IMPORTANCE OF NATURAL ENEMIES FOR STINK BUG CONTROL IN GEORGIA: 2007

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Introduction

A complex of stink bug species has become a very serious problem in Georgia cotton production. The problem is exacerbated by the widespread distribution of stink bugs across the landscape, the numerous host plants available to them for feeding and reproduction, and the difficulties associated with finding them in cotton and characterizing their damage. The dominant stink bug species in Georgia are the southern green stink bug, *Nezara viridula*, the green stink bug, *Acrosternum hilare*, and the brown stink bug, *Euschistus servus*. In addition to these species, several other species have become increasingly abundant including the red banded stink bug, *Piezodorus guildinii*, and *Euschistus quadrator*, both of which seem to be more abundant in the southernmost portions of the state (pers. observ.).

Various natural enemies have been reported attacking stink bugs in various regions of the world (e.g., Yeargan 1979, Jones 1988, Ehler 2002), but the natural enemy complex in the southeastern United States has been poorly defined. The purpose of this project is to characterize the suite of stink bug natural enemies present in Georgia and to determine their efficacy.

Methods

Parasitoid and Pathogen Survey. Cotton (Bollgard II, DPL434), Group 5 soybeans, and Group 7 soybeans were planted in Sumter County, Tift County, and Decatur County, Georgia. These crops were sampled regularly for stink bug populations (see Table 1 for sampling dates at each location), and all stink bugs collected in the samples were returned to the laboratory and held for parasitoid emergence. Collected bugs were held in 50 mL sample cups and provided with pieces of green bean pods and sunflower kernels as food. Bugs were checked daily for survival and parasitoid emergence. Bugs were held in an environmentally controlled rearing room at 24°C in the photoperiod of 14 hours. Dead bugs were dissected to evaluate the presence of pathogens and parasitoids. Bugs were considered to be parasitized if they met one or more of the following three criteria: (1) parasitoid egg(s) present on the bug cuticle, (2) parasitoid emerged from the bug, and/or (3) parasitoid immatures present in bug at the time of host death.

Predation of Stink Bug Egg Masses. In addition to assessing parasitism of nymphs and adults, egg masses were occasionally placed in the field to evaluate parasitism of

eggs. Available egg masses were placed in eight cotton plots, four of which were treated with hydramethylnon ant bait (Amdro®) to exclude the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae). Each plot was 0.5 acres. Egg masses were placed on the center row of each plot, with 1.5 m between placement sites, radiating out from the center of the plot. The number of egg masses placed and the duration of their tenures in the field varied among trials. All plots were planted with Bt cotton (DPL 555BR) on 4 June 2007. Plots were separated from one another by open gaps of 3 m of bare soil tilled at regular intervals. The plots were arranged in 4 blocks, each containing one *S. invicta* inclusion plot and one *S. invicta* exclusion plot. Plots were approximately square, and a 10x10m area in the center of each plot was designated for sampling. *Solenopsis invicta* exclusion plots were treated with Amdro at a rate of 1.1 kg of formulated bait per ha on 28 June, 16 July, 4 August, and 22 August 2007 to eliminate *S. invicta*. To assess the exclusion treatment, ant detection tests were conducted on 6 August and 2 September. This test consisted of placing three 33-ml test tubes containing a small piece (5 gm) of hotdog in each plot. After 1 hour all tubes were recovered and sealed, and transported back to the lab where the tubes were emptied and the number of ants was tallied.

Predation trials were conducted using egg masses of two species of stink bug: *P. guildinii*, and *N. viridula*. Eggs of *N. viridula* were obtained from a lab colony, whereas eggs of *P. guildinii* were obtained from field-collected adults. Three separate predation trials were conducted during the 2007 field season (11-14 July, 24-27 July, and 21-25 September); however, the number of eggs obtained for each trial varied due to inconsistent egg availability, causing the amount of replication used during the trials to fluctuate. During the 11 July trial a total of 8 egg masses of *N. viridula* were placed in cotton foliage of two treatment plots (four masses per plot) – one excluding ants and one including them. Thirty-two egg masses of *P. guildinii* were also placed in cotton foliage of the same two treatment plots (16 masses per plot). One egg mass was stapled to the lower surface of the uppermost, expanded leaf per cotton plant (total of 40 plants utilized). Five egg masses were placed on plants in each of 4 rows of cotton, which were separated from one another by three rows. One egg mass of *N. viridula* and four egg masses of *P. guildinii* were placed in each row. All egg masses were collected on 14 July and egg counts were not conducted between egg deployment and collection (3-day period). During the 24 July trial 20 egg masses of *N. viridula* were divided evenly among four plots (two ant exclusion plots and two inclusion plots). These eggs were similarly attached to the underside of the uppermost, fully expanded leaf. After deployment, these eggs similarly remained in the field for a 3-day period during which no egg counts were conducted until the final collection day (27 July). During the September predation trial, 34 egg masses were placed evenly among all eight treatment-plots (four or five per plot). Egg counts were then made at 1, 72 and 96 hours after all eggs had been deployed. The activity of predators on egg masses was observed during each egg mass count period. Predators were identified to species in the field and were recorded either preying upon or simply occupying egg masses.

Data Analyses. Survey results are reported without analysis at this point because we are still gathering data in the laboratory from more than 100 stink bugs that are still

alive. Due to the varying availability of stink bug egg masses data were analyzed using a one-way analysis of variance for each sampling period. Data from the 11 July sampling period were analyzed using one-way ANOVA (Proc GLM) with *S. invicta* status (presence or absence) as the treatment factor using SAS for Windows version 8. Data from the 24 July and 21 September sampling periods were similarly analyzed using one-way ANOVA (proc GLM); however, due to a high degree of variability across treatment plots, comparisons were made between plots from individual treatment blocks in order to search for significant differences that would not have been apparent across the entire field site.

Results and Discussion

Parasitoid and Pathogen Survey. A total of 1559 stink bugs of all life stages were collected in the survey, with the predominant species being *N. viridula* (Table 2), which accounted for 1165 of all individuals collected. The majority of bugs were collected from soybeans at each location because they were much more abundant in this crop than in cotton or peanuts. Overall parasitism of nymphal and adult bugs was low, and the majority of the parasitism (82.3%) was concentrated on the adult stage. Only 31 individuals were parasitized in the nymphal stages, and only in the 4^{th} and 5^{th} instars.

Parasitism of stink bug adults and nymphs was heavily dominated by a single species, the tachinid fly *Trichopoda pennipes.* This fly lays external eggs on the bugs, from which fly larvae bore into the host to become internal parasites. One adult specimen of *E. servus*, which had three fly eggs on its membranous wing, yielded an adult of the tachinid fly *Euthera tentatrix.* A possible third fly species was also obtained from a few bug specimens, based on differences in the puparial structure, but no adults emerged from these puparia. Two bugs were parasitized by an unidentified braconid wasp that produced a white cocoon. One of the bugs was collected on soybeans in Plains on 18 September as a fifth instar (*N. viridula*), from which the parasitoid emerged while the bug was still a nymph. The other wasp emerged from the adult stage of a *E. servus* collected as a fifth-instar nymph on soybeans in Tifton on 12 September.

Tachinid eggs on the bodies of the stink bugs were most commonly found on the ventral surface of the body, and most typically on the thorax (Fig. 1), with the total number per bug ranging from 1 to 7 eggs. As others have noted (e.g., McPherson et al. 1982), however, the presence of eggs may not be a particularly good indicator of parasitism, as many of the eggs fail to translate into larvae developing in the host (Fig. 2). In our study, of the 165 bugs encountered with external eggs only 52.7% produced parasitoids (including those that were dissected from hosts that died). However, the probability of successful parasitism increased with the number of eggs placed on a host, although the majority of bugs had only a single egg placed on them (Fig. 2). Further, an additional 28 bugs produced fly parasitoids without having external eggs on them -- about 1/3 as many as emerged from bugs with eggs on the integument. Some of these bugs may have been parasitized as nymphs, and could have lost the external egg during the molts preceding the adult stage. Regardless, external eggs are poor predictors of actual parasitism and mortality rates.

Male *N. viridula* were more heavily attacked by tachinids than were females, with 30.2% of males being parasitized compared to 22.7 females. This corresponds with what other studies have found, and appears to be due to parasitoid attraction to the sex pheromone released by males as they signal for mates (Harris and Todd 1980).

Two adult bugs were infected with an entomopathogenic fungus. Both were collected in soybeans. The first was an adult female *E. servus* collected in Tifton on 24 September. The second was an adult male *E. servus* collected in Plains on 10 October. Both individuals died in the lab, and dissections revealed dense mycelial mats occupying the abdomens of the cadavers. Both specimens were sent to Dr. Donald Steinkraus at the University of Arkansas for determination. Unfortunately, because the cadavers were not sporulating, Dr. Steinkraus was unable to give a definitive identification, but indicated that both specimens represented species of the fungal order Entomophthorales, an important group of entomopathogenic fungi. This represents the first record of fungal infection of *Euschistus* in North America, and may provide opportunities to further examine the pathogen in the future for developing biological control programs.

Predation of Stink Bug Egg Masses. Surprisingly, egg masses of *P. guildinii*, in cotton foliage suffered no attrition by predators, including *S. invicta*. Laboratory observations confirmed that although *S. invicta* workers investigated and manipulated egg masses of *P. guildinii* with their mandibles, they do not eat the eggs. Unlike the eggs of *P. guildinii*, eggs of *N. viridula* were observed in the field to be readily fed upon by *S. invicta* and the big eyed bug *Geocoris punctipes* (Hemiptera: Geocoridae). Actual egg removal rates, however, varied greatly among dates as well as treatment blocks. During the 11 July stink bug egg trial stink bug loss rates did not differ between ant exclusion plot one and ant inclusion plot one after three days ($F_{1, 6}$=2.95, P=0.13). During the 24 July trial there were similarly no differences between exclusion and inclusion plots for either block one or block two ($F_{1, 8}$=1.95, P=0.2; $F_{1, 8}$=3.6, P=0.09). Predation rates did, however, differ significantly during the September stink bug egg mass trial (Table 3). Although there were no significant differences in predation rates between inclusion and exclusion plots from blocks one, two or three, there were significantly more eggs absent from *S. invicta* inclusion plots 72 hours post-deployment ($F_{1, 8}$=6, P=0.04). At 96 hours post-egg deployment, significantly more eggs were found with their contents removed (shell left intact) in *S. invicta* exclusion plots than in inclusion plots ($F_{1, 8}$=5.69, P=0.04); however, total predation (proportion of eggs removed, emptied and chewed) was significantly higher in inclusion plots than exclusion plots ($F_{1, 8}$=11.52, P=0.009, Table 3).

This study is among the first to assess the impact of *S. invicta* predation on eggs of stink bugs (see also Krispyn and Todd 1982). Predation on stink bug eggs by *S. invicta* varied considerably among treatment blocks. Ehler (2002) observed that although predators readily fed upon nymphs of *N. viridula*, they rarely fed upon *N. viridula* eggs. In the current study we observed predation on eggs of *N. viridula* by both *S. invicta* and *G. punctipes*; however, eggs of *P. guildinii* were left untouched for the duration of egg predation trial (3 days). As noted above, the avoidance of *P. guildinii* eggs by predators

has also been observed in laboratory feeding trials (Ruberson, unpubl. data) and suggests that within the Hemiptera there may be defensive chemicals secreted onto the surface of eggs, some of which deter predation. Bundy & McPherson (2000) observed a great deal of variation in the surface architecture of stink bug eggs which may also influence the ability of predators to feed on the eggs of particular species. These factors may have strong implications for pest management given that *P. guildinii*, originally from South and Central America, appears to be expanding its range in the southern US, and is becoming a significant pest of US soybeans (Panizzi & Slansky 1985, J. Temple, Louisiana State Univ., personal comm.).

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Table 1. Stink bug sample dates and protocols for the respective locations, 2007.

Location	Dates sampled	Crops sampled	Sampling procedure
Attapulgus, Decatur Co., Georgia	25 July	Soybeans (Group V)	240 sweeps
	15 August	*Peanuts*	200 sweeps
		Cotton	200 sweeps
		Soybeans (Group V)	300 sweeps
	30 August	Cotton	300 sweeps
		Soybeans (Group V)	300 sweeps
	7 September	Cotton	300 sweeps
	20 September	Soybeans (Group VII)	280 sweeps
	9 October	Soybeans (Group VII)	500 sweeps
		Cotton	300 sweeps
		Peanuts	300 sweeps
Plains, Sumter Co., Georgia	27 July	Soybeans (Group V)	240 sweeps
		Cotton	480 sweeps
	1 August	Soybeans (Group V)	270 sweeps
		Soybeans (Group VII)	270 sweeps
		Cotton	400 sweeps + 10 m shakes
	7 August	Soybeans (Group V)	280 sweeps
		Soybeans (Group VII)	280 sweeps
		Cotton	300 sweeps + 10 m shakes
	14 August	Soybeans (Group V)	280 sweeps
		Soybeans (Group VII)	280 sweeps
		Cotton	300 sweeps + 10 m shakes
	21 August	Soybeans (Group V)	280 sweeps + 10 m shakes
		Soybeans (Group VII)	280 sweeps + 10 m shakes
		Cotton	500 sweeps + 10 m shakes
	28 August	Soybeans (Group V)	280 sweeps + 10 m shakes
		Soybeans (Group VII)	280 sweeps + 10 m shakes
	4 September	Cotton	442 sweeps
		Soybeans (Group V)	280 sweeps + 10 m shakes
		Soybeans (Group VII)	280 sweeps + 10 m shakes

	11 September	Soybeans (Group V)	280 sweeps + 10 m shakes
		Soybeans (Group VII)	280 sweeps + 10 m shakes
	18 September	Soybeans (Group V)	280 sweeps + 10 m shakes
		Soybeans (Group VII)	280 sweeps + 10 m shakes
	4 October	Soybeans (Group V)	500 sweeps
		Soybeans (Group VII)	300 sweeps
Tifton, Tift Co., Georgia	22 August	Soybeans (Group V)	250 sweeps
	29 August	Cotton	250 sweeps
	12 September	Soybeans (Group V)	250 sweeps
		Soybeans (Group VII)	250 sweeps
	19 September	Soybeans (Group V)	280 sweeps
		Soybeans (Group VII)	280 sweeps
	2 October	Millet	2 hours of searching heads
		Soybeans (Group V)	280 sweeps
		Soybeans (Group VII)	280 sweeps
	11 October	Soybeans (Group V)	280 sweeps
		Soybeans (Group VII)	280 sweeps
	23 October	Millet	2 hours of searching heads
	30 October	Millet	2 hours of searching heads

Table 2. Numbers of stink bugs collected, and number parasitized (in parentheses beneath), by location. Numbers are pooled across sample dates and host plants (cotton, soybeans, and millet).

Species	Life stage	Location			Totals
		Attapulgus	Plains	Tifton	
Nezara viridula	1st instar	0	8	0	8 (0)
	2nd instar	1	48	1	50 (0)
	3rd instar	0	86	8	94 (0)
	4th instar	20	74 (3)	64 (4)	158 (7)
	5th instar	6	207 (18)	143 (5)	356 (23)
	Adult male	15 (1)	147 (49)	85 (32)	247 (82)
	Adult female	3	168 (36)	81 (21)	252 (57)
Euschistus servus	2nd instar	0	7	0	7 (0)
	3rd instar	1	21	6	28 (0)
	4th instar	4	24	7	35 (0)
	5th instar	2	66	24 (1)	92 (1)
	Adult male	3	29	5	37 (0)
	Adult female	8	52 (1)	7	67 (1)
Acrosternum hilare	4th instar	0	18	5	23 (0)
	5th instar	0	5	2	7 (0)
	Adult male	0	20 (2)	0	20 (2)
	Adult female	0	19 (2)	0	19 (2)
Piezodorus guildinii	5th instar	2	0	3	5 (0)
	Adult male	7	0	3	10 (0)
	Adult female	2	0	9	11 (0)
Euschistus tristigmus	Adult male	3	0	4	7 (0)
	Adult female	7	2	3	12 (0)
Euschistus quadrator	Adult male	1	1	2	4 (0)
	Adult female	10	0	0	10 (0)

Table 3. Proportion (±SE) of *Nezara viridula* eggs preyed upon in fire ant inclusion and exclusion plots of each cotton block at 72 and 96 hours after eggs were initially deployed (on 24 July 2007). Predation type refers to the method by which eggs were fed upon. In cases where egg contents were removed the eggshell remained in place. Asterisks denote significant differences between inclusion and exclusion plots at the indicated time interval, based on one-way analysis of variance (ANOVA, *P<0.05).

Trial location	Proportion of eggs preyed upon at specified observation time:			
	Time since deployment (hrs)	Predation type	*Ant inclusion*	Ant exclusion
Block 1	72	Eggs removed	0.005 ± 0.005	0.019 ± 0.010
	96	Eggs removed	0.015 ± 0.010	0.013 ± 0.008
Block 2	72	Eggs removed	0.270 ± 0.240	0
	96	Eggs removed	0.039 ± 0.020	0
Block 3	72	Eggs removed	0.065 ± 0.060	0.035 ± 0.030
	96	Eggs removed	0.065 ± 0.060	0.047 ± 0.040
Block 4	72*	Eggs removed	0.600 ± 0.240	0.072 ± 0.070
	96*	Eggs removed	0.800 ± 0.200	0.081 ± 0.070
	96*	Only egg contents removed	0	0.013 ± 0.005

Fig. 1. Relative abundance and distribution of tachinid eggs on the bodies of parasitized stink bugs. Solid portions of the bars indicate the ventral surface, and stippled portions indicate the dorsal surface of the bug's integument.

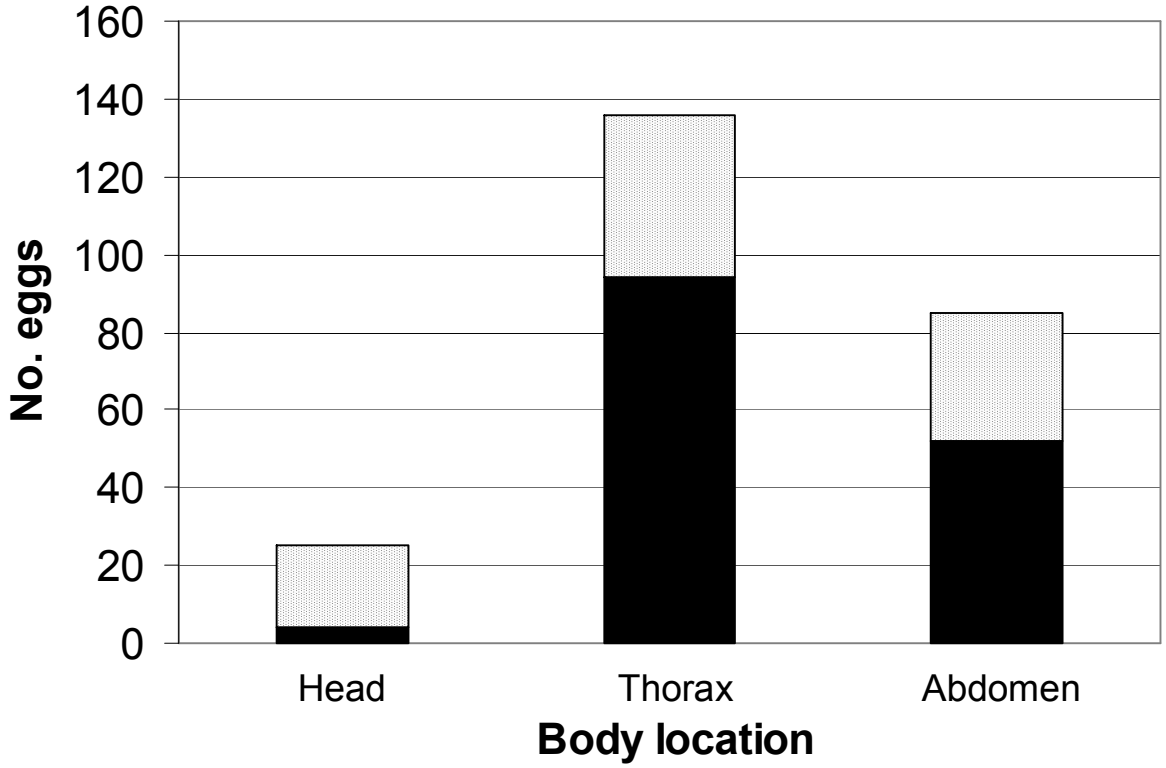

Fig. 2. Numbers of tachinid eggs per stink bug body (solid bars) in relation to successful stink bug parasitism (line). Parasitism is successful if a parasitoid was able to develop within the host to at least the second larval instar.