IMPORTANCE OF NATURAL ENEMIES FOR STINK BUG CONTROL

John R. Ruberson[1], Russell J. Ottens[1], Melissa D. Thompson[1], Scott R. Shaw[2] ,Dawn M. Olson[3], Scott Brown[4], Phillip Edwards[4], Edd Harrison[4], and Eddie McGriff[4]
[1]Department of Entomology, University of Georgia, Tifton
[2]Department of Renewable Resources, University of Wyoming, Laramie
[3]USDA-ARS-CPRMU, Tifton
[4]University of Georgia Cooperative Extension Service

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, the sporadic and unpredictable occurrence of their populations, 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 (but see below). 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. 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 exotic braconid wasp (*Aridelus rufotestaceus*) from nymphs of the southern green stink bug and an adult brown stink bug, *Euschistus servus*, in 2007 and 2008. These studies were continued in 2010 to obtain further information on the role and diversity of stink bug natural enemies.

Materials and Methods

Stink Bug Nymphal Mortality. A colony of Southern green stink bugs was established in the laboratory in Fall of 2009 and to a limited degree in Spring of 2010 that were to be used for placing eggs and nymphs in the field to evaluate mortality by predation, loss, and parasitism. The colony was maintained at 24 $\pm$ 1°C (L:D 14:10) with snap beans and shelled sunflower seeds as food sources, and water-saturated cotton for moisture. Forty whole-plant cages for the nymphal survival studies (20 total exclusion and 20 partial exclusion) were prepared using 50-gallon plastic garbage cans, with large openings cut in the garbage can frames. All openings on the total exclusion cages were

covered with fine mesh to exclude natural enemies. Half of the openings on the partial exclusion cages were covered with fine mesh. The partial exclusion cages were to serve as cage controls that allowed natural enemies to enter, while exposing the stink bugs to a cage environment. One acre of cotton (DP 143B2RF) each was planted in Attapulgus (Decatur County), Tifton (Tift County), and Plains (Sumter County) in May in preparation for the whole-plant cage studies of nymphal survival in cotton. Cotton plots were maintained using standard methods, but without use of insecticides.

The colony growth was much slower than expected due to low numbers of unparasitized bugs in the spring and throughout the summer. As a result, only a single set of stink bug nymphs was placed in the field – in Attapulgus, from 20-26 July. The cotton was squaring, with 11-12 nodes. Twelve plants were designated for the study, and adjacent plants were removed to discourage nymphs from moving from the target plant. Thirty 2nd-instar nymphs were placed on each plant on 20 July. Four plants were covered by total exclusion cages, four other plants were covered with partial exclusion cages, and the remaining four were left uncaged. Numbers of nymphs remaining on plants were counted on 26 July.

Parasitoid Surveys. Stink bugs of all life stages were surveyed weekly in corn, cotton, peanuts, and soybeans in selected fields in Coffee, Colquitt, Irwin, and Mitchell Counties. Beginning on 21 June with corn and peanuts, and adding cotton and soybeans on 6 July, and ending all sampling on 12 October, 12 commercial fields (three each of corn, cotton, peanuts, and soybeans) were sampled in each of two counties each week. Locations were alternated so that one week we sampled in Colquitt and Mitchell Counties, and the next in Coffee and Irwin. Twenty-four fields (6 of each crop) were sampled each week for stink bugs. Sampling was conducted along two transects in each field, each extending 120 meters into the field perpendicular to a tree line. Fifteen sample points were designated along each transect, with 10 samples in the first 60 meters (spaced at 6-meter intervals) and the remaining 5 points placed at 12–meter intervals. Sampling in corn consisted of visual examinations on each side of a 2-meter section of row at each sampling point. Samples in soybeans and cotton consisted of shake-cloth samples of 1.5-meter row sections at each point. Peanut was sampled with a Vortis suction sampler, sampling 2 meters of row at each sample location. Different rows were sampled at each sample date to reduce significant prolonged population disturbance. All stink bugs observed in samples were identified to species and stage, and returned to the laboratory where they were held with snap beans and shelled sunflower seeds to monitor for parasitism. All bugs from which no parasitoids had emerged were dissected upon death to assess the presence of mature parasitoid larvae (small larvae are much more difficult to detect).

Biology of the Parasitoid *Aridelus rufotestaceus*. Individual newly emerged female *A. rufotestaceus* were provided thirty 3rd-instar nymphs of the Southern green stink bug each day from the day of emergence until death. Nymphs were removed daily and placed individually in cups with pieces of bean pods and sunflower seeds for food. Fresh beans and seeds were provided every other day. Wasps and bugs were held at

25 ± 1°C, L:D 14:10, and were checked daily. Nymphs were checked for molting and for parasitoid emergence. Bugs that died prematurely were dissected and examined for the presence of parasitoid larvae to assess parasitism. Female parasitoids were checked daily for mortality. Offspring sex ratio was also evaluated.

A cohort of 60 Southern green stink bug nymphs for each of three instars (2, 3, and 4) was stung by female *A. rufotestaceus* and the stung nymphs were placed at 15 ± 1°C, L:D 14:10 to assess parasitoid development and survival at this temperature. Nymphs were placed individually in cups with pieces of bean pods and sunflower seeds for food. Fresh beans and seeds were provided every other day. Nymphs were checked for molting and for parasitoid emergence. Bugs that died were dissected and examined for presence of parasitoid larvae to assess parasitism.

Results and Discussion

Stink Bug Nymphal Mortality. No nymphs were recovered from the field study in any of the treatments 6 days after placement on plants. The cause of the complete loss of nymphs is unclear. Fire ants were found on 9 of the 12 plants, and may have been a major factor in the loss of nymphs. Other predators also were observed on inoculated plants (3 *Theridion* spiders, 2 *Cheiracanthium* spiders, 2 salticid spiders, 1 *Orius* nymph, and 1 *Geocoris punctipes* adult), but they were not as abundant as the ants. Temperature also may have played a significant role in stink bug mortality. The average daily high (in the plant canopy, measured with WatchDog® monitor) during the study was 99.8°F, and the canopy of the crop was still open, so heat may have been a problem for the nymphs. Because of limited stink bug numbers noted above, we were unable to run additional tests at this or the other locations, which might have better elucidated the results of this one trial.

Parasitoid Surveys. Seven stink bug species were collected, of which three were collected in sufficient numbers to yield meaningful numbers (Table 1). Southern green stink bug numbers were exceptionally low throughout the season, following high rates of parasitism on individuals of this species on spring weeds (personal observation).

The factors that led to the low populations are unknown, but the result was that Brown stink bugs predominated in all of the crop landscapes. A total of 1,757 bugs of all life stages were collected, of which only 31 were parasitized. As is typical of stink bug parasitism by tachinid flies, all parasitism was concentrated in the 5th instar and adult stages. The dominant parasitoid species of the Brown stink bug, *E. servus*, was *Cylindromyia binotata*, followed by *Euthera tentatrix*, and a single specimen of an unidentified tachinid fly species (believed to be a *Gymnosoma* sp.). All parasitism of the Southern green (*N. viridula*) and Green (*A. hilare*) stink bugs was by the tachinid *Trichopoda pennipes* and overall parasitism rates were low (1.2% for Green stink bugs and 5.8% for Southern green stink bugs). The parasitism rates observed for *E. servus* in this study (1.7% out of 1147, or 2.5% of the 753 5th-instar nymphs and adults) were low and similar to rates observed in other studies for this species. For reasons not entirely

clear, this stink bug species suffers little parasitism beyond the egg stage. Therefore, parasitoids of nymphs and adults probably have little impact on *E. servus* populations. Egg parasitoids and predators may, however, significantly impact populations of the brown stink bug. More work is needed to examine this possibility, particularly if brown stink bugs continue to be dominant pests.

Table 1. The most common bug species collected (by life stages) in each crop, and number parasitized (2010). Numbers are seasonal totals. All parasitoids were tachinid flies. Bugs were collected in Coffee, Colquitt, Irwin and Mitchell Counties.

	Nymphal Instars					
Crop	2	3	4	5	Adult	No. Parasitized (life stage)
Brown stink bugs, *Euschistus servus*						
Corn	19	12	19	19	75	2 (Ad)
Cotton	20	11	6	11	88	6 (Ad)
Peanuts	15	35	24	18	200	4 (Ad)
Soybeans	67	83	83	136	206	1 (N5), 6 (Ad)
Total	121	141	132	184	569	19
Southern green stink bugs, *Nezara viridula*						
Corn	0	8	8	3	7	3 (Ad)
Cotton	3	11	4	2	3	1 (N5), 1 (Ad)
Peanuts	13	11	0	3	2	0
Soybeans	1	8	5	3	9	1 (Ad)
Total	17	38	17	11	21	6
Green stink bugs, *Acrosternum hilare*						
Corn	0	1	0	0	0	0
Cotton	32	12	8	12	24	1 (Ad)
Peanuts	0	1	1	0	2	0
Soybeans	108	87	48	89	81	2 (N5), 3 (Ad)
Total	140	101	57	101	107	6

Biology of the Parasitoid *Aridelus rufotestaceus*. The average longevity of female *A. rufotestaceus* was 23.3 ± 14.45 days (at 25°C), and average fecundity was 141.7 ± 57.06. Interestingly, almost 90% of offspring were produced in the first 10 days after female adult emergence (Fiure 1). Therefore, females continue to live for almost two weeks after they have produced the majority of their offspring. Sex ratio of offspring was 98.4% female, characteristic of thelytokous parasitoids and corresponding with the preponderance of females observed in field collections and rearing. The few males that have been observed appear to be non-functional. They make no effort to mate with females, nor is mating necessary for females to produce viable offspring. Most of the sons were produced after the adult females had produced most of their offspring, suggesting that some factor changes later in life, affecting the sex ratio. Sex ratios of thelytokous wasp species are often regulated by bacterial symbionts (Stouthamer et al.,

1999), and it is possible that as the female wasp ages that the bacterial symbiont level declines, allowing more sons to be produced (Van Meer 1999).

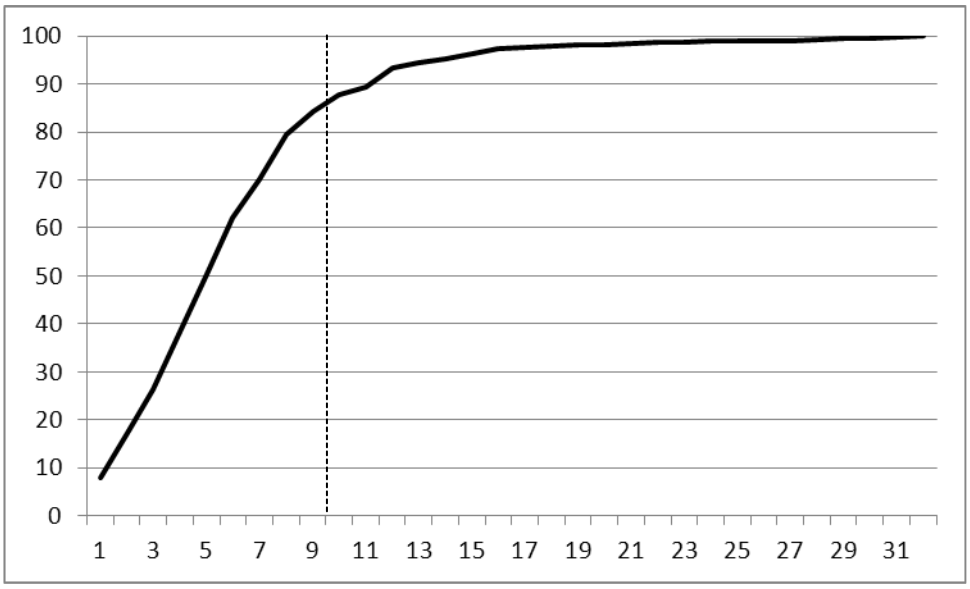

Figure 1. Cumulative percent progeny production (y axis) of female *Aridelus rufotestaceus* in relation to female adult age (25 ± 1C). Vertical dashed line indicates 10 days after adult

No parasitoids emerged at 15°C from any of the nymphal instars. Rather, all stink bug nymphs died within 24 days of being placed in the chamber. The Southern green stink bug appears to be limited in its geographic range by poor tolerance to low temperatures, and it appears that constant 15°C is too low to support survival of the bugs in immature stages. Although many of the stink bugs were successfully parasitized at 15°C (based on dissections of dead bugs), the inability of the hosts to survive at this temperature likely also limits the development and survival of the parasitoid. The prolonged development times of this wasp in a more suitable temperature range for the stink bugs (outlined in previous studies) of ca. 62 days at 20°C, 40 days at 25°C, and 35 days at 30°C limit the population growth rate of the parasitoid on the one hand, but also synchronize the wasp's development very well with the development of the southern green stink bug so that the host is in the proper stages when adult parasitoids emerge. Further, the parasitoid nearly always kills the host before the host can reach the adult stage and reproduce, unlike the adult parasitoids that have been the focus of many biological control studies targeting stink bugs. Therefore, the wasp may make valuable contributions to reducing stink bug populations if the wasp populations are sufficiently abundant and widespread. The question that is unclear is how abundant these parasitoids are in Georgia. Surveys have suggested that they are relatively rare, but many stink bug nymphs die after field collections, and some or many of the nymphs that die may be parasitized.

The importance of natural enemies in managing stinkbug populations is still unclear, although there are hints they may be very important. For example, the complete disappearance of the stink bug nymphs in cotton six days after inoculation suggests that there are factors that are limiting growth of stink bug populations at least in pre-squaring cotton. Similarly, Southern green stink bugs were common in the spring of 2010, but their populations diminished significantly by mid-summer. These dramatic population shifts may be due to enemies or weather, a combination of both, or other yet unclear factors. However, if these same factors are at work in other crops and non-cultivated hosts, they may exert considerable influence on stink bug populations and their manipulation may hold promise for managing these pests.

Acknowledgements

We gratefully acknowledge the assistance of Melissa Thompson, Russ Ottens, Casey Jo Huang, Robin Padick, Tiffany Beck, Rebekah Estes, and Patrick Strickland in conducting these studies.

References

Ehler L.E. 2002. An evaluation of some natural enemies of *Nezara viridula* in northern California. Biocontrol 47:309-325.

Jones, W.A. 1988. World review of the parasitoids of the southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae). Ann. Entomol. Soc. Am. 81:262-273.

Stouthamer R, Breeuwer AJ, HurstGDD.1999.*Wolbachia pipientis*: microbial manipulator of arthropod reproduction. *Annu. Rev. Microbiol.* 53:71–102

Van Meer, M.M.M. 1999. Phylogeny and host symbionts interactions of thelytoky inducing *Wolbachia* in Hymenoptera. PhD Thesis, Wageningen University, Wageningen, The Netherlands. 117 pp.

Yeargan, K.V. 1979. Parasitism and predation of stink bug eggs in soybean and alfalfa fields.