the fixed effect was treatment; weekly counts were too low to examine. Random effects were replicate and residual error. Means were back transformed using the ILINK option in the LSMEANS statement and compared using Tukey's honestly significant difference (HSD).

Results

In peanut, nymphs of 3 stink bug species, *E. tristigmus*, *E. servus*, and *C. hilaris*, were captured in stink bug traps baited with *Euschistus* pheromone. The frequency of occurrence for stink bug nymphs in pheromone-baited traps was higher for the 2 *Euschistus* species than for *C. hilaris* (Table 1). In orchards, nymphs of 4 stink bug species, *E. servus*, *E. tristigmus*, *E. ictericus*, and *T. c. custator*, were captured in these traps. *Euschistus servus* was the most prevalent stink bug nymph captured in pheromone-baited traps whereas the frequency of capture was low for the other species (Table 1).

In peanut, *E. servus* 4th and 5th instars were more prevalent than earlier instars in pheromone-baited traps (Table 2). In orchards, 5th instars were the most prevalent instar captured. In peanut, *E. tristigmus* 3rd through 5th instars were more prevalent than 2nd instars. In contrast, *C. hilaris* 2nd instars were the most prevalent ones captured in stink bug traps. For traps without lures, *E. servus* 4th and 5th instars were captured in traps in peanut, and only 5th instars were captured

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Table 1. Frequency of occurrence of nymphs of stink bug species captured in pheromone-baited traps in peanut and pecan/peach orchards in Georgia.

Stink bug species	Frequency of occurrence (%)		χ^2	df	*P*
	Peanut (739)[a]	Orchards (82)[a]			
Euschistus servus	35.0	87.9			
Euschistus tristigmus	48.4	8.5			
Euschistus ictericus	—	2.4			
Chinavia hilaris	16.6	—			
Thyanta custator custator	—	1.2			
	Frequency comparisons				
Peanut: all species			112.9	2	0.0001
Peanut: *Euschistus* species			16.2	1	0.0001
Orchards: all species			173.5	3	0.0001

[a]Total number of stink bug nymphs captured.

in traps in orchards. In peanut, only 5th instars of *E. tristigmus* and 2nd instars of *C. hilaris* were captured.

In peanut, trap capture of *E. servus* nymphs was significantly influenced by treatment ($F_{1,102}$ = 46.72, P < 0.0001) but not week ($F_{2,1}$ = 5.00, P = 0.3014) or location ($F_{2,1}$ = 24.83, P = 0.1405). Capture of *E. servus* adults also was significantly affected by treatment ($F_{1,102}$ = 226.20, P < 0.0001) but not week ($F_{2,1}$ = 44.91, P = 0.1049) or location ($F_{2,1}$ = 28.16, P = 0.1321). For *E. tristigmus*, capture of nymphs was significantly affected by treatment ($F_{1,102}$ = 67.79, P < 0.0001) but not week ($F_{2,1}$ = 13.85, P = 0.1867) or location ($F_{2,1}$ = 29.96, P = 0.1281). Similarly, capture of adults of this stink bug species was significantly influenced by treatment ($F_{1,102}$ = 112.03, P < 0.0001) but not week ($F_{2,1}$ = 1.89, P = 0.4571) or location ($F_{2,1}$ = 5.93, P = 0.2788). Trap capture of *C. hilaris* nymphs was significantly influenced by treatment ($F_{1,102}$ = 33.12, P < 0.0001) but not week ($F_{2,1}$ = 11.61, P = 0.2032) or location ($F_{2,1}$ = 44.99, P = 0.1048). At orchard locations, capture of *E. servus* nymphs was significantly affected by treatment ($F_{1,250}$ = 28.27, P < 0.0001). Capture of *E. servus* nymphs and adults in peanut and orchard locations and *E. tristigmus* nymphs and adults and *C. hilaris* nymphs in peanut was higher in traps with MDD lures than in those without MDD lures (Table 3). In peanut, capture of nymphs and adults was similar each week and for each location for each of the 3 stink bug species captured (Tables 4 and 5). *Chinavia hilaris*, mainly 2nd instars, was numerically higher near the peanut–cotton interface.

For traps with lures, the percentage of traps in which both *E. servus* nymphs and adults were captured (53.7%) in peanut was similar to the percentage of traps in which either nymphs (no adults) or adults (no nymphs) were captured (46.3%) (χ^2 = 1.03, df = 1, P = 0.3098). Likewise, for *E. tristigmus*, the percentage of traps with both adults and nymphs (48.0%) was similar to that for traps with either nymphs (52.0%) or adults (46.3%) (χ^2 = 0.27, df = 1, P = 0.6008). Along orchards, the percentage of traps in which either *E. servus* nymphs or adults were captured (76.9%) was significantly higher than the percentage of traps containing both nymphs and adults (23.1%) (χ^2 = 34.92, df = 1, P < 0.0001). Similarly, for *C. hilaris* in peanut, the percentage of traps in which only nymphs or adults were captured (92.2%) was significantly higher than the percentage of traps that captured both development stages (7.8%) (χ^2 = 34.92, df = 1, P < 0.0001). These results strongly in-

Table 2. Frequency of occurrence of instars of stink bug species captured in pheromone-baited traps in peanut and pecan/peach orchards in Georgia.

Stink bug species		Frequency of occurrence (%)		χ^2	df	*P*
		Peanut	Orchards			
Euschistus servus	2nd	8.7 (253)[a]	—			
	3rd	14.6	2.8 (70)[a]			
	4th	39.5	24.3			
	5th	37.2	72.9			
Euschistus tristigmus	2nd	15.1 (351)[a]				
	3rd	27.9				
	4th	26.8				
	5th	30.2				
Chinavia hilaris	2nd	69.2 (117)[a]				
	3rd	24.8				
	4th	5.1				
	5th	0.9				
		Frequency comparisons				
Peanut: *E. servus*, all instars				74.1	3	0.0001
Peanut: *E. servus*, 4th and 5th instars				0.19	1	0.6667
Orchards: *E. servus*				54.0	2	0.0001
Peanut: *E. tristigmus*, all instars				19.2	3	0.0002
Peanut: *E. tristigmus*, 3rd to 5th instars				0.75	2	0.6867
Peanut: *C. hilaris*				173.5	3	0.0001

[a]Total number of instars captured in parentheses.

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Table 3. Numbers of *Euschistus servus* and *E. tristigmus* nymphs and adults and *Chinavia hilaris* nymphs in yellow pyramid traps with and without *Euschistus* pheromone in peanut and pecan/peach orchards in Georgia.

		Mean (SE) per trap			
		Nymphs		Adults	
Crop	Species	Pheromone	No pheromone	Pheromone	No pheromone
Peanut	*E. servus*	4.04 (0.55) a	0.03 (0.02) b	7.32 (0.98) a	0.67 (0.13) b
	E. tristigmus	5.75 (0.59) a	0.05 (0.03) b	4.72 (0.31) a	0.23 (0.07) b
	C. hilaris	1.11 (0.19) a	0.02 (0.01) b	—	—
Orchards	*E. servus*	0.45 (0.15) a	0.02 (0.01) b	7.85 (0.61) a	0.38 (0.06) b

For nymphs and adults, least square means within a row followed by the same letter are not significantly different for treatment (Tukey's HSD, $P > 0.05$).

dicate that the presence of adults in traps did not affect the presence of nymphs of the same species in traps, i.e., nymphs were not drawn to traps by adults.

Discussion

Euschistus species nymphs were the most frequently captured stink bug species in MDD-baited traps in peanut and at orchard locations in Georgia. Similar to our results for nymphs, a previous study showed that more *E. servus* than *E. tristigmus* adults were captured in MDD traps in pecan orchards, and it was these 2 species that were predominantly captured in traps (Cottrell et al. 2000). Also, density of *E. servus* and *E. tristigmus* nymphs in MDD traps was numerically similar in an earlier experiment in peanut (Tillman & Cottrell 2016).

Yellow pyramid traps baited with the *Euschistus* aggregation pheromone captured significantly more nymphs of 2 *Euschistus* species, *E. servus* and *E. tristigmus*, as well as *C. hilaris*, than non-baited traps regardless of the crop with which traps were associated (*E. servus* only) or the type of location in which traps were placed in peanut. This result confirms that these nymphs were not randomly entering traps but were actually selecting traps containing the synthetic aggregation pheromone. In 2 field tests, 90.2% (n = 1,290) of 5th instars of *N. viridula* were caught on the outside of small field cages containing male *N. viridula* rather than on cages with females or on control cages, strongly indicating that these nymphs were attracted to the male-specific pheromone of this stink bug species (Harris & Todd 1980). Unfortunately, the data were not statistically analyzed for 5th instars separate from adults. In field experiments, pyramid traps baited with lures containing the aggregation pheromone of *Halyomorpha halys* (Stål) captured significantly more nymphs of this stink bug species than control traps. This finding demonstrates that this aggregation pheromone was attractive to nymphs (Khrimian et al. 2014). The aggregation pheromone produced by male *Murgantia histrionica* (Hahn) also was attractive to nymphs and adults of both sexes in field bioassays using plants baited or not baited with lures (Weber at al. 2014). Beyond stink bugs, a synthetic blend of active components of the male-produced aggregation pheromone of *Riptortus pedestris* (=*clavatus*) (Heteroptera: Alydidae) attracts nymphs as well as adults (Leal et al. 1995).

Aggregation pheromones draw stink bug adults and nymphs to plants where both feeding and mating occur. The attraction of nymphs to aggregation pheromones supports the idea that they are associated with food because it is not expected that nymphs are seeking mates. In peanut–cotton farmscapes, *E. servus* and *N. viridula* adults and late instars disperse into cotton at the interface of the 2 crops as cotton fruit becomes available (Tillman et al. 2009). Peanut is an unlikely host plant for *C. hilaris* (Tillman 2013). So capture of *C. hilaris* 2nd instars in MDD-baited traps indicates that they were seeking suitable food even very early in their development. Sugie et al. (1996) identified the aggregation pheromone, methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate (MDT), produced by males of *Plautia stali* Scott. In Japan, *P. stali* largely reproduces in plantation forests of Japanese cypress and uses their cones as a food source during the summer and autumn (Yamada & Noda 1985). Although this stink bug does not develop and reproduce on fruit crops (Shiga & Moriya 1984), depletion of cypress cones causes starving adults and nymphs to depart from this food source into cultivated fields (Tsutsumi 2001). Toyama et al. (2015) used sticky traps with lures containing the *P. stali* aggregation pheromone to trap nymphs of this species in cypress. A morphological indicator of nutritional status showed that nymphs attracted to the pheromone were starving. Toyama et al. (2015) concluded that the pheromone-baited sticky trap could be a useful tool for predicting infestation of orchards by *P. stali*.

Pyramid traps baited with aggregation pheromone also can be used as monitoring tools to assess the presence and seasonal development of certain stink bug pest species on crop and non-crop host plants. For example, traps baited with the *H. halys* aggregation pheromone in association with MDT were used to monitor season-long activity of adults and nymphs around areas of crop production, including fruit orchards, vegetables, ornamentals, and row crops, across many parts of the U.S. (Leskey et al. 2015). In a recent study, traps with MDD lures were used to monitor stink bugs along field borders between peanut or cotton and woodland habitats (Tillman &

Table 4. Weekly number of *Euschistus servus* and *E. tristigmus* nymphs and adults and *Chinavia hilaris* nymphs in yellow pyramid traps in peanut in Georgia.

	Mean (SE) per trap				
	E. servus		*E. tristigmus*		*C. hilaris*
Week	Nymphs	Adults	Nymphs	Adults	Nymphs
1	0.32 (0.12) a	3.85 (0.59) a	0.41 (0.13) a	0.32 (0.12) a	1.29 (0.20) a
2	0.47 (0.18) a	2.03 (0.33) a	0.76 (0.23) a	0.47 (0.18) a	0.94 (0.16) a
3	0.31 (0.12) a	1.38 (0.24) a	0.48 (0.15) a	0.31 (0.12) a	1.00 (0.17) a

Least square means within a column followed by the same letter are not significantly different for week (Tukey's HSD, $P > 0.05$).

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Table 5. Numbers of *Euschistus servus* and *E. tristigmus* nymphs and adults and *Chinavia hilaris* nymphs in yellow pyramid traps in peanut at 3 sites in Georgia.

	Mean (SE) per trap				
	E. servus		*E. tristigmus*		*C. hilaris*
Site	Nymphs	Adults	Nymphs	Adults	Nymphs
Cotton edge	0.64 (0.24) a	3.42 (0.53) a	0.35 (0.11) a	0.64 (0.24) a	0.77 (0.14) a
Shrub edge	0.37 (0.14) a	1.60 (0.27) a	0.91 (0.28) a	0.37 (0.14) a	1.31 (0.21) a
Woodland edge	0.19 (0.08) a	1.97 (0.32) a	0.47 (0.15) a	0.19 (0.08) a	1.15 (0.19) a

Least square means within a column followed by the same letter are not significantly different for site (Tukey's HSD, $P > 0.05$).

Cottrell 2016). The presence of *E. servus* adults and nymphs in early-season traps, well before crops were susceptible to stink bug attack, strongly indicated that *E. servus* initially was present and developing on non-crop hosts.

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