IMPORTANCE OF NATURAL ENEMIES FOR STINK BUG CONTROL IN GEORGIA

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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*, with the southern green stink bug generally dominating by a significant margin. 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. This project was initiated in 2007 to characterize the suite of stink bug natural enemies present in Georgia and to determine their efficacy. In 2007, we found that the parasitoid complex attacking stink bugs was primarily active against adult stink bugs, and had little impact on immatures. However, we obtained a few specimens of an unrecognized wasp from nymphs of the southern green stink bug and an adult brown stink bug. These studies were continued in 2008 to obtain further information on the role and diversity of stink bug natural enemies.

Materials and Methods

Parasitoid and Pathogen Survey. Cotton (Bollgard II, DPL143RF), Group 5 soybeans (DP5915R), and Group 7 (Asgrow H1242R) soybeans were planted in Sumter County (2 June), Tift County (16 June), and Decatur County (12 June), Georgia. These crops were sampled 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 with a photoperiod of 14:10 (L:D). 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 four criteria: (1) parasitoid egg(s) present on the bug cuticle, (2) parasitoid emerged from the bug, (3)

parasitoid immatures present in bug at the time of host death, and/or (4) the presence of a tracheal funnel in the stink bug, signifying that a parasitoid larva had completed development in the host and departed (see Fig. 1).

Predation of Stink Bug Egg Masses. In addition to assessing parasitism of nymphs and adults, egg masses of the southern green stink bug were placed in a set of eight 0.5-acre experimental cotton plots (DPL143B2/RF; planted 16 June) to evaluate egg predation and parasitism. Four of the plots were treated to exclude red imported fire ants, *Solenopsis invicta*. Egg masses were placed on plants in the center of the plot, with 2 m between placement sites, in a 2x3 or 2x4 layout (either 3 or 4 egg masses placed on each of the two rows). The number of egg masses placed in the field varied among trials (Table 3). 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 fire ant inclusion plot and one fire ant exclusion plot. Plots were approximately square, and a 10x10m area in the center of each plot was designated for sampling. Fire ant exclusion plots were treated with hydramethylnon ant bait (Amdro®) at a rate of 1.1 kg of formulated bait per ha on 18 June, 8 July, 22 July, 4 August, and 4 September 2008 to eliminate fire ants. To assess the exclusion treatment, ant detection tests were conducted on 8 July, 9 August, and 16 September. This test consisted of placing six 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 recorded.

Predation trials were conducted using southern green stink bug egg masses. The egg masses were obtained from a lab colony maintained on green bean pods and shelled sunflower seeds. Eggs were placed in the field on multiple occasions (see Table 3 for dates). Each egg mass was stapled to the lower surface of the uppermost expanded leaf. Three to four egg masses were placed on plants in each of two rows of cotton, which were separated from one another by six rows. All egg masses were collected after 72 hours of exposure to enemies. Egg counts were then made at 1, 6, 18, 24, 48, and 72 hours after all eggs had been deployed by digitally photographing each egg mass. This minimized disturbance of the egg mass and allowed us to make more accurate counts on the computer. The activity of predators at each observation period also was recorded on the digital images of the egg masses. Predators were identified to species in the field or from the images 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. We are also still processing the egg mortality data, so summary statistics are presented here for dates that have been processed.

Results and Discussion

Parasitoid and Pathogen Survey. A total of 1604 stink bugs of all life stages of four species were collected in the survey, with the predominant species being the southern green stink bug (Table 2), which accounted for 961 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 nymphs and adult bugs was low, and the majority of the parasitism (76.2%) was concentrated on the adult stage, as we found in 2007. Only 41 individuals were parasitized in the nymphal stages out of 751 nymphs collected (0.5%), and only in the 4^{th} and 5^{th} instars. In contrast, 120 out of 822 adults collected were parasitized (14.6%). Most parasitism was found in the southern green stink bug (6.3% of nymphs and 27.0% of adults). Parasitism was much lower in the other species collected.

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 1 to 10 eggs per host in the present survey), from which fly larvae bore into the host to become internal parasites. Nine bugs were parasitized by a braconid wasp that produced a white cocoon (Fig. 2), which has since been identified as *Aridelus rufotestaceus*, a species native to the Sino-Russian region (Shaw et al. 2001), and recorded for the first time in the Americas in the present studies. Two of these wasps were reared from stink bugs in 2007. The wasps in 2008 were obtained from stink bugs in soybeans in Tifton (5^{th} instar southern green stink bug; 10 October) and the remainder were collected in Plains (two 4^{th} instar southern green stink bugs, five 5^{th} instar southern green stink bugs, and one 5^{th} instar brown stink bug; all from 17-25 September). It is encouraging to encounter a few more in 2008 than in 2007, and in two locations, although all cases were found late in the season.

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. 3). The data also suggest that antagonism may occur among competing parasitoids if the number of eggs placed on a host is too high (e.g., >4), leading to reductions in successful parasitism. Further, an additional 26 bugs produced fly parasitoids without having external eggs on them (16.0% of all parasitism). 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 not particularly good predictors of actual parasitism and mortality rates.

Male southern green stink bugs were more heavily attacked by tachinids than were females, with 30.5% of males being parasitized compared to 23.8 of 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 Plains. Both were adult male brown stink bugs, one collected in cotton on 2 October and the other collected in soybeans on 25 September. 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. These observations and the two specimens collected in 2007 comprise 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. However, we must first identify the fungus and induce it to sporulate before we can conduct further studies.

Predation of Stink Bug Egg Masses. The Amdro treatments were effective in suppressing fire ant populations. Fire ants were found in 50 (76.3 ants per tube), 62.5 (95.9 ants per tube), and 70.8% (106.8 ants per tube) of the 24 tubes placed in the ant inclusion plots on 8 July, 9 August, and 16 September, respectively. In contrast, ants were obtained in only 1 out of 24 tubes on both 8 July and 9 August, and each tube contained a single ant. Predation of stink bug eggs by chewing predators after 72 hours ranged from 7.8 to 51.5% of all eggs in cotton plants with fire ants present (overall mean of 17.85%; Table 3). In contrast, predation by chewing predators in plots without ants ranged from 0 to 6.3% after 72 hours (overall mean of 0.20%). Sucking predators had limited impact on stink bug egg mortality, accounting for an overall mean of only 0.15% when fire ants were present, and 0.20% when they were absent (Table 3). Ant presence had no apparent effect on sucking predation. Several species of sucking predators were found feeding on egg masses, with the big-eyed bug *Geocoris punctipes,* dominating the complex. Other species that were observed feeding on stink bug eggs were the cotton fleahopper, *Pseudatomoscelis seriatus*; the plant bug *Spanagonicus* sp.; brown stink bug nymphs; and the big-eyed bug *Geocoris uliginosus*. Actual egg removal rates, however, varied greatly among dates as well as treatment blocks (Table 3), although the differences were more pronounced in the fire ant inclusion plots because of the much greater range of mortality in these plots.

This study is among the first to assess the impact of fire ant predation on eggs of stink bugs (see also Krispyn and Todd 1982). Predation on stink bug eggs by fire ants varied considerably among treatment blocks. Ehler (2002) observed that although predators readily fed upon southern green stink bug nymphs, they rarely fed upon the eggs. In the current study we observed predation on eggs of southern green stink bugs by fire ants, *G. punctipes*, larval green lacewings, *Chrysoperla rufilabris*, and several other species observed infrequently. Egg loss was quite variable, but it is obvious that fire ants are the most important predators of stink bug eggs, accounting for loss as high as 50% on one occasion.

The growth of conservation tillage in cotton may contribute to increased fire ant populations, and enhanced predation of stink bug eggs in cotton. Further, expanded distribution of *Aridelus rufotestaceus* may contribute to large-scale partial suppression, as this wasp is currently parasitizing about 20% of southern green stink bug nymphs in some areas of Italy (Shaw et al. 2001).

Acknowledgments

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Table 1. Stink bug sample dates and protocols for the respective locations, 2008.

Location	Dates sampled	Crops sampled	Sampling procedure
Live Oak, Florida	15 April	Potatoes, weeds	3 hours of searching plants
Attapulgus,	5 June	Peach trees (fruiting)	3 hours of searching plants
Decatur Co., Georgia	15 July	Soybeans (Group V)	300 sweeps
	24 July	Soybeans (Group V)	300 sweeps
	31 July	Cotton	300 sweeps
	20 August	Cotton	300 sweeps + 30 shakes
	16 September	Cotton	300 sweeps + 30 shakes
	26 September	Cotton	300 sweeps + 30 shakes
		Soybeans (Group VII)	300 sweeps
Plains,	23 July	Soybeans (Group V)	300 sweeps
Sumter Co., Georgia	30 July	Soybeans (Group V)	300 sweeps
	6 August	Soybeans (Group V)	480 sweeps
		Cotton (2nd week of flower)	320 sweeps + 16 shakes
	15 August	Soybeans (Group (V)	300 sweeps
		Cotton	320 sweeps + 16 shakes
	21 August	Soybeans (Group (V)	300 sweeps
		Cotton	320 sweeps + 16 shakes
	28 August	Soybeans (Group (V)	300 sweeps
		Cotton	320 sweeps + 16 shakes
	11 September	Soybeans (Group (V)	300 sweeps
		Cotton	320 sweeps + 16 shakes
	17 September	Soybeans (Group V/VII)	300 sweeps
		Cotton	320 sweeps + 16 shakes
	25 September	Soybeans (Group V/VII)	300 sweeps
		Cotton	320 sweeps + 16 shakes
	2 October	Soybeans (Group VII)	300 sweeps
		Cotton	320 sweeps + 16 shakes
	10 October	Soybeans (Group VII)	300 sweeps
		Cotton	320 sweeps + 16 shakes
	16 October	Soybeans (Group VII)	300 sweeps
		Cotton	320 sweeps + 16 shakes
Tifton,	29 April	Crimson clover	270 sweeps
Tift Co., Georgia		Wheat	450 sweeps
	2 May	Crimson clover	400 sweeps
	8 May	Crimson clover	400 sweeps
	27 May	Flowering wild mustard	300 sweeps
	29 May	Flowering wild mustard	400 sweeps
	31 July	Soybeans (Group V)	300 sweeps
	9 September	Soybeans (Group V)	300 sweeps
	17 September	Soybeans (Group V)	300 sweeps
	2 October	Soybeans (Group (V/VII)	300 sweeps each
	10 October	Soybeans (Group (V/VII)	300 sweeps each

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	2nd instar	16 (0)	8 (0)	9 (0)	33 (0)
	3rd instar	4 (0)	29 (0)	53 (0)	86 (0)
	4th instar	10 (0)	55 (1)	36 (1)	101 (2)
	5th instar	16 (0)	186 (31)	105 (1)	307 (32)
	Adult male	27 (13)	75 (31)	95 (16)	197 (60)
	Adult female	29 (10)	86 (32)	91 (7)	206 (49)
Euschistus servus	2nd instar	1 (0)	0	1 (0)	2 (0)
	3rd instar	0	4 (0)	2 (0)	6 (0)
	4th instar	1 (0)	6 (0)	3 (0)	10 (0)
	5th instar	7 (0)	38 (0)	24 (0)	69 (0)
	Adult male	24 (0)	17 (0)	18 (0)	59 (0)
	Adult female	22 (1)	25 (0)	25 (1)	72 (2)
Acrosternum hilare	3rd instar	3 (0)	5 (0)	3 (0)	11 (0)
	4th instar	0	5 (0)	15 (1)	20 (1)
	5th instar	5 (0)	23 (6)	44 (1)	72 (7)
	Adult male	9 (0)	17 (2)	40 (2)	66 (4)
	Adult female	3 (0)	21 (2)	28 (3)	52 (5)
Piezodorus guildinii	5th instar	0	0	34 (0)	34 (0)
	Adult male	24 (0)	0	55 (0)	79 (0)
	Adult female	42 (0)	0	49 (0)	91 (0)
					1573 (162)

Also collected stink bugs in Live Oak, Florida: 17 adult female *Nezara viridula* (1 parasitized), and 14 males (10 parasitized)

Table 3. Proportion (±SE) of *Nezara viridula* eggs preyed upon in fire ant inclusion and exclusion plots of cotton at 24, 48, and 72 hours after eggs were initially deployed. Predation type refers to the method by which eggs were fed upon. In cases where egg contents were sucked out, the eggshell remained in place. Chewed eggs were either removed, or fragments of eggshells were left attached to the substrate.

Trial start date/ No. egg masses	Proportion of eggs preyed upon (chewed/sucked out) at specified observation time:					
	Ants present			Ants absent		
	24 h	48 h	72 h	24 h	48 h	72 h
9 July N = 48	14.1/0	19.6/0	20.9/0	0/0	0/0	0/0.1
22 July N = 48	40.1/0	47.5/0	51.5/0	2.5/0	3.2/0	4.2/0.1
6 Aug N = 48	7.6/0	7.8/0	7.8/0	0/0	0/0	0/0
14 Aug N = 48	15.8/0	20.3/0	20.3/0	0.4/0	0.5/0	0.5/0
28 Aug N = 48	2.4/0.1	8.4/0.1	13.0/0.1	0.02/0	0.02/0	0.02/0
3 Sept N = 48	6.6/0	10.7/0	10.7/0	0.1/0/0	0.1/0	0.1/0.3
9 Sept N = 64	0/0.1	0.1/0.1	7.9/0.1	0.8/0,3	3.0/0.8	6.3/0.9
16 Sept N = 64	6.0/1.0	8.0/1.0	10.7/1.0	0.1/0.2	0.1/0.2	0.8/0.2
Mean % chewed	11.60	15.30	17.85	5.13	0.87	1.49
Mean % sucked out	0.15	0.15	0.15	0.06	0.13	0.20

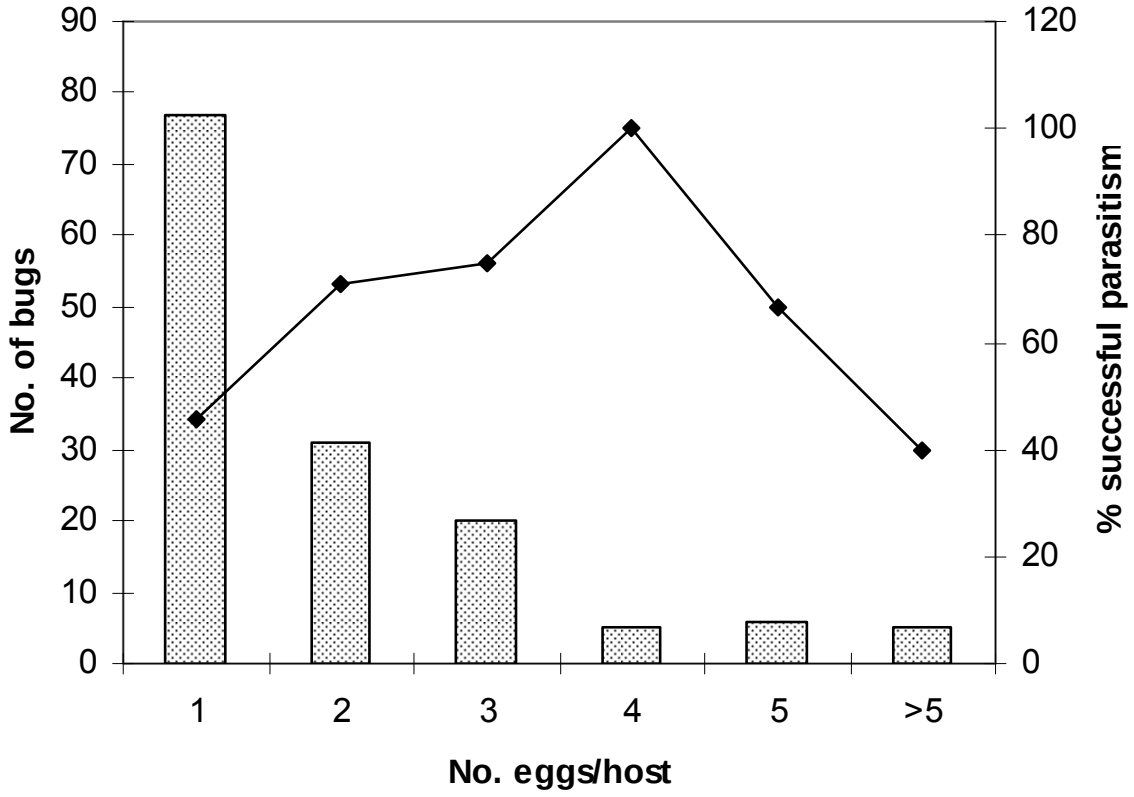

Fig. 3. Numbers of tachinid eggs per stink bug body (solid bars) in relation to successful stink bug parasitism (line). Parasitism is successful if a parasitoid was able to develop within the host to at least the second larval instar.