IMPORTANCE OF NATURAL ENEMIES FOR STINK BUG CONTROL

John R. Ruberson[1], Melissa D. Thompson[1], James D. Harwood[2] and Kacie J. Athey[2]
[1]Department of Entomology, University of Georgia, Tifton
[2]Department of Entomology, University of Kentucky, Lexington, KY

Summary

Studies were conducted to characterize which predators in cotton and soybeans attack Southern green and Brown stink bugs. It is relatively easy to demonstrate loss of stink bugs in cotton and other crops, but it is far more complex to determine who or what is responsible for the loss so that the appropriate natural enemies can be conserved. If we can determine particular natural enemy species that are especially active in consuming stink bugs, then we can target those natural enemies for conservation or enhancement. We applied molecular methods to address this issue. DNA primers were developed at the University of Kentucky to allow us to assay the gut contents of predators for the presence of stink bug DNA. This provides positive evidence for predation, and allows us to determine which predator species are feeding on stink bugs in the field. Two primers were developed – one for the Southern green stink bug, *Nezara viridula,* and the other for the Brown stink bug, *Euschistus servus*. We evaluated stink bugs and predators in three crops: 1) cotton (B2RF), 2) soybeans MG5 and 3) soybeans MG7. All three crops were planted at three locations: the Belflower Farm, Tifton, GA; the Attapulgus Research and Education Center, Attapulgus, GA; and the Southwest Research and Education Center, Plains, GA. All crops were sampled by sweep net and all arthropods taken in the samples were counted, and all predators and stink bugs were placed in 100% ethanol in preparation for DNA testing. Collected samples were sent to the University of Kentucky for processing. Due to the high number of specimens needing to be processed, we are still running DNA analyses and, therefore, we can only present preliminary conclusions here. All assays should be completed by early March, but thus far 629 out of the 1,873 collected predators have been assayed for the presence of stink bug DNA. So far, five predator species have tested positive for stink bug DNA – four species for Southern green stink bugs, and only one (an assassin bug, *Zelus* sp.) tested positive for Brown stink bug. The species positive for Southern green stink bug were the big-eyed bug *Geocoris punctipes*, the pirate bug *Orius insidiosus*, the maculate lady beetle *Coleomegilla maculata*, and the hooded beetle *Notoxus monodon*. Of these species, the big-eyed bugs had the highest populations in the field, of which 4.5% were positive for stink bug DNA. Hooded beetles were less common, but 6.8% of them tested positive. This is a new record for stink bug predation by this species, and provides direction for future work. Only 1.9% of pirate bugs were positive. Forty percent of the maculate lady beetles were positive, but only five individuals have been assayed so far, and this species was relatively uncommon in the field. Our preliminary data indicate that predation of stink bugs is occurring, but perhaps at low rates, and that the rates of predation may differ with stink bug species (Southern green higher than Brown).

Completion of the assays will provide us much more insight into the role of predation and which predator species are most important for stink bug suppression.

Introduction

A complex of stink bug species has become a serious and persistent problem in Georgia cotton production. The problem is exacerbated by the widespread distribution of stink bugs across the landscape and their mobility, 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, *Chinavia hilaris*, and the brown stink bug, *Euschistus servus*, with the Southern green stink bug historically dominating by a significant margin, followed by the Brown stink bug. 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.), and the former appears to have limited interest in cotton, feeding chiefly in soybeans.

Various natural enemies have been reported attacking stink bugs in different regions of the world (e.g., Yeargan 1979, Jones 1988, Ehler 2002, Eubanks 2001) and in some cases have been found to be very important (e.g., Kiritani 1964, Nishida 1966), but the natural enemy complex in the southeastern United States has been poorly defined. This overall project was initiated in 2007 with the support of the Georgia Cotton Commission and Cotton Incorporated to characterize the suite of stink bug natural enemies present in Georgia and to determine their efficacy. In previous studies we found that the parasitoid complex attacking stink bugs was primarily active against adult stink bugs, and had little impact on immatures. We also found previously that the eggs of stink bugs are susceptible to predation in cotton, but that predation was not particularly high (typically less than 25%). In the present study we are applying molecular techniques to field sampling to determine which predators in the field are consuming stink bugs in cotton. Once we have a better idea of which predators attack stink bugs in cotton, we can pursue in more detail the stink bug life stages they attack, and better determine how much of an impact they are having. This study represents a significant step in that direction. The project is still underway due to logistical bottlenecks that slowed progress. As a result, what is presented here will by necessity be a preliminary report of the progress and outcomes.

Materials and Methods

Primer Development: DNA primers were developed at the University of Kentucky to examine the gut contents of stink bug natural enemies for the presence of stink bug DNA indicating predation on stink bugs by the assayed predator. Specimens of the

Southern green stink bug (*Nezara viridula*) and the Brown stink bug (*Euschistus servus*) were collected from lab colonies and various field locations in Tifton, GA, in May 2011. In addition, we collected 127 non-target species from the field locations and transported them to the University of Kentucky for further processing. Each specimen was preserved in the field in 95% ethanol and stored in the freezer until extraction.

DNA was extracted from all specimens using Qiagen DNeasy Blood and Tissue Kits©. Following extraction, *N. viridula, E. servus* and non-target DNA was amplified using general 16S primers 16Sbr-H (5'- CCG GTC TGA ACT CAG ATC ACG T -3') and 16Sar-L (5'- CGC CTG TT ATC AAA AAC AT -3'). Following amplification, the bands were visualized on 2% agarose gels. The PCR product was then sent off for sequencing at AGTC (University of Kentucky, Lexington, KY). Sequences were editing using Geneious© (Biomatters Ltd) and aligned using MUSCLE (http://www.ebi.ac.uk/Tools/msa/muscle/). Primer design occurred using Primer3 (http://frodo.wi.mit.edu/). Upon receiving the primers, targets and non-targets were amplified using a temperature gradient to determine melting temperature. Following this, the primers were tested against a variety of non-targets. The primers were used to identify stink bug species in the gut contents of predators once specificity was established. Predators could be assayed for both stink bug species simultaneously.

Field Sampling: In order to obtain a broad base of information on stink bug predators, we used three crops for the studies: 1) cotton (DP1034B2RF), 2) soybeans MG5 (Asgrow 568RR) and 3) soybeans MG7 (Asgrow AG6931RR). All three crops were planted at three locations: the Belflower Farm, Tifton, GA (on 2 June 2011); the Attapulgus Research and Education Center, Attapulgus, GA (on 31 May 2011); and the Southwest Research and Education Center, Plains, GA (on 6 June 2011). The initial planting of cotton and soybeans did not received adequate water, and they were re-planted on 17 June. Aldicarb was applied in furrow at planting at 3.93 kg/ha (3.5 lbs/acre). No other insecticides were applied to the crops throughout the season. On each sample date we made 200 sweeps per crop plot (along two different rows in the plot separated from one another by 6 rows) with a 31 cm diameter net (15” diameter). Sweeping was initiated 5 meters into the crop and along rows at least 5 rows from the plot edge to reduce edge effects. Different rows were sampled on each sample date to prevent prolonged disruption of sampling rows. All arthropods taken in the samples were counted, and all predators and stink bugs were placed in 100% ethanol in preparation for DNA testing. Collected samples were sent to the University of Kentucky for processing. Sampling was initiated in Attapulgus and Plains on 29 July, and on 18 August in Tifton, and was conducted approximately weekly (weather permitting). Sampling in all three locations was terminated by 7 October. Due to the high number of specimens needing to be processed, we are still running DNA analyses and, therefore, we can only present preliminary conclusions here. All assays should be completed by early March.

Results and Discussion

DNA Primer Development and Predator Assays. The *N. viridula* primers were NV-334F:5'- TTTTTATTATTTATTTGGGTTG-3'and NV-566R: 5'-GTCGAACAGACCTAGAAC-3'. The *E. servus* primers were ES-43F: 5'-GTCTGATGTTATTTATATCAGATTTAA-3' and ES-295R: -5'-AATAAATATTAACAATTTAACCAAAAC-3'. Once specificity was established, we tested predator gut contents for presence of *N. viridula* and *E. servus* DNA, indicating predation on these species. The bands were visualized on a 2% agarose gel to determine presence of either species of stink bug in the gut of the predator. To date, 629 (out of the 1,873 collected; see below) Arthropods have been assayed from the three crop treatments. Predators from the following sample dates have been assayed thus far: 29 July, 26 August, 8 September, 12 September, and 16 September. Table 1 presents the results to date for predator groups in which positive results were obtained.

It is clear from Table 1 that the frequency of predation on stink bugs in the assayed Arthropods was low. This is not surprising, given the overall low populations of stink bugs observed in the fields for most of the season and locations. However, it is also apparent that predation is occurring and that a complex of species is responsible. The highest number of positive responses was in the big-eyed bug, with 5 of 112 bugs testing positive for the presence of Southern green stink bug DNA so far. This was no surprise, as we and others (Ragsdale et al. 1981, Stam et al. 1987) have previously observed big-eyed bugs feeding on stink bug eggs in the field. However, this is a first record for stink bug predation by hooded beetles, and the positive rate for this species was relatively high. It is unclear which stage(s) the hooded beetle attacked, but this observation warrants additional study as hooded beetles can be very common in cotton fields.

Only a single positive predation event has been found for the Brown stink bug thus far, and that was in an assassin bug, and is a first record for *Zelus* spp. attacking stink bugs in the US. It is possible that the disparity in positive response between the Southern green stink bug and Brown stink bug is due to differential population sizes for the two species (with the Brown stink bug being less abundant), but this cannot be clarified until all of the samples are processed. However, the Brown stink bug appears to be much less susceptible to adult parasitoids than is the Southern green stink bug, and it is possible that this differential susceptibility also may extend to predators of the Brown stink bug. We will be able to address this more clearly when the specimens are all examined.

Table 1. Predators surveyed to date and frequency of positive responses.

Predator species	No. evaluated	No. positive	% positives	Stink bug species
Geocoris punctipes – Big-eyed bug	112	5	4.5	*Nezara viridula*
Coleomegilla maculata – Spotted lady beetle	5	2	40	*Nezara viridula*
Notoxus monodon – Hooded beetle	44	3	6.8	*Nezara viridula*
Orius insidiosus – Pirate bug	53	1	1.9	*Nezara viridula*
Zelus sp. – Leafhopper assassin bug	10	1	10	*Euschistus servus*

Stink Bug and Predator Surveys. Stink bug populations and species were highly variable among locations. Populations in all crops were relatively low until September, when populations in soybeans increased, especially in Attapulgus (Fig. 1). The Southern green stink bug was the most abundant, although its numbers were low throughout most of the season in most plots. The Green stink bug, *Chinavia hilaris*, was also observed with regularity, but at lower numbers than either the Southern green or Brown stink bugs, and it is not shown here. Further, we did not develop primers to assess predation on Green stink bugs.

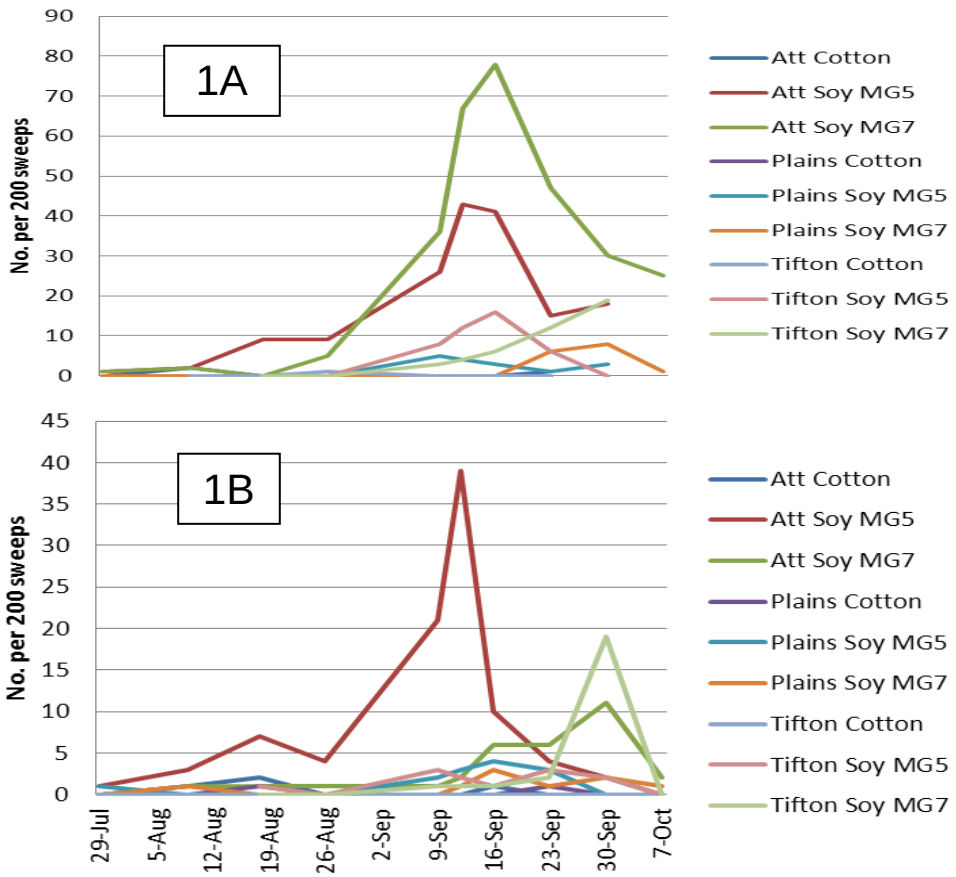

Fig. 1. Abundance of Southern green stink bugs (A) and Brown stink bugs (B) (nymphs and adults) in relation to location and crop. Note the different scales on the y-axes.

We collected a total of 1,873 predators over the period from 29 July to 7 October 2011.Of these, 578 were collected in cotton, 667 in MG5 soybeans, and 569 in MG7 soybeans. Total numbers by crop and location are presented in Fig. 2. The predator complex was dominated by spiders (510 collected), followed by big-eyed bugs (*Geocoris punctipes* and *Geocoris uliginosus*; 327 collected), and pirate bugs (*Orius insidiosus*; 221 collected). Of the two relatively abundant species with the highest positive rates for stink bug DNA, big-eyed bugs were more abundant later in the season, coinciding with the increased stink bug populations in all crops, whereas the hooded beetles were of varying abundance throughout the season. Spiders were abundant throughout the season, but few spiders have been assayed for stink bug DNA as of the present date.

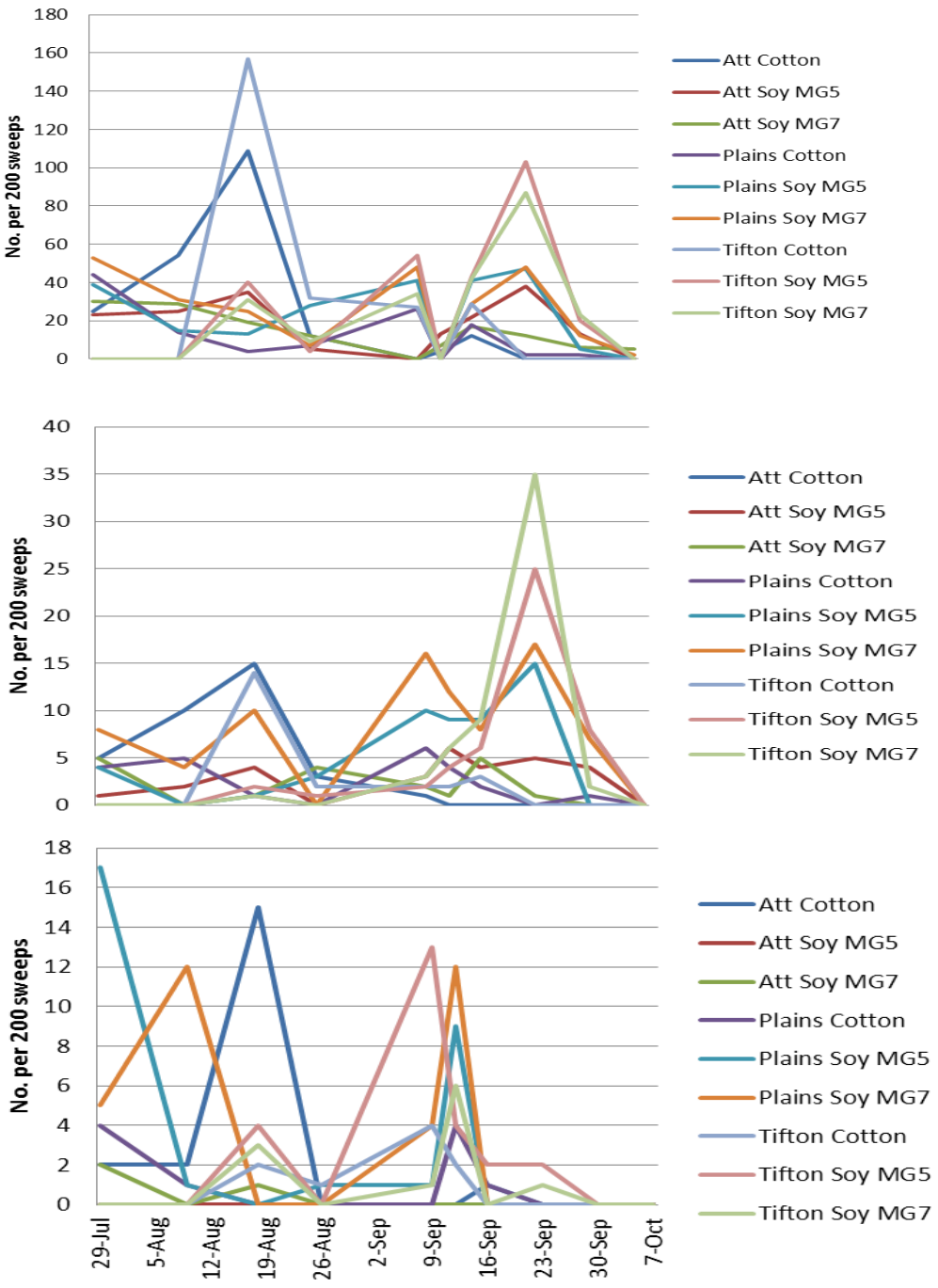

Fig 2. Number of all (Fig. 2A) predatory Arthropods, (Fig. 2B) big-eyed bugs (*Geocoris* spp.), and (Fig. 2C) hooded beetles (*Notoxus monodon*) by location and crop type throughout the sampling period, 2011. Note the different scales on the y-axes.

Conclusions

Although our data are preliminary, it is clear that some abundant predators in cotton attack Southern green and Brown stink bugs, although predation on Brown stink bugs may be reduced relative to that of Southern green stink bugs. Relative predation should be clarified with additional assays. The observation that hooded beetles fed on stink bugs is novel, and provides fodder for additional studies. This beetle is commonly observed in cotton and is known to be a generalist predator, but there has been a lack of clarity on its target prey. The frequency of consumption of stink bugs by big-eyed bugs also is promising, as these predators tend to increase late in the season when stink bugs are building in cotton, and can be quite abundant. Additional studies will elucidate life stages attacked by positive predators and the extent of attack on these life stages. The use of DNA to identify predators of stink bugs is a promising approach to addressing this thorny issue.

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