Stink Bugs (Heteroptera: Pentatomidae) and their Natural Enemies in Alfalfa in South Georgia[1]

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Abstract The objective of this 3-yr study was to determine species composition and abundance of stink bugs (Heteroptera: Pentatomidae) and their natural enemies in alfalfa, *Medicago sativa* L., in Georgia. Six species of phytophagous stink bugs, *Nezara viridula* (L.), *Euschistus servus* (Say), *Thyanta custator custator* (F.), *Euschistus quadrator* Rolston, *Oebalus pugnax pugnax* (F.), and *Chinavia hilaris* (Say), and one predatory species, *Podisus maculiventris* (Say), occurred in alfalfa. Generally, density of *N. viridula*, the predominant stink bug species, was high in seeding alfalfa in the late spring-early summer, and parasitization of this stink bug by *Trichopoda pennipes* (F.) could reach high levels as density of the pest increased in the crop. *Euschistus servus* and *T. c. custator* together comprised the second most predominant group of stink bugs in this crop. Based on seasonal occurrence of nymphs (all 5 instars) and adults, nymphal development time, ovarian development time, and reproductive status of adults (*N. viridula* only), alfalfa is a reproductive host for *N. viridula* and *E. servus*, and perhaps for *T. c. custator*. Mainly adults of *E. quadrator*, *O. p. pugnax*, and *C. hilaris* were present in the crop. Stink bug predators included *Geocoris* spp., *Orius insidiosus* (Say), spiders, lady beetles, nabids, reduviids, and *Solenopsis invicta* Buren.

Key Words *Nezara viridula*, *Euschistus servus*, *Medicago sativa*, *Trichopoda pennipes*

Alfalfa, *Medicago sativa* L., is a high-yielding, perennial legume that is well suited to hay, silage, pasture, and seed production (Hancock et al. 2011). Breeding efforts at the University of Georgia have greatly improved stand-life of alfalfa in south Georgia, and production of the crop has steadily increased in the state (Hancock et al. 2011). Stink bugs (Heteroptera: Pentatomidae), including the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euschistus servus* (Say), are pests in conventional and organic production of major agronomic and vegetable crops in Georgia. Previous researchers reported that *E. servus* readily fed on the vegetative growth and developing seed of alfalfa in Arizona (Russell 1952, Wene and Sheets 1964). Similarly, *Euschistus conspersus* Uhler fed on alfalfa with seed in California (Bacon et al. 1971). In light of the recent increase in alfalfa production and the relative lack of information on stink bugs in this crop, the objective of this 3-yr experimental-farm project was to determine species composition and abundance of stink bugs and their natural enemies in alfalfa in Georgia.

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Materials and Methods

Study site. Over the 3-yr study, 4 separate alfalfa plots (70.1 m × 3.7 m) were sampled at the USDA, ARS experimental farm (≈ 20 ha) in Tifton, GA. All recommended agricultural practices for production of alfalfa (Hancock et al. 2011) were followed except alfalfa was cut only in the late fall of 2000 and 2001, and no insecticides were applied. The Amerigraze 702 variety of alfalfa was planted at a seedling rate of 22.4 kg/ha on 22 November 1999.

Sampling. Alfalfa was examined usually, but not always, on a weekly basis for the presence of stink bugs from 22 March through 25 October in 2000, from 11 April through 8 August in 2001, and from 8 March through 28 June in 2002. Insects were sampled using sweep nets (38 cm diam). A sweep sample consisted of 25 sweeps, and a total of 10 random sweep samples was obtained per plot per sampling date. Once a sweep sample was collected, it was placed in a 3.8-L self-sealing plastic bag which was placed in a cooler and transported to the laboratory where insect species and developmental stage were identified and recorded. In 2001, *N. viridula* females were dissected under a dissecting microscope on 17 May and each sampling date in June and July to visually examine the ovaries for oocytes. *Nezara viridula* adults with at least one *Trichopoda pennipes* (F.) egg on the exoskeleton were considered to be parasitized by the parasitoid, but subsequent mortality of *T. pennipes* developmental stages was not assessed. In 2000, parasitization of *N. viridula* by this parasitoid was not determined on 3, 24, and 31 May and after 12 July. Parasitization of this pest by this parasitoid was not observed until 2 May in 2001 and 7 June in 2002. Parasitization of *E. servus* adults was not determined. Voucher specimens of all insects are stored in the USDA-ARS, Crop Protection & Management Research Laboratory in Tifton, GA.

Statistical analyses. Density data for phytophagous stink bug adults and stink bug predators over the study were compared using the PROC MIXED procedure of the Statistical Analysis System (SAS Institute 2008). The fixed effects were species, year, and species × year. Random effects were residual error. Least squares means were separated by least significant difference (LSD, $P < 0.05$) (SAS Institute 2008) where appropriate. To construct seasonal graphs of populations of *E. servus* and *N. viridula*, means for stink bug nymphs and adults per sample were obtained for each of these 2 stink bug species for each sampling date using PROC MEANS (SAS Institute 2008). Percent parasitization of *N. viridula* adults by *T. pennipes* was calculated for each sampling date these data were obtained.

Results and Discussion

Stink bug species. Six species of phytophagous stink bugs, *N. viridula*, *E. servus*, *Thyanta custator custator* (F.), *Euschistus quadrator* Rolston, *Oebalus pugnax pugnax* (F.), and *Chinavia hilaris* (Say), were collected from alfalfa in this 3-yr study in Georgia. On several occasions, adults of *N. viridula*, *E. servus*, and *T. c. custator* were observed feeding on alfalfa seed. A significant species × year interaction was detected for overall stink bug density ($F = 10.23$; df = 8, 17,000; $P < 0.0001$). *Nezara viridula* was the predominant stink bug species over all years of the study, and only *N. viridula* populations varied significantly by year with density highest in 2000 and lowest in 2001 (Table 1). Density was similar for *E. servus* and *T. c. custator*, and together they comprised the second most predominant group of stink bugs in this crop. All 5

Table 1. Least squares means for number of phytophagous stink bugs per sweep sample in alfalfa over all plots and dates in 2000, 2001, and 2002

Species	2000	%	2001	%	2002	%
Nezara viridula (L.)	0.36aA	79.5	0.18cA	63.6	0.29bA	71.6
Euschistus servus (Say)	0.04aB	9.0	0.06aB	21.2	0.04aB	10.2
Thyanta custator custator (F.)	0.05aB	11.5	0.02aB,C	6.8	0.03aB	7.4
Euschistus quadrator Rolston	.	.	0.01aC	2.5	0.04aB	9.1
Oebalus pugnax pugnax (F.)	.	.	0.01aC	4.2	0.004aB	1.0
Chinavia hilaris (Say)	.	.	0.004aC	1.7	0.003aB	0.7

Least squares means within a row followed by the same lowercase letter are not significantly different among years for a single stink bug species. Least squares means within a column followed by the same uppercase letter are not significantly different among stink bug species for a single year. (PROC MIXED, LSD, $P > 0.05$, SE = 0.014).

nymphal stages of *N. viridula*, *E. servus*, and *T. c. custator* were collected from alfalfa. Overall percentage of females in the population ranged from 50 - 60% for these 3 stink bug species. Mostly adults of *E. quadrator*, *O. p. pugnax*, and *C. hilaris* were collected. Recently, all developmental stages of the invasive stink bug species, *Piezodorus guildinii* (Westwood), also have been found in alfalfa in Georgia (unpubl. data).

Seeding alfalfa has been previously reported as a host plant for *E. servus*, *E. conspersus*, and *Thyanta pallidovirens* (Stål) in Arizona and California (Russell 1952, Wene and Sheets 1964, Bacon et al. 1971). Very little information has been reported on *N. viridula* in alfalfa. McBrien et al. (2001) mentioned collecting nymphs and adults of this stink bug in alfalfa in California to establish a colony of the insect. Researchers in Iran reported that this stink bug species was present, though rare, in alfalfa (Mirab-balou et al. 2007). There are no published reports of *O. p. pugnax* and *E. quadrator* on alfalfa. Yeargan (1979) stated that *E. servus*, *Euschistus variolarius* (Palisot de Beauvois), and *C. hilaris* were the most common stink bugs in alfalfa in Kentucky. The lack of literature on stink bug species in alfalfa may be more a reflection of alfalfa production practices (i.e., cutting for hay versus growing for seed) than on the ability of seeding alfalfa to serve as a host plant. Not surprisingly, stink bug species composition in alfalfa appears to vary by locality or local composition of stink bug species although *Euschistus* spp. were present in most reports on stink bugs on this crop.

Stink bug natural enemies and insect pollinators. A significant species × year interaction was detected for overall predator density ($F = 63.86$; df = 14, 23,000; $P < 0.0001$). The predominant predators in alfalfa were *Geocoris* spp., *O. insidiosus*, and spiders (Table 2). Lady beetles, nabids, reduviids, *Solenopsis invicta* Buren, and the predatory stink bug *Podisus maculiventris* (Say) also inhabited alfalfa. The tachinid *T. pennipes* parasitized *N. viridula* late-instar nymphs and adults. Two species of stink bug egg parasitoids, *Trissolcus basalis* (Wollaston) and *Telenomus podisi* Ashmead, were collected from alfalfa from midApril through late June. Each of these natural enemy species has been reported as a significant biocontrol agent of stink bugs (Ragsdale et al. 1981, Yeargan 1979, Tillman 2006, Tillman 2011). Insect pollinators, particularly honey bees and native bees, were found in alfalfa from midApril through midJuly.

Seasonal occurrence of *N. viridula* in alfalfa. Each year of the study, *N. viridula* adults entered flowering alfalfa in early to midApril (Fig. 1). Stink bugs were not present in alfalfa on March sampling dates (not shown on figures). Based on presence of young nymphs, *N. viridula* females began ovipositing on alfalfa as soon as, or soon after, the first fruit appeared on the crop. Then the density of *N. viridula* adults and nymphs increased until peaking in seeding alfalfa. In 2000 and 2001, density of *N. viridula* decreased on the crop once most of the seed were mature (Fig. 1 A, B). This decrease in reduction in adult density was probably due to 3 factors: (1) reduction in adult food (seed) and subsequent dispersal to host plants with suitable food (not examined, but other susceptible crops such as cotton were present in the location), (2) high rates of parasitization by *T. pennipes*, and (3) predation of nymphs (although not quantified). In 2001, overall percentage of unparasitized *N. viridula* females that were reproductive (oocytes were present) was 67% on 17 May, 42% in June, and 63% in July. Normal development from eggs to adults for *N. viridula* requires about 35 d in the field (Drake 1920, Harris and Todd 1980). Also, the first 10 d of adulthood for *N. viridula* females are used for oocyte development and maturation (Fortes et al. 2011).

Table 2. Least squares means for number of stink bug predators per sweep sample in alfalfa over all plots and dates in 2000, 2001, and 2002

Species	2000	2001	2002
Geocoris spp.*	2.2aA	1.84bB	0.95cB
Orius insidiosus (Say)	1.1bB	2.45aA	0.51cC
Spiders**		1.09bC	1.66aA
Lady beetles†	0.5aC	0.55aD	0.44aC
Nabidae/Reduviidae††	0.03bD	0.23aE,F	0.09a,bD
Solenopsis invicta Buren	0.02aD	0.06aF,G	0.04aD
Podisus maculiventris (Say)	0.005aD	0.02aG	0.005aD

Least squares means within a row followed by the same lowercase letter are not significantly different among years for a single stink bug species. Least squares means within a column followed by the same uppercase letter are not significantly different among stink bug species for a single year. (PROC MIXED, LSD, $P > 0.05$, SE = 0.0617).

* *Geocoris punctipes* (Say) and *G. uliginosus* (Say).

** *Peucetia viridans* (Hentz), *Oxyopes salticus* Hentz, *Misumena vatia* (Clerck), *Phidippus* spp., *Tetragnatha* spp., and unknown spiders.

† *Hippodamia convergens* Guérin-Méneville, *Coccinella septempunctata* (L.), *Coleomegilla maculata* De Geer, and *Harmonia axyridis* (Pallas).

†† *Nabis* spp., *Zelus renardii* Kolenati, and *Sinea diadema* (F.), and unknown species.

Thus, the gradual drop in percentage of reproductive females in June indicates that new (nonreproductive) females that had developed on alfalfa were present on alfalfa at that time. Presence of nymphs in July in 2000 and 2001 suggests that at least some of the females that developed on alfalfa laid eggs on this crop at this time. In 2000, nymphs were found on alfalfa in August, but density of *N. viridula* adults remained very low in this crop after early August.

In 2000 and 2001, *T. pennipes* apparently began parasitizing *N. viridula* adults soon after stink bugs dispersed into alfalfa (Fig. 1 A, B). In 2000, 3rd-instar *T. pennipes* emerged from around 10% of the parasitized *N. viridula* 1 - 2 d after being collected on 17 and 24 May. So, at least some *N. viridula* adults were parasitized by *T. pennipes* as early as 3 May, for development time for *T. pennipes* larvae is approx. 2 wks (Worthley 1924). This tachinid parasitoid also parasitizes *N. viridula* adults in other crops and geographical locations (McPherson et al. 1982, Jones 1988). As *N. viridula* increased in fruiting alfalfa, levels of parasitization of this stink bug by *T. pennipes* reached high levels (Fig. 1 A-C). Heightened levels of parasitization in aggregations of stink bug hosts have been observed for *Trichopoda* spp. on other vegetation (Liljesthröm and Rabinovich 2004, Tillman 2006).

Seasonal occurrence of *E. servus* in alfalfa. *Euschistus servus* adults entered alfalfa while still flowering or just beginning to fruit (Fig. 2). In 2001 and 2002, nymphs were present on seeding alfalfa indicating eggs were oviposited in this crop. Inexplicably, nymphs were not found in alfalfa in 2000 until July. Density of nymphs was highest in August in 2000 and in July in 2001. The presence of nymphs in alfalfa in June and July in 2001 and in August, September, and October in 2000 indicates that females that developed on alfalfa would then lay eggs on the crop.

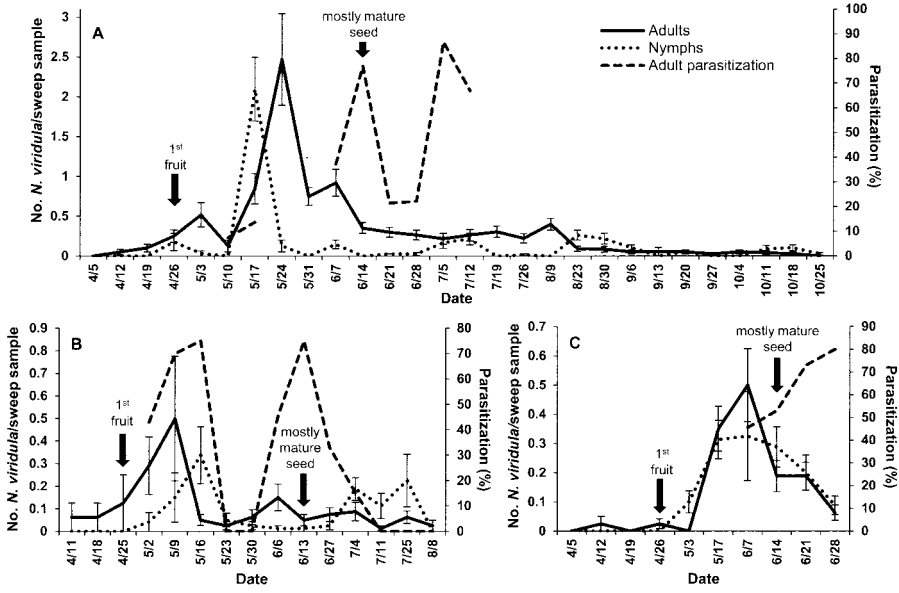

Fig. 1. Mean number of *N. viridula* nymphs and adults per sweep sample and percentage parasitization of adults by *T. pennipes* in alfalfa for sampling dates in A) 2000, B) 2001, and C) 2002. Error bars represent SEM.

Altogether these results indicate that alfalfa is a reproductive host for *N. viridula* and *E. servus*, and perhaps for *T. c. custator*. Adults of each of these stink bug species fed on alfalfa seeds, the preferred food for stink bug species (Patel et al. 2006, Mizell et al. 2008). Generally, oviposition requires mating in *N. viridula* (Fortes et al. 2011), and based on the sex ratios, each of the 3 stink bug species likely mated in the alfalfa. Incidence of all 5 nymphal stages of each species on alfalfa indicated that females oviposited on the crop and resulting nymphs fed and developed on this crop. Based on occurrence of nymphs and adults over time, nymphal development time, ovarian development time, and reproductive status of adults (*N. viridula* only), *N. viridula* and *E. servus* nymphs developed into new adults in alfalfa, and the new females could produce nymphs on the crop.

Previous researchers reported that *E. servus* dispersed into seeding alfalfa, remained in the crop until plants matured or it was harvested, and then migrated into cotton (Russell 1952, Wene and Sheets 1964). If a second seed crop of alfalfa was produced in late summer or early fall, this stink bug dispersed from cotton into alfalfa. Similarly, *E. conspersus* only dispersed into cotton when alfalfa seed became unattractive due to hardening or until harvest (Toscano and Stern 1976). Density of nymphs was higher in seed alfalfa compared with cotton suggesting that alfalfa was a better host plant for nymphal development. Apparently, *Euschistus* spp. prefer seeding alfalfa to cotton. This preference of stink bugs for alfalfa over cotton and the ability of alfalfa to regrow after being cut suggests that this crop possibly could serve as a full-season trap crop for stink bugs in agricultural

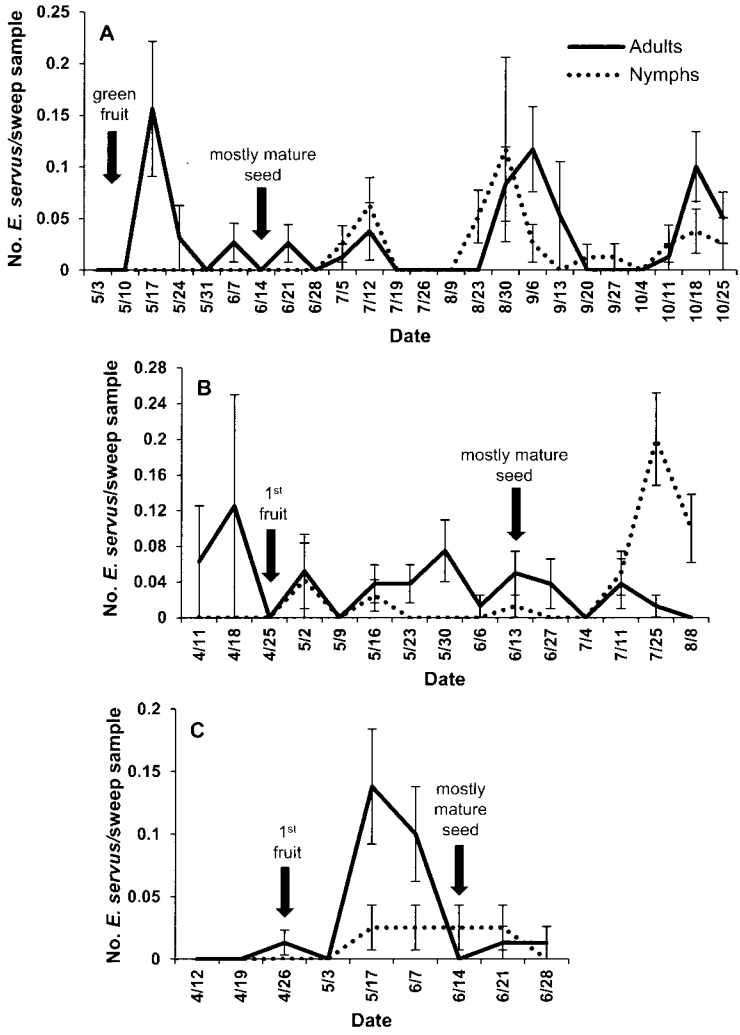

Fig. 2. Mean number of *E. servus* nymphs and adults per sweep sample in alfalfa for sampling dates in A) 2000, B) 2001, and C) 2002. Error bars represent SEM.

crops. In addition, alfalfa produces a large amount of nectar (Kropacova 1963, McGregor and Todd 1952) and thus could be a source of food to stink bug parasitoids on the farm.

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