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Predation of stink bugs (Hemiptera: Pentatomidae) by a complex of predators in cotton and adjoining soybean habitats in Georgia, USA

P. Glynn Tillman[1,], Matthew H. Greenstone[2], and Jing S. Hu[2]*

Abstract

Stink bugs (Hemiptera: Pentatomidae) are economic pests of cotton and soybean. This study was conducted to examine predation on stink bugs by arthropod predators in cotton and adjoining soybean habitats. Gut-content analysis based on polymerase chain reaction (PCR) was used to detect stink bug deoxyribonucleic acid (DNA) in predators collected from both crops over a 5 wk period. *Nezara viridula* (L.), *Euschistus servus* (Say), *Chinavia hilaris* (Say), and *Euschistus quadrator* Rolston were detected on soybean and cotton. *Piezodorus guildinii* (Westwood) and *Thyanta custator custator* (F.) were detected only on soybean whereas *Euschistus tristigmus* (Say) was detected only on cotton. Over both crops, 13 predators screened positive for a variety of stink bug species DNA by PCR analysis: *Geocoris punctipes* (Say) and *Geocoris uliginosus* (Say) (Hemiptera: Geocoridae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* (Pallas) (cotton), and *Scymnus* sp. (cotton) (Coleoptera: Coccinellidae), *Oxyopes salticus* Hentz and *Peucetia viridans* (Hentz) (cotton) (Araneae: Oxyopidae), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), *Mecaphesa asperata* (Hentz) (Araneae: Thomisidae), *Zelus renardii* Kolenati (Hemiptera: Reduviidae), and *Notoxus monodon* (F.) (cotton) (Coleoptera: Anthicidae). In soybean, the percentage of *G. punctipes* and *G. uliginosus* screening positive for *N. viridula* was high, 87.3%, whereas the percentage screening positive for *E. servus* was moderately high, 60.3%. In cotton, the percentage of *N. viridula* DNA in gut-contents of *O. insidiosus* was high, 91.6%. Detection of *P. guildinii* and/or *T. c. custator* DNA in predators in cotton and of *E. tristigmus* DNA in predators in soybean demonstrated predator dispersal between soybean and cotton. In soybean, the percentage of *P. guildinii* DNA in gut contents of *G. punctipes*, *G. uliginosus*, and *O. insidiosus*, including those individuals in cotton that dispersed from soybean, was high. We conclude that a complex of arthropod predators prey on a complex of stink bugs in both cotton and adjoining soybean while foraging in and between these crops.

Key Words: PCR-based gut-content analysis; *Euschistus*; *Nezara*; *Geocoris*; *Orius*; crop-to-crop dispersal

Resumen

Los chinches (Hemiptera: Pentatomidae) son plagas económicas de algodón y soja. Se realizó este estudio para examinar la depredación sobre chinches de depredadores artrópodos en los hábitats del algodón y soja adyacentes. Se utilizó el análisis del contenido del estómago basado en la reacción en cadena de la polimerasa (PCR) para detectar el ácido desoxirribonucleico (ADN) del chinche en los depredadores recolectados de ambos cultivos durante un período de 5 semanas. Se detectaron *Nezara viridula* (L.), *Euschistus servus* (Say), *Chinavia hilaris* (Say), y *Euschistus quadrator* Rolston en la soja y el algodón. Se detectaron *Piezodorus guildinii* (Westwood) y *Thyanta custator custator* (F.) sólo en la soja mientras que se detectó *Euschistus tristigmus* (Say) sólo en el algodón. De ambos cultivos, 13 depredadores examinados fueron positivos para el ADN de una variedad de especies de chinches por el análisis de PCR: *Geocoris punctipes* (por ejemplo) y *Geocoris uliginosus* (Say) (Hemiptera: Geocoridae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), *Hippodamia convergens* Guérin-Méneville, *Harmonia axyridis* (Pallas) (algodón), y *Scymnus* sp. (algodón) (Coleoptera: Coccinellidae), *Oxyopes salticus* Hentz y *Peucetia viridans* (Hentz) (algodón) (Araneae: Oxyopidae), *Solenopsis invicta* Buren (Hymenoptera: Formicidae), *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), *Mecaphesa asperata* (Hentz) (Araneae: Thomisidae), *Zelus renardii* Kolenati (Hemiptera: Reduviidae) y *Notoxus monodon* (F.) (algodón) (Coleoptera: Anthicidae). En soja, el porcentaje de *G. punctipes* y *G. uliginosus* que resultaron positivos para *N. viridula* fue alto, el 87.3%, mientras que el porcentaje resultaron positivos para *E. servus* fue moderadamente alto, 60.3%. En el algodón, el porcentaje de ADN de *N. viridula* en el contenido del intestino de *O. insidiosus* fue alto, 91.6%. La detección de ADN del *P. guildinii* y/o *T. c. custator* en depredadores en algodón y de ADN de *E. tristigmus* en depredadores en soja demostró una dispersión de depredadores entre la soja y el algodón. En la soja, el porcentaje de ADN de *P. guildinii* en el contenido estomacal de *G. punctipes, G. uliginosus*, y *O. insidiosus*, incluyendo los individuos en el algodón que dispersaron a partir de soja, fue alto. Llegamos a la conclusión de que hay un complejo de artrópodos depredadores que se aprovechan de un complejo de chinches en algodón y soja contigua mientras se alimentan en y entre estos cultivos.

Palabras Clave: análisis del contenido intestinal basado en PCR; *Euschistus*; *Nezara*; *Geocoris*; *Orius*; dispersión de cultivo al cultivo

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, 130,905 bales of cotton nationwide were lost to stink bug pests in 2014 (Williams 2015). Until recently, the 3 main stink bug pest species in soybean (*Glycine max* [L.] Merr.; Fabales: Fabaceae) and cotton

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(*Gossypium hirsutum* L.; Malvales: Malvaceae) in the southeastern USA have been the southern green stink bug, *Nezara viridula* (L.), the brown stink bug, *Euschistus servus* (Say), and the green stink bug, *Chinavia hilaris* (Say). Other species include *Euschistus quadrator* Rolston, *Euschistus tristigmus* (Say), and *Thyanta custator accerra* McAtee (Bundy & McPherson 2000; McPherson & McPherson 2000). The redbanded stink bug, *Piezodorus guildinii* (Westwood), is native to Brazil and countries in the Caribbean Basin (Stoner 1922; Panizzi et al. 2000). In 1960, it was detected in soybean in the southeastern USA but was not known to cause economic damage (McPherson & McPherson 2000). During the last decade, *P. guildinii* has become a serious pest attacking soybean in the southern region of the USA (Musser et al. 2010; Temple et al. 2011; Vyavhare et al. 2014). In cotton, stink bugs feed on developing seeds and lint, causing shedding of young bolls, yellowing of lint, yield reduction, and transmission of a bacterial pathogen (Barbour et al. 1990; Medrano et al. 2009). In soybean, pod feeding by stink bugs results in reduction in oil content and yield (McPherson et al. 1995).

A complex of generalist arthropod predators, including *Geocoris punctipes* (Say) and *Geocoris uliginosus* (Say) (Hemiptera: Geocoridae), *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), *Podisus maculiventris* (Say) (Hemiptera: Pentatomidae), *Oxyopes salticus* Hentz and *Peucetia viridans* (Hentz) (Araneae: Oxyopidae), *Zelus renardii* Kolenati and *Sinea diadema* (F.) (Hemiptera: Reduviidae), *Nabis roseipennis* Reuter and *Nabis capsiformis* Gernar (Hemiptera: Nabidae), *Orius insidiosus* (Say) (Hemiptera: Anthocoridae), and *Solenopsis invicta* Buren (Hymenoptera: Formicidae), are relatively common and abundant in cotton and soybean (Bell & Whitcomb 1963; Whitcomb & Eason 1967; Pitre et al. 1978). Many of these generalist predators or closely related species have been reported attacking *N. viridula* and *E. servus* in various crops (Kiritani 1964; Ehler 2002; Tillman 2008, 2010, 2011).

Adults of *C. hilaris*, *N. viridula*, and *E. servus* exhibit dispersal into crops at field edges, especially at crop-to-crop interfaces, as they colonize cotton (Tillman et al. 2014). Indeed, an edge effect in dispersal of colonizing stink bugs into cotton was detected up to ~8.2 m (9 rows) from peanut-to-cotton interfaces. Hence, strategic placement of a trap cropping system for these pests at crop-to-crop interfaces can be the foundation for a successful stink bug management strategy in an agricultural system. For example, the addition of a habitat of sorghum in combination with *Euschistus* species pheromone traps at crop-to-crop interfaces suppressed dispersal of *E. servus* into cotton (Tillman & Cottrell 2012). Because *C. hilaris*, *E. servus*, and *N. viridula* prefer soybean to cotton (Bundy & McPherson 2000), this host plant may serve as a trap crop for stink bugs in cotton. In addition to being a sink, soybean may harbor more predators and therefore exhibit higher predation rates than cotton. Nectar feeding can be important for survival and development of arthropod predators (Lundgren 2009). For sample, extrafloral nectar increased the survival and fecundity of the lady beetle *Coleomegilla maculata* (De Geer) (Coleoptera: Coccinellidae) (Lundgren & Seagraves 2011). Flowers of buckwheat (*Fagopyrum esculentum* Moench; Polygonales: Polygonaceae) secrete nectar composed of sucrose, fructose, and glucose (Cawoy et al. 2008). Thus, combining buckwheat with soybean may enhance predation of stink bugs.

Two technological approaches have dominated molecular gut-content analysis: serological assays such as enzyme-linked immunosorbent assay (ELISA) and immunodot with either conventional or monoclonal antibody-based assays to detect prey antigenic determinants (Greenstone 1996); and polymerase chain reaction (PCR) amplification of prey deoxyribonucleic acid (DNA) sequences (Symondson 2002; Sheppard & Harwood 2005). Gut remains of habitat-specific prey are definitive evidence of where a mobile predator recently has been (Greenstone 1983; Opatovsky et al. 2013). In a 3 yr study on seasonal abundance of stink bugs in cotton–soybean ecosystems in Georgia, USA, *P. guildinii* was found exclusively in soybean (Bundy & McPherson 2000). So, detection of *P. guildinii* DNA in predators in cotton may provide insights on dispersal activity of these predators. Therefore, our specific objectives for this paper were to use PCR gut-content analysis to (1) document predation on stink bugs by arthropod predators in cotton and adjoining soybean with and without buckwheat and in cotton alone, and (2) detect remains of crop-specific prey in predators' guts as evidence of predator dispersal between cotton and adjacent soybean.

Materials and Methods

SITE DESCRIPTION

The study was conducted on a grower's peanut–cotton farmscape in Irwin County, Georgia, USA (31°35'41.4"N, 83°18'11.1"W) in 2011. The peanut field was ~9 ha, and the cotton field was ~22 ha. The farmscape was isolated from other crops by woodlands, 3 ponds, and wetlands. The nearest cotton field was ~500 m away, and the closest soybean field was ~1.7 km away. There were no alfalfa fields within a 2.0 km radius from the farmscape. The only known non-crop host of *E. tristigmus* in the woodlands surrounding this farmscape was elderberry (*Sambucus nigra* subsp. *canadensis* [L.] R. Bolli; Dipsacales: Adoxaceae); the elderberry patch consisted of around 10 plants ~250 m away from soybean. Cotton (DP 1050 B2RF) was planted on 31 May, peanut (Georgia-06G) was planted on 30 May, and soybean (Southern States RT 5160N; maturity Group V) was planted on 22 Jun. Rows were planted 0.91 m apart for each crop. None of the crops received insecticide treatments during the study.

EXPERIMENT

Treatment plots were established at the crop-to-crop interface of the farmscape. The 3 treatments were (1) cotton plus 2 rows of soybean with a row of buckwheat on each side of soybean; (2) cotton plus 2 rows of soybean; and (3) cotton without soybean. Each experimental plot was 22.9 m long (along the interface) and 271 rows (~247.8 m) wide. For each of the 3 treatments, cotton was 267 rows (~244.2 m) wide. Soybean with buckwheat (treatment 1) or soybean alone (treatment 2) was placed in 4 field rows (~3.6 m) between cotton and peanut. There was a 3.7 m alley between each interface plot. Each treatment was assigned randomly to a plot within a block for each of 4 blocks in a randomized complete block design.

For each cotton and soybean sample, all plants within a 1.83 m length of row were shaken over a drop cloth. The sampling technique does not harm the plants, but only gently shakes the predators from them. In general, stink bug eggs and 1st instars are not detected using this method. In cotton, 3 samples per row were obtained per plot. Cotton rows 1, 2, 5, 9, 16, 33, 133, 200, and 267 were sampled, covering the cotton field. For soybean, 2 samples were taken along each row per plot sampled. In peanut, sweep nets (38 cm in diameter) were used to capture stink bugs. The peanut canopy within a 7.31 m length of row of was swept for stink bugs. One sample per row was obtained per plot. Peanut rows 1, 2, 9, 16, and 33 were sampled, covering the peanut field.

Cotton for the 3 treatments with this crop and soybean for the 2 treatments with this crop were sampled once a week for 5 wk. Six years of previous studies on stink bugs in agricultural farmscapes in this region revealed that stink bug adults disperse into cotton at field edges as they colonize cotton, and dispersal of stink bugs into cotton was detected mainly 9 rows from peanut-to-cotton interfaces (Tillman et al. 2009a, 2014). Thus in cotton, predators were collected for PCR

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gut-content analysis only from rows 1, 2, 5, and 9. On 1 Sep, predators were collected from each of these 4 rows. On subsequent collection dates, predators were collected only from rows 1 and 2 because few predators were detected in rows 5 and 9. In soybean, predators for PCR gut-content analysis were collected from both rows. In order to quickly transport collected predators from soybean and cotton to the laboratory, we had to forego collecting predators from peanut for gut-content analysis. Each sampling week, a single crop (i.e., cotton or soybean) was sampled per day in order to quickly preserve collected predators. In the 1st week of the study, predators sampled in either cotton or soybean were collected from all treatment replicates on the same sampling date (see below); stink bugs also were sampled from all replicates on these dates. For each remaining week, predators were sampled and collected from 2 replicates per crop on 2 sampling dates (see below) to rapidly preserve predators; stink bugs also were sampled from 2 replicates per crop on 2 sampling dates. In cotton, sampling/collection dates for predators and sampling dates for stink bugs, respectively, were as follows: 1 Sep (week 1), 6 and 8 Sep (week 2), 12 and 15 Sep (week 3), 19 and 22 Sep (week 4), and 26 and 29 Sep (week 5). In soybean, sampling/collection dates for predators and sampling dates for stink bugs, respectively, were as follows: 2 Sep (week 1), 7 and 9 Sep (week 2), 13 and 16 Sep (week 3), 20 and 23 Sep (week 4), and 27 and 30 Sep (week 5). Predators were captured using soft forceps previously soaked in 10% sodium hypochlorite for 10 min in the laboratory to destroy any contaminating DNA. Collected predators were placed immediately in 80% ethanol in 0.5 or 1.5 mL Eppendorf safe-lock tubes (Eppendorf, Hauppauge, New York, USA), and then the tubes were placed immediately on ice in a cooler which was transported to the laboratory, where the tubes were stored at −80°C until DNA extraction. Insect species in cotton and soybean were identified and recorded in the field using an HP iPAQ rx1950 pocket personal computer (Hewlett-Packard Co., Palo Alto, California, USA). Stink bugs collected from peanut were identified to species in the laboratory. All field observations of arthropod predators feeding on stink bugs were noted. Even though our study concentrated on populations of stink bugs in crops, elderberry was randomly searched visually to determine the presence of stink bug species on this plant. Stink bug species were identified using the keys in McPherson & McPherson (2000). Identification of stink bug species and predators, except for spiders, *Scymnus* species, and *S. invicta*, were based on rearing these insects in the laboratory; spiders were identified by the second author (M.H.G.). Voucher specimens of all insects are held in the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Crop Protection & Management Research Unit in Tifton, Georgia, USA.

MOLECULAR GUT-CONTENT ANALYSIS

To meet the goals of this project, we designed species-specific PCR primers to detect DNA of 7 stink bugs, *N. viridula*, *E. servus*, *C. hilaris*, *E. quadrator*, *P. guildinii*, *Thyanta custator custator* (F.), and *E. tristigmus*, in arthropod predators. Preliminary PCRs (50 mL) for nucleotide sequencing of cytochrome oxidase I (*COI*) were performed in Buffer B (Promega, Madison, Wisconsin, USA) with 10 mL clear 5′ GoTaq Flexi buffer, 1.0 mL dNTPs mix (10 mM) (Promega), 1.0 mL primers LCO1490 and HCO2198 (20 nM) (Folmer et al. 1994), 0.25 unit mL^{-1} Taq polymerase (Promega), and 3.0 mL $MgCl_2$ (25 mM) (Promega). Initial denaturation was for 3 min at 95 °C, followed by 44 cycles of 45 s at 95 °C, 2 min at 40 °C, and 2 min at 72 °C; 5 min at 72 °C completed the program. Conditions for species-specific amplifications were the same as for preliminary amplifications except that the reaction volume was 25 µl, extension time was 1.5 min, and annealing temperatures were between 53 and 55 °C depending on the species to be amplified. Species-specific primers were designed with MegAlign (DNASTAR Lasergene, Madison, Wisconsin, USA). Primer sequences and GenBank Accession Nos. for stink bugs are given in Table 1. The predators collected at the study site by the first authors (P.G.T.) have the following Accession Nos: *G. punctipes*, KJ000388-90; *G. uliginosus*, KJ000384-87; *O. insidiosus*, KF941151-58; *Scymnus* sp., KJ002069-78; *S. invicta*, KF941170-81; *Notoxus monodon* (F.) (Coleoptera: Anthicidae), KF941141-50; *Z. renardii*, KF941182-84; *Ox. salticus*, KF941160-67; *P. viridians*, KF941168-69; and *Mecaphesa asperata* (Hentz) (Araneae: Thomisidae), KJ002066-68. Earlier, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) (Accession No. JF296003-11) was collected by James Harwood in Fayette County, Kentucky, USA, and *H. convergens* (Accession No. JF296012) and *P. maculiventris* (Accession No. DQ459378) were collected by the second author (M.H.G.) in Beltsville, Maryland, USA.

The success of each reaction was checked by electrophoresis of 6 mL of the PCR/Stop reaction in 1.5% agarose (preliminary reactions) or 2.25% agarose (species-specific reactions) in 1.0× Tris-acetate-EDTA (TAE) buffer. The remainder of the reaction was loaded and the fragments for sequencing were excised from 2.25% NuSieve agarose (Cambrex Bio Science Rockland Inc., Rockland, Maine, USA) run in 1× TAE modified to have a final EDTA concentration of 0.1 mM. Sequencing was done by BigDye terminator v3.1 kits on an ABI 3100 sequencer

Table 1. Species-specific primer sequences and GenBank Accession Nos. for stink bugs.

Species	Primer name	Primer sequence	GenBank Accession Nos.
Nezara viridula	NvF1	TGA ACT AGG ACA ACC CGG A	JX548492-95
	NvR2	GAA GGG TCA AAG AAT GAT GT	
Euschistus servus	EsF1	GAA CTA GGA CAA CCA GGA	JX548507-09
	EsR1	CGG TCA GTT AAT AGT ATG GTG A	
Chinavia hilaris	ChF2	ATG TAG TAG TTA CCG CTC AC	JX548471-74
	ChR2	AAT CGG ATC TCC TCC TCC TGA T	
Euschistus quadrator	EqF2	CGT AGT TGT AAC CGC CCA TGC A	JX548475-77
	EqR1	TTC CGG TCA GTT AAT AGT	
Euschistus tristigmus	EtF2	TGT TGT AGT TAC TGC TCA CGC A	JX548478-82
	EtR1	GTA TTA AAG CGA TCG	
Piezodorus guildinii	PgF1	GAA TTA GGT CAA CCT G	JX548496-501
	PgR1	CTA TTA AAG TTG CGG TCT G	
Thyanta custator custator	TcF2	ATG TAG TAG TTA CAG CAC AT	JX548502-06
	TcR2	AAT AGG ATC TCC CCC TGA A	

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(Applied Biosystems, Foster City, California, USA). Editing, alignments, and primer design were performed with Lasergene (DNAStar, Madison, Wisconsin, USA). In addition to DNA-free (water) and target-species (positive) controls, each primer pair was run against a minimum of 3 individuals of each of the other species in the predator and prey complex.

DATA ANALYSES

With one exception, stink bug density data for cotton and soybean were examined for each stink bug species detected in a crop and for a combination of all stink bug species in a crop; density data for *E. tristigmus* in cotton were not examined because this species was detected at very low densities in this crop. For predators, density was examined for only 6 species: *G. punctipes* and *G. uliginosus* combined, *O. insidiosus*, *H. convergens*, *Ox. salticus*, and *S. invicta*, because for these predator species at least 35 individuals were collected for PCR analyses in 1 or both crops throughout the season, and they were present for more than 2 wk in both crops. For cotton and soybean, predator and stink bug count data were modeled by a Poisson distribution. The analyses were done using Proc GLIMMIX (SAS 9.3; SAS Institute 2010). Model fit was evaluated by use of the chi-square and df statistic provided by Proc GLIMMIX (Littell et al. 2006). Fixed effects were treatment, sampling week, and treatment by sampling week. Random effects were replicate and residual error. The treatment by sampling week interaction was not significant for all density data; the interaction therefore was dropped from the model, and the model was rerun. For *N. viridula*, *C. hilaris*, and *E. quadrator* in cotton and *O. insidiosus*, *P. guildinii*, and *T. c. custator* in soybean, the model was rerun with only treatment as the fixed effect because density was too low to examine weeks. Subsamples (3 per row in cotton, 2 per row in soybean) were pooled. Means were back-transformed using the ILINK option in the LSMEANS statement and compared using Tukey's honestly significant difference (HSD) (SAS 9.3; SAS Institute 2010). In cotton, density was examined for insects only in rows from which predators were collected for PCR analyses because no stink bugs were detected in the remaining rows (i.e., rows 16, 33, 133, 200, and 267). For *O. insidiosus*, only the first 4 sampling weeks were examined because no individuals were detected in either crop in the last sampling week. For each predator species, the percentage of individuals screening positive for stink bug DNA was determined for each crop.

Results

Adults and nymphs of *N. viridula*, *E. servus*, *C. hilaris*, and *E. quadrator* were found on soybean, cotton, and peanut. Adults and nymphs of *P. guildinii* and *T. c. custator* were found exclusively on soybean, and adults and nymphs of *E. tristigmus* were found solely on cotton. Stink bugs were detected in both rows of soybean. Stink bugs on cotton were detected in rows 1, 2, 5, and 9 from the crop-to-crop boundary. Even though egg masses and 1st instars were not detected, stink bugs laid egg masses on their respective crop(s), because 2nd through 5th instars were detected. Ten arthropod predator species, *G. punctipes*, *G. uliginosus*, *O. insidiosus*, *H. convergens*, *Ox. salticus*, *P. viridians*, *M. asperata*, *P. maculiventris*, *S. invicta*, and *Z. renardii*, were detected in soybean, cotton, and peanut. Three additional predator species, *H. axyridis*, *Scymnus* sp., and *N. monodon*, were detected in cotton and peanut. *Chinavia hilaris*, *E. tristigmus*, and *N. viridula* nymphs feeding on elderberry fruit from mid-Jun through Jul developed into adults by early to mid-Aug.

For the 6 stink bug species detected on soybean, density was not influenced by treatment (Tables 2 and 3). For cotton, *E. servus* density was significantly higher on cotton without soybean than on cotton with

Table 2. Mixed model of variance statistics to test for the effect of treatment (cotton + soybean + buckwheat, cotton + soybean, and cotton alone) and sampling week on stink bug and predator density in soybean and cotton.

			Soybean			Cotton		
Insect group	Fixed effect	Species	df	*F*	*P*[a]	df	*F*	*P*[a]
Stink bugs	Treatment	*Euschistus servus*	1, 71	0.07	0.7876	2, 116	8.03	**0.0005**
	Week		4, 71	2.37	0.0602	4, 116	2.41	0.0528
	Treatment	*Nezara viridula*	1, 71	1.31	0.2566	2, 120	0.32	0.7742
	Week		4, 71	4.91	**0.0015**	—	—	—
	Treatment	*Chinavia hilaris*	1, 71	2.53	0.1161	2, 120	2.01	0.1380
	Week		4, 71	3.14	**0.0196**	—	—	—
	Treatment	*Euschistus quadrator*	1, 71	0.16	0.6904	2, 120	0.56	0.5752
	Week		4, 71	3.37	**0.0140**	—	—	—
	Treatment	*Piezodorus guildinii*	1, 78	0.01	0.9724	n/a	n/a	n/a
	Treatment	*Thyanta custator custator*	1, 78	0.01	0.9824	n/a	n/a	n/a
	Treatment	All species	1, 71	0.05	0.8253	2, 116	9.31	**0.0002**
	Week		4, 71	0.12	0.9757	4, 116	2.65	**0.0367**
Predators	Treatment	*Geocoris* species[b]	1, 71	7.65	**0.0072**	2, 116	1.59	0.2088
	Week		4, 71	8.04	**0.0001**	4, 116	3.30	**0.0133**
	Treatment	*Orius insidiosus*	1, 59	0.33	0.5677	2, 1	1.65	0.4821
	Week		—	—	—	3, 96	5.05	**0.0027**
	Treatment	*Hippodamia convergens*	1, 71	1.47	0.2297	2, 116	0.17	0.8460
	Week		4, 71	0.90	0.4686	4, 116	4.10	**0.0038**
	Treatment	*Oxyopes salticus*	1, 71	2.51	0.1176	2, 116	4.08	**0.0193**
	Week		4, 71	7.52	**0.0001**	4, 116	9.95	**0.0001**
	Treatment	*Solenopsis invicta*	1, 71	1.60	0.2104	2, 116	1.80	0.1696
	Week		4, 71	7.76	**0.0001**	4, 116	9.71	**0.0001**

[a] Significant *P* values in bold.
[b] *Geocoris* species are *G. punctipes* and *G. uliginosus*.

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Table 3. Density of stink bug species in soybean for the cotton plus soybean with buckwheat treatment (1) and cotton plus soybean treatment (2).

	Mean (SE) per 1.83 m length of row					
Treatment	*Euschistus servus*	*Nezara viridula*	*Chinavia hilaris*	*Euschistus quadrator*	*Piezodorus guildinii*	*Thyanta custator custator*
1	1.51 (0.19) a	0.27 (0.10) a	0.66 (0.16) a	1.03 (0.18) a	0.04 (0.04) a	0.08 (0.04) a
2	1.44 (0.19) a	0.39 (0.13) a	0.43 (0.12) a	1.12 (0.19) a	0.04 (0.04) a	0.08 (0.04) a

Least squares means followed by the same letter in the same column are not significantly different (Tukey's HSD, $P > 0.05$).

soybean (Tables 2 and 4). However, in no case was the density of the other species on cotton influenced by treatment. Overall mean density of *E. tristigmus* was very low, 0.002 ± 0.002 per 1.83 m length of row, on cotton.

All primer pairs were prey-species-specific, with no instances of false positives against other prey species or any of the predator species. In total, 427 arthropod predators were collected from soybean and 726 from cotton. Over both crops, 11 species of native generalist predators screened positive for stink bug DNA: *Geocoris* species (91.7% *G. punctipes* and 8.3% *G. uliginosus*), *O. insidiosus*, *H. convergens*, *Scymnus* sp., *P. maculiventris*, *Ox. salticus*, *P. viridians*, *M. asperata*, *N. monodon*, and *Z. renardii* (Table 5). Two exotic predator species, *S. invicta* and *H. axyridis*, also were positive for stink bug DNA.

Geocoris punctipes and *G. uliginosus* fed on each of the 6 stink bug species in soybean and on *N. viridula*, *E. servus*, and *C. hilaris* in cotton (Table 5). Presumably, they also fed on *E. tristigmus* in cotton, because some *Geocoris* individuals in soybean were positive for DNA of this stink bug. *Geocoris punctipes* and *G. uliginosus* were observed preying mainly on stink bug eggs and 1st instars. The percentage of both *Geocoris* species screening positive for stink bug DNA was high, 87.3%, for *N. viridula* and moderately high, 60.3 %, for *E. servus* in soybean (Table 5). Over all stink bug species detected in a crop, the percentage of *G. punctipes* and *G. uliginosus* positive for stink bug DNA was relatively higher in soybean than cotton.

On soybean, density of all stink bug species was not affected by treatment or week (Table 2). Combined *G. punctipes* and *G. uliginosus* density was significantly higher in soybean for the treatment with buckwheat than for the treatment without buckwheat (Table 2, Fig. 1A), and it was significantly lower for week 1 compared with the other weeks (Table 2, Fig. 1B). Nevertheless, the percentage of both *Geocoris* species positive for stink bug DNA appeared to be similar between treatments and across weeks.

On cotton, density of all stink bug species was significantly higher for cotton without soybean compared with cotton with soybean (Table 2, Fig. 1A), and it was significantly higher in week 5 than in weeks 1 and 2 (Table 2, Fig. 1B). Combined *G. punctipes* and *G. uliginosus* density was not influenced by treatment, but it was significantly lower in week 5 compared with the other weeks. Even though the percentage of individuals positive for stink bug DNA tended to be lower in weeks 1 and 4 than in the other weeks, insect density did not appear to influence the percentage of individuals positive for stink bug DNA. The percentage of both *Geocoris* species screening positive for stink bug DNA was similar across treatments in cotton.

For *G. punctipes* and *G. uliginosus* positive for stink bug DNA, the percentage of individuals positive for a combination of stink bug species DNA was high in soybean and moderately high, 78.6%, in cotton (Table 6). The percentage of both *Geocoris* species positive for *P. guildinii* and/or *T. c. custator* DNA alone and in combination with other stink bug species DNA was high for individuals positive for stink bug DNA in cotton. However, the percentage of both *Geocoris* species positive for *E. tristigmus* DNA alone and in combination with other stink bug species DNA was low in soybean.

In fruiting cotton, *O. insidiosus* was observed preying on *N. viridula* eggs and thrips. The percentage of *N. viridula* DNA in gut contents of *O. insidiosus* was high in cotton and soybean (Table 5). However, *O. insidiosus* also fed on a wider range of native prey species in cotton than in soybean, possibly because we could collect more individuals in cotton. Presumably, this predator also fed on *P. guildinii* and *T. c. custator* in soybean, because individuals in cotton were positive for DNA of these stink bugs.

In cotton and soybean, *O. insidiosus* density was not influenced by treatment (Table 2, Fig. 2). The percentage of *O. insidiosus* positive for stink bug DNA appeared to be similar across weeks and treatments in both crops. Of those *O. insidiosus* positive for stink bug DNA in cotton, greater than 50% tested positive for a combination of stink bug species (Table 6). The percentage of *O. insidiosus* positive for *P. guildinii* and/or *T. c. custator* DNA was high in cotton. In contrast, the percentage of *O. insidiosus* positive for *E. tristigmus* DNA was low.

Over both crops, *H. convergens* fed on each of the native stink bug species and on *P. guildinii* (Table 5). It was observed preying on 1st instar stink bugs, but it also preyed on aphids in cotton. In soybean, *H. convergens* density was not influenced by treatment or week (Table 2, Fig. 3A). Nevertheless, the percentage of *H. convergens* positive for stink bug DNA appeared to drop after week 1 in this crop.

In cotton, *H. convergens* density was not influenced by treatment (Table 2, Fig. 3A), but it was significantly higher in week 2 than in weeks 1, 4, and 5 (Table 2, Fig. 3B). The percentage of individuals positive for stink bug DNA tended to be higher in weeks 2 and 5 than in the other weeks. Predator density may have influenced the percentage of individuals positive for stink bug DNA in week 2. The percentage of *H. convergens* positive for stink bug DNA was relatively low across weeks and treatments in this crop. The percentage of *H. convergens* positive

Table 4. Density of stink bug species in cotton for the cotton plus soybean with buckwheat treatment (1), cotton plus soybean treatment (2), and cotton without soybean treatment (3).

	Mean (SE) per 1.83 m length of row			
Treatment	*Euschistus servus*	*Nezara viridula*	*Chinavia hilaris*	*Euschistus quadrator*
1	0.10 (0.05) b	0.07 (0.04) a	0.02 (0.02) a	0.17 (0.06) a
2	0.20 (0.07) b	0.05 (0.03) a	0.12 (0.05) a	0.14 (0.06) a
3	0.54 (0.15) a	0.09 (0.05) a	0.19 (0.07) a	0.23 (0.08) a

Least squares means followed by the same letter in the same column are not significantly different (Tukey's HSD, $P > 0.05$).

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Table 5. Percentage of predators screening positive for stink bug species DNA alone or in combination with other stink bug DNA for predator species in soybean and cotton.

		Predators screening positive for stink bug DNA (%)							
Crop	Predator species (n^a)	NV	ES	CH	EQ	ET	PG	TC	SB
Soybean	*Geocoris* species (189)	87.3	60.3	29.6	1.6	14.3	31.2	18.5	91.5
	Orius insidiosus (8)	100	0	0	0	0	0	0	100
	Hippodamia convergens (15)	13.3	33.3	0	13.3	6.7	6.7	0	46.7
	Podisus maculiventris (30)	43.3	3.3	0	0	0	0	3.3	49.9
	Oxyopes salticus (70)	4.3	12.9	5.7	1.4	5.7	2.9	1.4	21.4
	Solenopsis invicta (104)	3.9	6.7	5.0	2.9	1.0	1.0	0	10.6
	Mecaphesa asperata (5)	0	20.0	0	0	0	0	0	20.0
	Zelus renardii (4)	0	25.0	0	0	25.0	0	0	50.0
Cotton	*Geocoris* species (94)	40.4	20.2	3.2	0	0	53.2	8.5	56.4
	Orius insidiosus (149)	91.6	1.3	2.6	0	1.9	85.7	1.3	91.6
	Hippodamia convergens (104)	4.8	3.9	1.9	1.9	0	23.4	25.0	13.5
	Harmonia axyridis (17)	5.9	5.9	0	0	0	11.8	0	11.8
	Scymnus sp. (72)	2.8	1.4	0	4.2	0	2.8	0	8.4
	Podisus maculiventris (33)	12.1	9.1	0	0	0	0	3.0	15.2
	Oxyopes salticus (33)	3.0	0	0	0	0	0	21.2	3.0
	Solenopsis invicta (185)	1.6	2.7	0	2.2	0.6	1.1	0	7.0
	Peucetia viridans (5)	0	20.0	0	0	20.0	0	0	20.0
	Mecaphesa asperata (18)	11.1	0	0	0	0	38.9	0	11.1
	Zelus renardii (2)	50.0	100	0	0	0	0	0	100
	Notoxus monodon (14)	7.1	7.1	7.1	0	0	0	0	21.4

CH, *Chinavia hilaris*; EQ, *Euschistus quadrator*; ES, *Euschistus servus*; ET, *Euschistus tristigmus*; NV, *Nezara viridula*; PG, *Piezodorus guildinii*; SB, all stink bug species detected in a crop; TC, *Thyanta custator custator*; *Geocoris* species are *G. punctipes* and *G. uliginosus*.

[a]Number of insects collected.

for *P. guildinii* and/or *T. c. custator* DNA was high for individuals positive for stink bug DNA in cotton (Table 6). However, the percentage of *H. convergens* positive for *E. tristigmus* DNA was low in soybean.

In cotton, overall density of *H. axyridis*, 0.02 ± 0.01 per 1.83 m length of row, and of *Scymnus* sp., 0.2 ± 0.03 per 1.83 m length of row, and the percentage of individuals positive for stink bug DNA were very low (Table 5). The percentage of *H. axyridis* positive for *P. guildinii* and/or *T. c. custator* DNA was low (33.3%) for individuals positive for stink bug DNA (Table 6). The percentage of *Scymnus* sp. positive for *P. guildinii* and/or *T. c. custator* DNA also was low (25%) for individuals positive for stink bug DNA (Table 6).

Immature spiders fed mainly on 1st instar stink bugs whereas larger spiders fed on larger nymphs. *Peucetia viridans* (mature females) was the only predator in the study that preyed on adult stink bugs. In soybean, *Ox. salticus* preyed on each of the 6 stink bug species (Table 5). In cotton, it preyed on *N. viridula* and presumably *E. tristigmus* (individuals in soybean were positive for *E. tristigmus* DNA). In addition, over all stink bug species detected on a crop, the percentage of *Ox. salticus* positive for stink bug DNA was relatively higher in soybean than cotton.

In soybean, *Ox. salticus* density was not influenced by treatment (Table 2, Fig. 4A), but it was significantly lower in week 1 compared with the other weeks (Table 2, Fig. 4B). In cotton, *Ox. salticus* density was higher in the treatment with soybean and buckwheat than in cotton without soybean (Table 2, Fig. 4A), and it was significantly higher in week 4 than in weeks 1, 2, and 5 (Table 2, Fig. 4B). The percentage of *Ox. salticus* positive for stink bug DNA was low in soybean and cotton regardless of insect density. For *Ox. salticus*, the percentage of individuals positive for *T. c. custator* DNA in cotton was high for individuals positive for stink bug DNA (Table 6). However, in soybean the percentage of individuals positive for *E. tristigmus* DNA was low.

Overall density of *M. asperata* in both soybean, 0.03 ± 0.02 per 1.83 m length of row, and cotton, 0.05 ± 0.01 per 1.83 m length of row, and the percentage of individuals positive for stink bug DNA were very low (Table 5). The percentage of *M. asperata* positive for *P. guildinii* DNA was high for individuals positive for stink bug DNA in cotton (Table 6). Overall density of *P. viridans* also was very low in both soybean, 0.01 ± 0.01 per 1.83 m length of row, and cotton, 0.02 ± 0.01 per 1.83 m length of row. Only 1 individual of this spider (in cotton) was positive for stink bug DNA (Table 5). The 3 *P. viridians* spiders collected in soybean were not positive for stink bug DNA. Even though *N. viridula* DNA was not detected in *P. viridans*, individuals were observed feeding on adults of this stink bug species.

On many occasions, *S. invicta* ants foraging in soybean and cotton were observed removing stink bug eggs from plants. This predator also fed on aphid honeydew and extrafloral nectar in cotton. The percentage of stink bug DNA in gut contents of *S. invicta* was relatively low for all stink bugs in both crops (Table 5). Over both crops, *S. invicta* preyed on each of the native stink bugs, except for *T. c. custator*, and on *P. guildinii*. One *S. invicta* individual screened positive for *E. tristigmus* DNA in soybean, and 2 individuals screened positive for *P. guildinii* DNA in cotton (Table 6).

In soybean, *S. invicta* density was not influenced by treatment (Table 2, Fig. 5A), but it was significantly higher in weeks 1, 4, and 5 than in weeks 2 and 3 (Table 2, Fig. 5B). In cotton, *S. invicta* density was not influenced by treatment (Table 2, Fig. 5A), but it was significantly higher in week 2 than in weeks 1, 3, and 5 (Table 2, Fig. 5B). In general, the percentage of individuals positive for stink bug DNA tended to be low across weeks in both crops and across treatments in cotton.

Podisus maculiventris preyed on *N. viridula*, *E. servus*, and *T. c. custator* (Table 5). It was observed feeding mainly on 4th and 5th instars but also on some 2nd and 3rd instars. Overall density of *P. maculiventris* was very low in both soybean, 0.1 ± 0.03 per 1.83 m length of row, and cotton, 0.03 ± 0.01 per 1.83 m length of row. In soybean, the percentage of *N. viridula* DNA in the gut contents of *P. maculiventris* was moderately high, 43.3% (Table 5). One *P. maculiventris* individual in cotton screened positive for *T. c. custator*.

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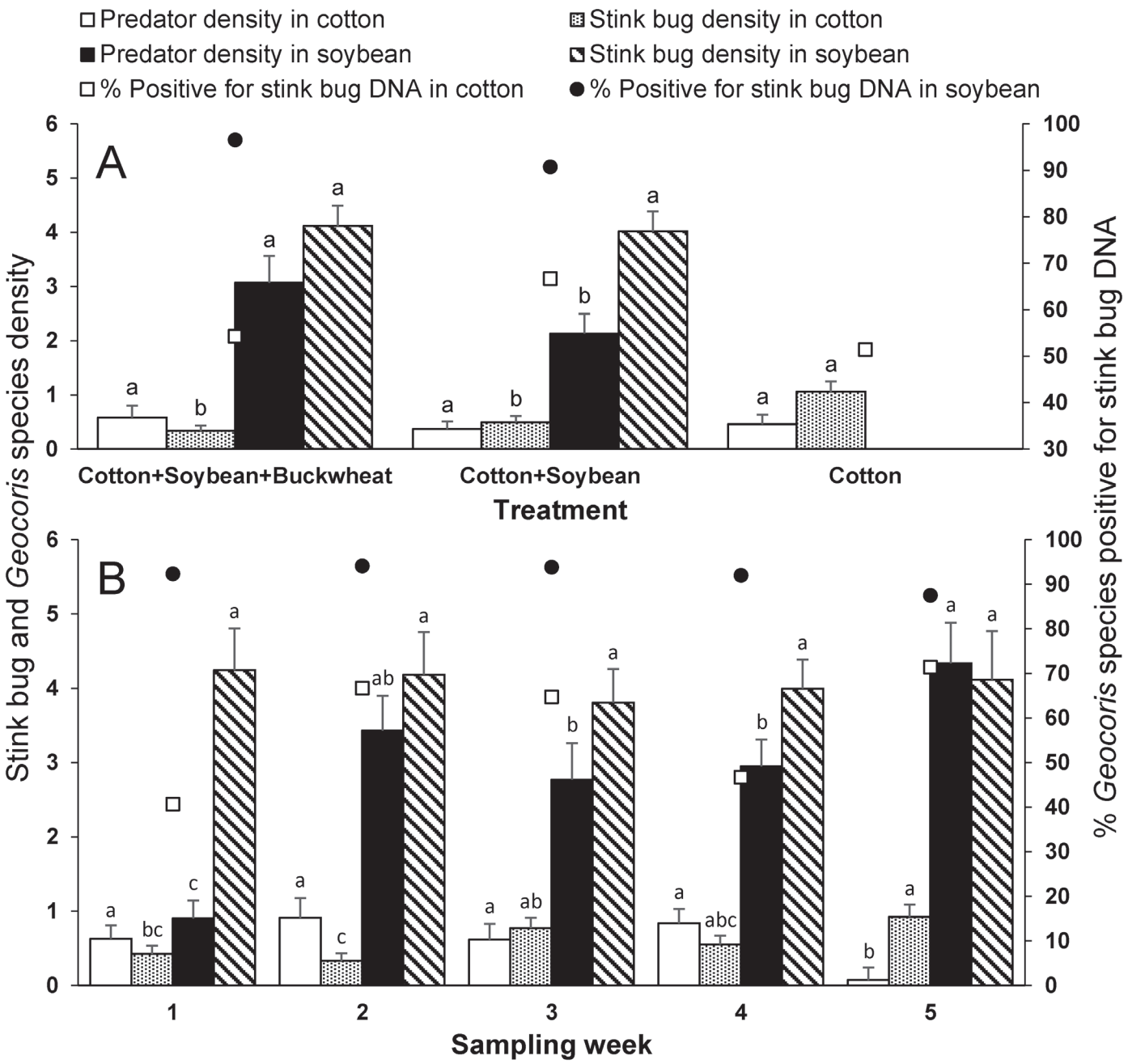


Fig. 1. Least squares means (± SE) for number of stink bugs (species combined) and *Geocoris* species (*G. punctipes* and *G. uliginosus*) per 1.83 m length of row and percentage of predators positive for stink bug DNA in (A) soybean and cotton by treatment and (B) soybean and cotton by sampling week. Within an insect group in a crop, treatment or week means with the same lowercase letter are not significantly different (Tukey's HSD, $P > 0.05$).

Overall density of *Z. renardii* was very low in both soybean, 0.03 ± 0.01 per 1.83 m length of row, and cotton, 0.03 ± 0.01 per 1.83 m length of row, and overall density of *N. monodon* was very low in cotton, 0.02 ± 0.01 per 1.83 m length of row. Only a few individuals of these 2 predator species screened positive for native stink bug DNA (Table 5). One *Z. renardii* individual screened positive for *E. tristigmus* in soybean.

Discussion

A complex of arthropod predators preyed on a complex of stink bug species in cotton and adjoining soybean habitats. A laboratory study on feeding behavior of these predators (except for *H. axyridis*, *Scymnus* sp., *M. asperata*, and *N. monodon*) showed that they feed on 1st instars of each of the stink bug species detected in cotton and soybean (P.G.T., unpublished). *Geocoris punctipes* and *G. uliginosus* also were observed attacking natural and sentinel eggs and early instars of *N. viridula* and *E. servus* in peanut, corn, and cotton (Tillman 2008, 2010, 2011). *Orius insidiosus* and *S. invicta* were observed attacking eggs whereas *Ox. salticus* and *P. maculiventris* preyed on nymphs of these 2 stink bug species in these crops. Even though prey density was low at times in our study, the detection in predator guts of low-density prey is common in molecular gut-content research (Harwood et al. 2007). As in our study, Ragsdale et al. (1981), employing stage-specific ELISA, determined that *G. punctipes*, *O. insidiosus*, *Ox. salticus*, *P. maculiven-*

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Table 6. Percentage of predators screening positive for a combination of stink bug DNA, for *Euschistus tristigmus* DNA, or for *Piezodorus guildinii* and/or *Thyanta custator custator* DNA for predators positive for stink bug DNA in soybean and cotton.

		Predators screening positive for stink bug DNA (%)		
Crop	Predator species (*n*[a])	Combination of stink bug DNA	ET[b]	PG/TC[c]
Soybean	*Geocoris* species (173)	78.6	15.6	34.1
	Hippodamia convergens (7)	42.9	14.3	14.3
	Oxyopes salticus (16)	31.3	25.0	12.5
	Solenopsis invicta (12)	25.0	8.3	8.3
Cotton	*Geocoris* species (69)	49.3	none	72.5
	Orius insidiosus (141)	69.2	2.1	84.6
	Hippodamia convergens (42)	28.6	none	85.7
	Podisus maculiventris (6)	16.7	none	16.7
	Oxyopes salticus (8)	none	none	87.5
	Solenopsis invicta (15)	none	6.7	13.3
	Mecaphesa asperata (8)	12.5	none	87.5
	Harmonia axyridis (3)	33.3	none	33.3
	Scymnus sp. (8)	none	none	25.0

Geocoris species are *G. punctipes* and *G. uliginosus*.
[a]Number of insects positive for stink bug DNA.
[b]*Euschistus tristigmus* DNA alone or in combination with other stink bug DNA.
[c]*Piezodorus guildinii* and/or *Thyanta custator custator* DNA alone or in combination with other stink bug DNA.

tris, *S. invicta*, and the lady beetle species *C. maculata* preyed on *N. viridula*. *Orius insidiosus* and *S. invicta* preyed on eggs, *G. punctipes* and *P. maculiventris* preyed on eggs and nymphs, and *Ox. salticus* preyed on nymphs. Stam et al. (1987) released radioactive phosphorus (^{32}P)-labeled *N. viridula* early instars to detect predation of this pest in soybean. They reported that *G. punctipes*, *G. uliginosus*, *P. maculiventris*, *C. maculata*, *N. roseipennis*, and a complex of spiders, including *Ox. salticus*, tested positive for ^{32}P. These authors also observed *G. punctipes* feeding on *N. viridula* eggs and 1st and 2nd instars and *S. invicta* feeding on eggs of this stink bug in soybean. Ehler (2002) observed *G. punctipes* and *Oxyopes* sp. feeding on *N. viridula* eggs and nymphs and *Z. renardii* feeding on nymphs of this stink bug in the laboratory. In laboratory studies, *P. maculiventris* fed on *N. viridula* eggs, nymphs, and adults (De Clercq et al. 2002). Therefore, *P. maculiventris* and *P. viridans* (observed in the current study) are the only predators in this study known to prey on stink bug adults.

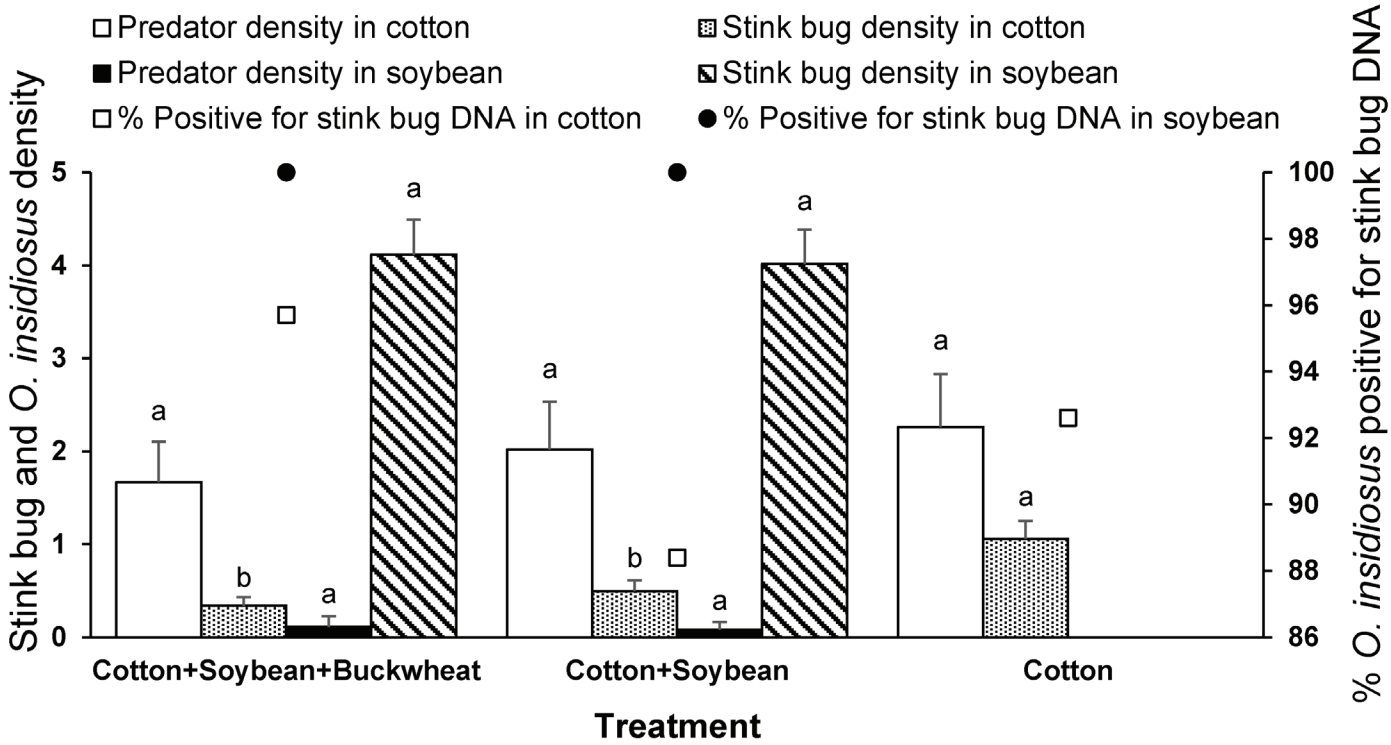


Fig. 2. Least squares means (± SE) for number of stink bugs (species combined) and *Orius insidiosus* per 1.83 m length of row and percentage of predators positive for stink bug DNA in soybean and cotton by treatment. Within an insect group in a crop, treatment means with the same lowercase letter are not significantly different (Tukey's HSD, $P > 0.05$).

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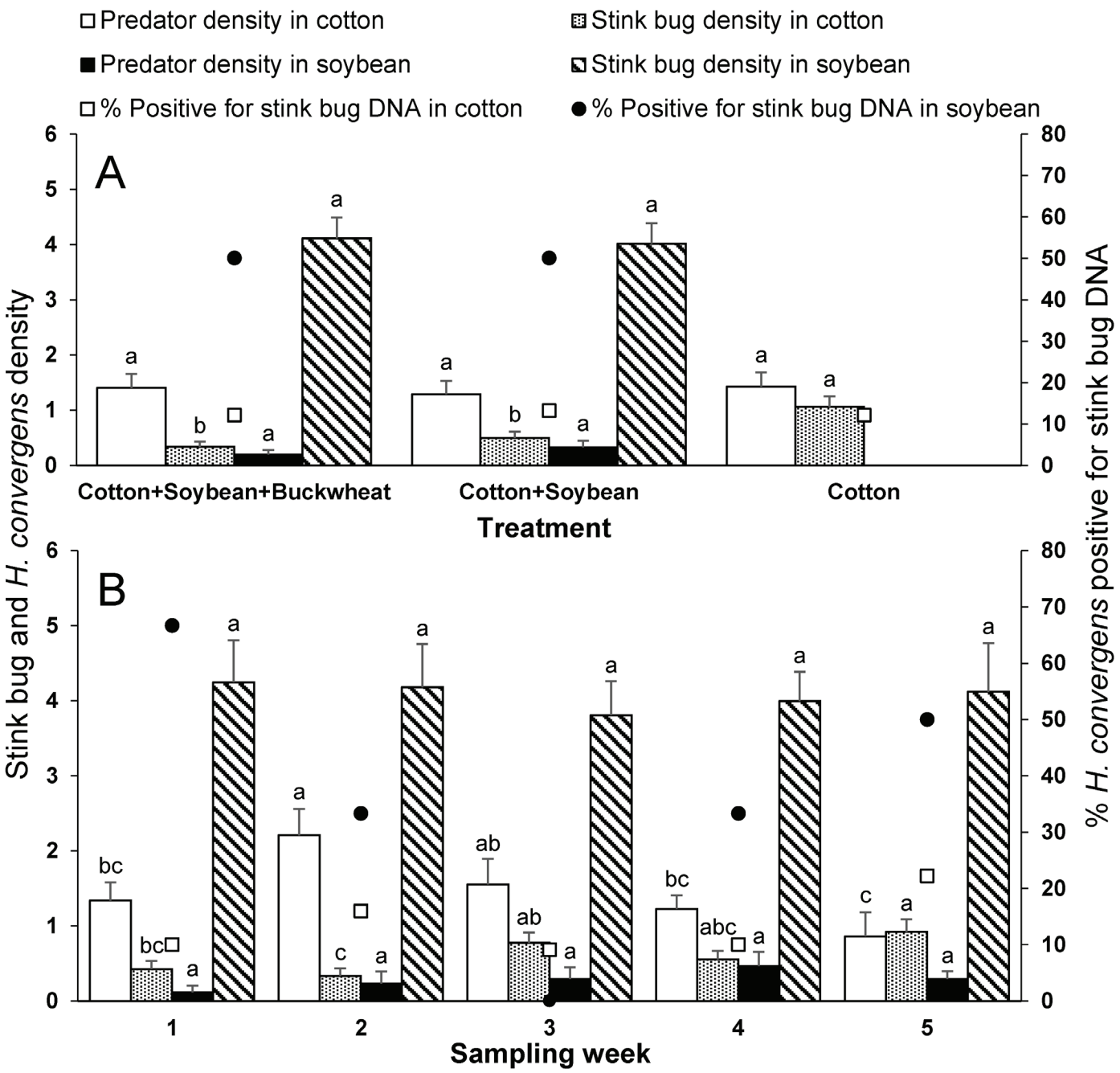


Fig. 3. Least squares means (± SE) for number of stink bugs (species combined) and *Hippodamia convergens* per 1.83 m length of row and percentage of predators positive for stink bug DNA in (A) soybean and cotton by treatment and (B) soybean and cotton by sampling week. Within an insect group in a crop, treatment or week means with the same lowercase letter are not significantly different (Tukey's HSD, $P > 0.05$). No predators were collected for PCR analyses in week 3 in soybean.

In this current study, we discovered that *G. punctipes* and *G. uliginosus* also prey on *E. servus*, *C. hilaris*, *E. quadrator*, *P. guildinii*, *T. c. custator*, and *E. tristigmus*, whereas *O. insidiosus* preys on *E. servus*, *C. hilaris*, and *E. tristigmus*. We determined that the lady beetle *H. convergens*, in addition to feeding on *N. viridula*, also preys on *E. servus*, *C. hilaris*, *E. quadrator*, and *P. guildinii*. We also discovered that *Ox. salticus* preys on *E. servus*, *C. hilaris*, *E. quadrator*, *P. guildinii*, *T. c. custator*, and *E. tristigmus* and that *S. invicta* feeds on *E. servus*, *C. hilaris*, *E. quadrator*, *E. tristigmus*, and *P. guildinii.* This is this first report of *P. maculiventris* preying on *E. servus* and *T. c. custator*; *Z. renardii* preying on *E. servus* and *E. tristigmus*; and *M. asperata* and *N. monodon* preying on stink bugs in agronomic crops. Furthermore, this is the first report of *P. viridans* from the field screening positive for *E. servus* and *E. tristigmus* DNA. However, Randall (1982) previously observed *P. viridans* feeding on *E. servus* in the field.

Detection of habitat-specific prey in predator's guts is definitive evidence for predator dispersal (Greenstone 1983; Opatovsky et al. 2013) and provided insight on dispersal activity of predators in cotton and adjoining soybean. In our study, *P. guildinii* and *T. c. custator* were specific to soybean. Bundy & McPherson (2000) found *P. guildinii* exclusively in soybean and *T. c. accerra*, not *T. c. custator*, in soybean and cotton in a 3 yr study on seasonal abundance of stink bugs in cot-

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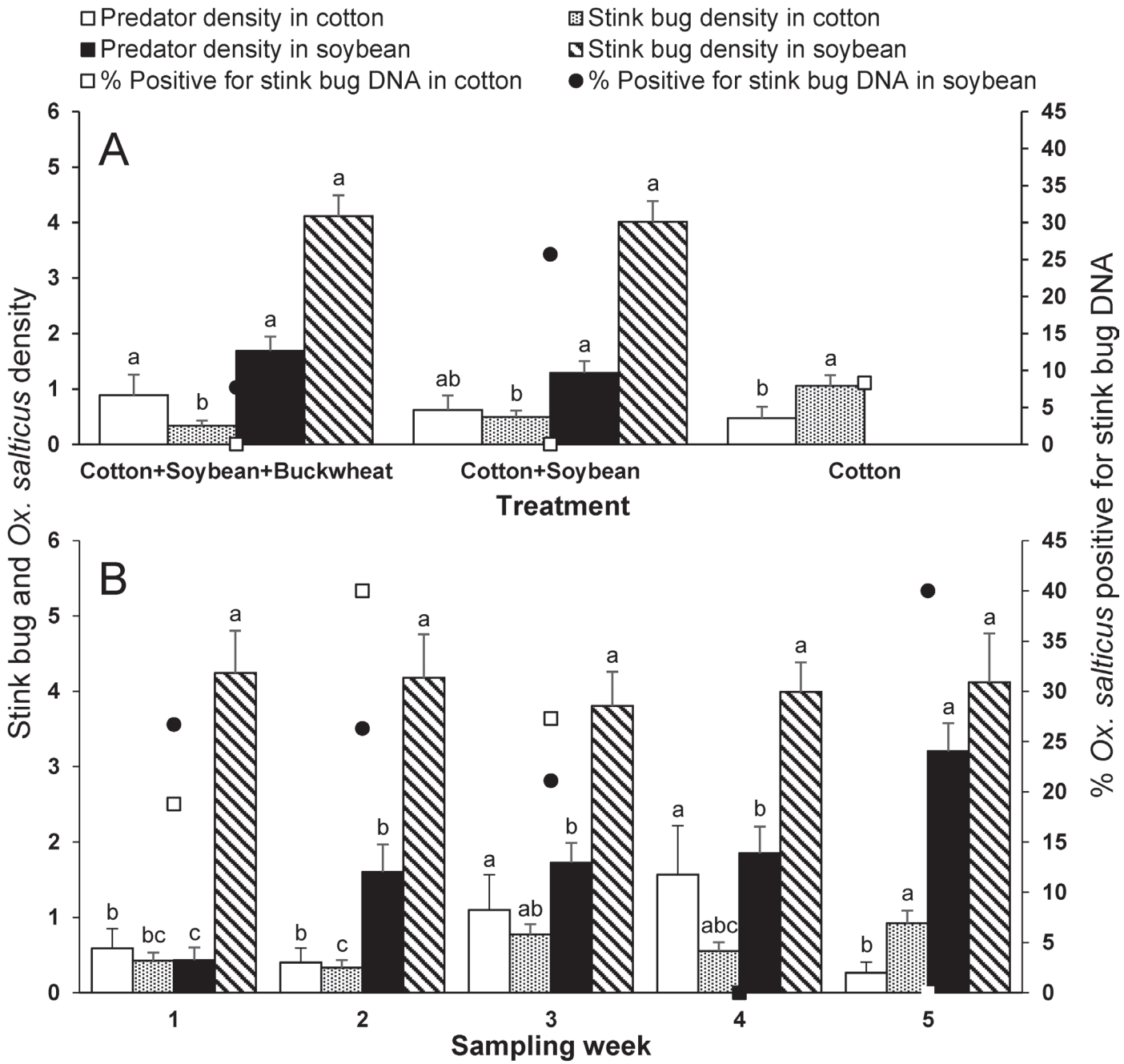


Fig. 4. Least squares means (± SE) for number of stink bugs (species combined) and *Oxyopes salticus* per 1.83 m length of row and percentage of predators positive for stink bug DNA in (A) soybean and cotton by treatment and (B) soybean and cotton by sampling week. Within an insect group in a crop, treatment or week means with the same lowercase letter are not significantly different (Tukey's HSD, $P > 0.05$). Only 1 *Ox. salticus* individual screened positive for stink bug (*Nexara viridula*) DNA in cotton. No predators were collected for PCR analyses in weeks 4 and 5 in cotton.

ton–soybean ecosystems in Georgia, USA. Because the peanut–cotton farmscape in our study was relatively isolated from other crops and the closest soybean was very distant from the farmscape, it is highly unlikely that predators dispersed from other soybean fields into our cotton plots. Both *P. guildinii* and *T. c. custator* can develop on alfalfa (Tillman 2013). However, no alfalfa was grown near this farmscape. In a previous 4 yr study, *P. guildinii* was not present in peanut in Georgia (Tillman 2008). Only *Thyanta* sp. adults were present in this crop at extremely low densities; they did not reproduce in peanut. In the current study, neither of these stink bug species was present in peanut. Thus, predators screening positive for DNA of these 2 stink bugs did not disperse from peanut into cotton even though some predators that were positive for *N. viridula*, *E. servus*, or *E. quadrator* DNA may have dispersed from peanut into the other 2 crops, because nymphs of these stink bug species were present on this crop. In our study, *E. tristigmus* was present only on cotton. In their 3 yr study in cotton–soybean ecosystems, Bundy & McPherson (2000) found *E. tristigmus* on soybean in low numbers in only one of the years. Previously, only *E. tristigmus* adults were detected in peanut at extremely low densities (Tillman 2008); they did not reproduce in this crop. Similar to the findings of Jones & Sullivan (1982) for *C. hilaris*, *E. tristigmus* was not present in elderberry by mid-Aug in the farmscape studied. Thus, it

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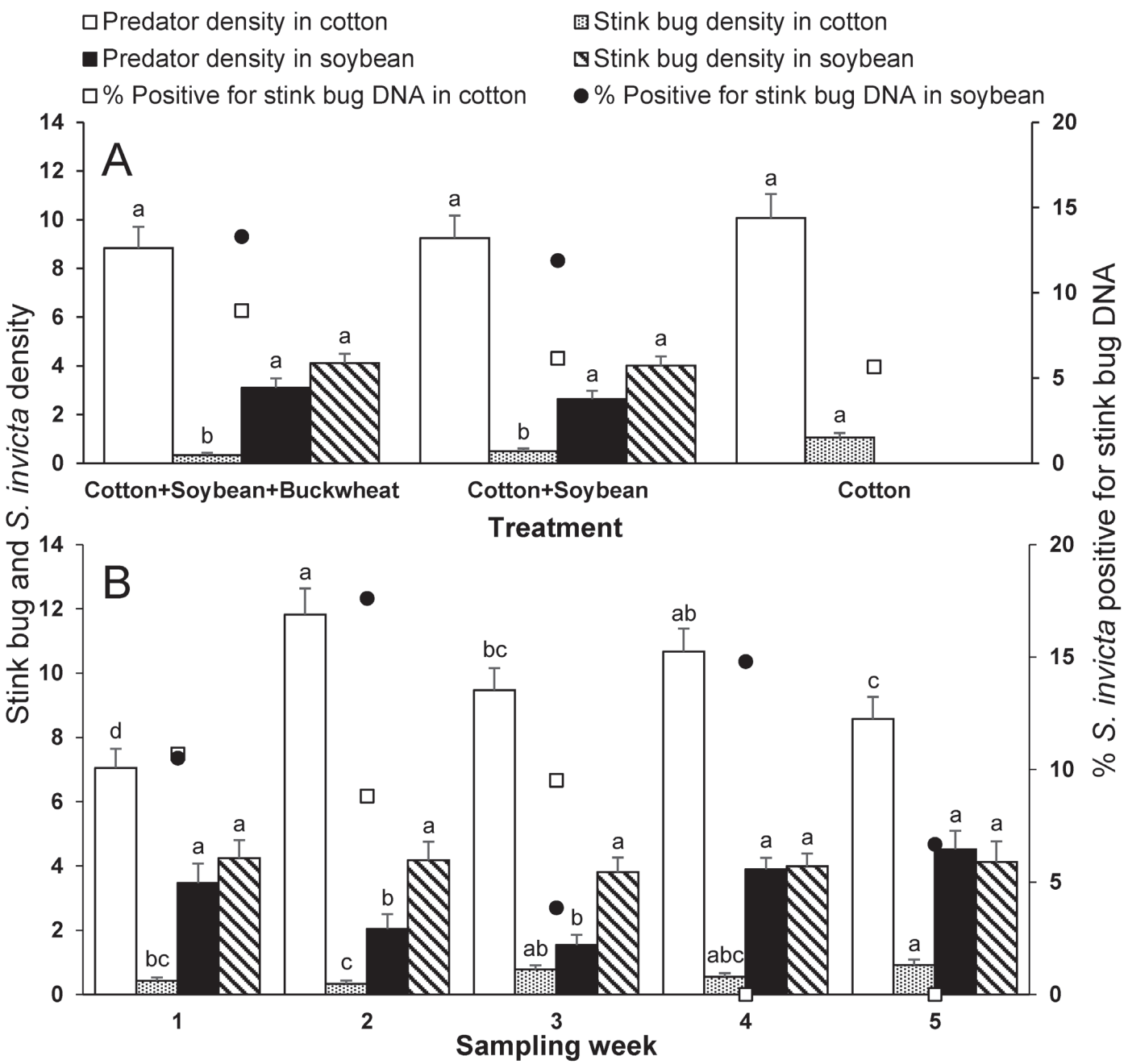


Fig. 5. Least squares means (± SE) for number of stink bugs (species combined) and *Solenopsis invicta* per 1.83 m length of row and percentage of predators positive for stink bug DNA in (A) soybean and cotton by treatment and (B) soybean and cotton by sampling week. Within an insect group in a crop, treatment or week means with the same lowercase letter are not significantly different (Tukey's HSD, $P > 0.05$).

is highly unlikely that any predators that may have dispersed ~250 m from this shrub into soybean habitats retained *E. tristigmus* DNA in their gut contents at least 2 wk later.

Because *P. guildinii*, *T. c. custator*, and *E. tristigmus* were habitat specific, detection of *E. tristigmus* DNA in gut contents of predators in soybean demonstrated that the predators had dispersed from cotton into soybean, and detection of *P. guildinii* and/or *T. c. custator* DNA in gut contents of predators in cotton demonstrated that the predators had dispersed from soybean into cotton. Thus, *G. punctipes*, *G. uliginosus*, and *Ox. salticus* dispersed between soybean and cotton and *O. insidiosus*, *H. convergens*, and *M. asperata* dispersed from soybean into cotton. In a previous study, seasonal occurrence and abundance of predators and percentage of stink bug egg predation indicated that these natural enemies exhibited crop-to-crop dispersal in farmscapes (Tillman 2011). This current study confirms that these predators disperse among crops in these farmscapes. Crop-to-crop dispersal may be necessary for survival of arthropod predators considering that not all plants of any crop generally have suitable stink bug prey at all times.

In diverse agricultural settings where crops are closely associated, some of the generalist arthropod predators in our study previously have been reported to exhibit crop-to-crop dispersal. Detection of remains of soybean-specific prey, namely *Megacopta cribraria* (F.) (Hemiptera:

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Plataspidae), in gut contents of *G. punctipes* in cotton demonstrated that this predator dispersed from soybean into adjacent cotton (Greenstone et al. 2014). Prasifka et al. (2001) used rubidium chloride (RbCl) marking of herbivorous insects to demonstrate movement of different predator taxa, including *Geocoris* spp., *O. insidiosus*, *H. convergens*, assorted spiders, *Scymnus loewii* Mulsant (Coleoptera: Coccinellidae), and *Notoxus* sp., between cotton and sorghum. Another rubidium-marking study provided evidence that *O. insidiosus* dispersed from corn into adjacent sorghum and from sorghum into adjacent cotton (Tillman et al. 2007).

In this study, *G. punctipes*, *G. uliginosus*, and *O. insidiosus* preyed on a variety of stink bug species. However, they frequently preyed on certain stink bug species in a crop. In soybean, the percentage of *N. viridula* and *E. servus* DNA in gut contents of *G. punctipes* and *G. uliginosus* was high, 87.3%, to moderately high, 60.3%. In addition, the percentage of *P. guildinii* DNA in gut contents of *G. punctipes*, *G. uliginosus*, and *O. insidiosus*, including those individuals in cotton that dispersed from soybean, was high. In cotton, the percentage of *N. viridula* DNA in the guts of *O. insidiosus* was high.

Fire ants, including *S. invicta*, collect solid food such as pest insects (Hays & Hays 1959) less frequently than liquid food (Tenant & Porter 1991). Nonetheless, in our study, *S. invicta* preyed on most stink bug species in both crops. Ragsdale et al. (1981) determined that this predator fed solely on stink bug eggs in soybean. We observed *S. invicta* individuals removing eggs from soybean and cotton plants in this study and from peanut and corn plants in previous studies (Tillman 2008, 2010). Also, removal of sentinel stink bug eggs from plants by *S. invicta* can be very intense in peanut and cotton (Tillman 2011). Nevertheless, the percentage of stink bug DNA in gut contents of *S. invicta* was relatively low in both crops. Nuessly & Sterling (1986) observed that *S. invicta* ants foraging on *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) eggs marked with ^{32}P captured their prey intact and then returned to the nest where eggs were disseminated by foraging ants to ants at the nest. This likely explains the low percentage of foraging ants screening positive for stink bug DNA and the presence of *E. tristigmus* DNA in *S. invicta* in soybean and *P. guildinii* DNA in this predator in cotton.

In cotton–soybean farmscapes, *C. hilaris*, *E. servus*, and *N. viridula* are highly attracted to fruiting soybean, more so than to fruiting cotton (Bundy & McPherson 2000). This likely explains why *E. servus* density and overall stink bug density were higher in cotton without soybean than cotton adjacent to soybean in the current study. In a recent study, soybean planted at peanut–cotton interfaces was an effective trap crop for stink bug pests, reducing both stink bug density in cotton and boll injury (P.G.T., unpublished). Even though densities of *N. viridula*, *C. hilaris*, and *E. quadrator* tended to be higher in cotton without soybean than in cotton with soybean, significant differences were not detected among treatments, likely due to the lower numbers of these 3 stink bug species in general compared with that of *E. servus*. The finding that *Ox. salticus* was more abundant in cotton adjacent to soybean than in cotton without soybean indicates that this predator is attracted more to soybean than cotton.

This study has shown the importance of these species of naturally occurring predators in the consumption of stink bug pests in soybean and cotton. Conservation of these natural enemies can play a significant role in an integrated pest management program for these pests. Currently, control practices for stink bug pests in conventionally grown crops are limited to the application of broad-spectrum insecticides that are equally toxic to the predator and pest (Tillman et al. 2003; Tillman & Mullinix 2004). Using selective insecticides could help conserve predators. For example, application of spinosad did not affect *G. punctipes* and *H. convergens* densities in cotton (Tillman & Mulrooney 2000). However, selective insecticides are not always effective against stink bug pests (Tillman et al. 2009b). Other tactics are needed for conserving these predators in both conventional and organic cropping systems. Perhaps a soybean habitat could serve as an insecticide-free refuge for predators near cotton. Nectar feeding can be important for survival and development of arthropod predators (Lundgren 2009). Nocturnal spiders, including thomisids and oxyopids, feed on extrafloral nectar of cotton (Taylor & Pfannenstiel 2008). De Lima & Leigh (1984) reported that nectar is essential for development of *Geocoris pallens* Stål (Hemiptera: Geocoridae) on cotton in the absence of prey. Higher numbers of *G. punctipes* and *G. uliginosus* in soybean with buckwheat than in soybean without this plant indicate that these 2 predator species were attracted to buckwheat, perhaps to the nectar. However, the percentage of both *Geocoris* species positive for stink bug DNA was only around 6% higher for predators collected from soybean with buckwheat compared with soybean alone.

Using molecular gut-content analysis, we have shown that generalist arthropod predators prey on stink bug species in cotton and soybean habitats. By detecting the remains of crop-specific prey in predators' guts, we have shown that these predators disperse between cotton and soybean. We conclude that a complex of arthropod predators prey on a complex of stink bugs in cotton and adjoining soybean while they forage in and between these crops.

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