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# Ecology and Management of Stink Bugs (Hemiptera: Pentatomidae) in Southeastern Farmscapes

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ECOLOGY AND MANAGEMENT OF STINK BUGS (HEMIPTERA:  
PENTATOMIDAE) IN SOUTHEASTERN FARMSCAPES

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A Dissertation  
Presented to  
the Graduate School of  
Clemson University

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In Partial Fulfillment  
of the Requirements for the Degree  
Doctor of Philosophy  
Entomology

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by  
Grant Lewis Pilkay  
August 2013

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Accepted by:  
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## ABSTRACT

A three-year study (2009-2011) was conducted to examine the spatial and temporal dynamics of stink bugs in three commercial farmscapes in South Carolina and Georgia. Crops included wheat, *Triticum aestivum* (L.), corn, *Zea mays* (L.), soybean, *Glycine max* (L.), cotton, *Gossypium hirsutum* (L.), and peanuts, *Arachis hypogaea* (L.). Farmscapes were sampled weekly using whole-plant examinations for corn, with all other crops sampled using sweep nets. The predominant pest species of phytophagous stink bugs were the brown stink bug, *Euschistus servus* (Say), the green stink bug, *Chinavia hilaris* (Say), and the southern green stink bug, *Nezara viridula* (L.). Chi-square tests indicated a departure from a normal distribution in 77% of analyses of the variance to mean ratio, with 37% of slopes of Taylor's power law and 30% of coefficient  $\beta$  of Iwao's patchiness regression significantly greater than one, indicating aggregated distributions. SADIE indices indicated aggregated patterns of stink bugs in 18% of year-end totals and 42% of weekly counts, with 80% of adults and nymphs positively associated using the SADIE association tool. Peak stink bug densities were linked to crop phenology, following the fruiting pattern of crops in the farmscape. Stink bugs exhibited higher densities in crops adjacent to soybean in Barnwell and Lee Counties, SC, compared with crops adjacent to corn or fallow areas.

Efficacy of applications of insecticide limited to the borders of fields to mitigate injury by stink bugs in cotton was evaluated from 2007 to 2011 in South Carolina and Georgia. Stink bug densities and boll injury were greater around the exterior compared with the interior portions of fields based on ANOVA models and interpolation maps of

SADIE aggregation indices. Border and whole-field applications had no significant effect ( $P > 0.05$ ) on average numbers of stink bugs, but boll injury was significantly lower ( $P < 0.05$ ) in both border and whole-field insecticide treatments compared with untreated controls. No significant difference ( $P > 0.05$ ) was found between injury levels in fields receiving border or whole-field treatments. Fields receiving no insecticide treatments exceeded economic thresholds 55% of the time. Fields receiving whole-field and border applications of insecticide exceeded thresholds 41% and 30% of the time, respectively. Treated area was 4.4-fold smaller in fields receiving border applications than in fields receiving whole-field applications, indicating substantial savings in insecticide. Results suggested that border treatments of insecticides provided protection from stink bug injury similar to whole-field insecticide treatments, but with considerable savings in application costs.

Studies of stink bugs in the field could be improved if movement could be monitored in real time. Harmonic radar tagging was investigated as a method for monitoring the movement of *N. viridula*. Because adhesive toxicity and tag weight limit the use of this technology, initial efforts focused on selection of the optimal adhesive and design of harmonic radar tags to reduce influence on movement of stink bugs. A design consisting of a 6-cm long 0.10-mm thick silver-plated copper monopole on the anode terminal of a three-contact Schottky barrier diode attached with a rubberized cyanoacrylate (Gorilla super glue) provided a compromise between unimpaired movement and tracking range, adding an additional 8% to the weight of the stink bug while not significantly ( $P > 0.05$ ) reducing walking or flying mobility in the laboratory.

Recovery of tagged stink bugs in cotton and fallow fields ranged from 10-75% after 24 hours, while marked stink bugs were recovered at rates of 0-35% using sweep-net or drop-cloth sampling. The distance dispersed in the field was not influenced ( $P > 0.05$ ) by crop, tagged status, or gender of the insect. Future research should examine improvements to the harmonic radar transceiver and the wire antenna to decrease encumbrance.

Laboratory studies were conducted to determine host preference of the tachinid parasitoid fly *Trichopoda pennipes* (F.) for *E. servus* and *N. viridula*. In choice and no-choice tests, 8-fold fewer eggs were laid on *E. servus*, compared with *N. viridula*. Twenty-four *T. pennipes* emerged from 100 *N. viridula*, whereas only two larvae emerged from 100 laboratory-parasitized *E. servus*. Post-mortem dissections of egg-bearing stink bugs without larval emergence revealed 20 *T. pennipes* larvae inside *N. viridula* but only one inside *E. servus*. These results confirmed that *T. pennipes* prefers *N. viridula* as a host and is likely an infrequent parasitoid of *E. servus*.

While gathering *T. pennipes* for the selection trials, *Cylindromyia euchenor* (Walker), previously found in *E. servus*, was collected. Unlike most tachinids, which deposit eggs on or near the hosts, members of the genus *Cylindromyia* have an ovipositor formed from an abdominal sternite, which, assisted by serrated curved claspers, implants eggs directly into hosts. No research has been done on the behavior or host preferences of *C. euchenor*. My observations were limited to three females over approximately two weeks. Female parasitoids directly injected eggs into *E. servus* exclusively, ignoring *N.*

*viridula*. The sequence of oviposition was recorded and described, demonstrating the ovipositional behavior for the first time and indicating a host preference for *E. servus*.

## DEDICATION

I would like to dedicate this work to the past, present, and future generations of my family who made it possible for me to do what hadn't been done before.

“A human being should be able to change a diaper, plan an invasion, butcher a hog, conn a ship, design a building, write a sonnet, balance accounts, build a wall, set a bone, comfort the dying, take orders, give orders, cooperate, act alone, solve equations, analyze a new problem, pitch manure, program a computer, cook a tasty meal, fight efficiently, die gallantly. Specialization is for insects.”

- Robert A. Heinlein

## ACKNOWLEDGMENTS

Thanking everyone involved in this endeavor would take a great deal more space than I have available, but I will try. First, I would like to thank my adviser, Dr. Francis Reay-Jones, who gave me the opportunity to advance my education and professional experience. I would also like to thank the members of my committee, Dr. Jeremy Greene and Dr. Peter Adler of Clemson University, and Dr. Michael Toews of the University of Georgia, who provided substantial assistance and advice. Thank you also to Dr. William Bridges of Clemson's Department of Mathematical Sciences who helped make sense of "what all those numbers mean". At Clemson, Mrs. Tammy Morton was essential in helping me have all the forms, papers, and statements required completed on time. She was the first person I met at Clemson, and I would never have been able to navigate the convoluted maze of the university forms system without her help. A very special thanks goes to Dr. William Bruce Ezell, Jr of UNC Pembroke. Dr. Ezell was the instructor of my first Entomology course when getting my Master's degree in Science Education. He encouraged me to advance my education to the highest levels and was first to suggest Clemson University's Entomology program when I expressed interest in doing so. Without his help and support, I would not have been able to accomplish the vast majority of what I set out to do.

My biggest thanks go to my family and friends, who put up with a constant barrage of panic, speculation, and my own weapons-grade paranoia while somehow managing to offer support and prayers. Thank you, everyone for giving me the flexibility to go to school out-of-state. It would not have been possible without you.



## TABLE OF CONTENTS

	Page
TITLE PAGE .....	i
ABSTRACT .....	ii
DEDICATION .....	vi
ACKNOWLEDGMENTS .....	vii
LIST OF TABLES .....	xi
LIST OF FIGURES .....	xii
PREFACE .....	xiv
 CHAPTER	
I. INTRODUCTION .....	1
General introduction to stink bugs .....	1
Taxonomy .....	1
Biology overview .....	2
Stink bug species of economic importance .....	5
Primary economic species in the southeastern U.S. ....	5
Southern green stink bug <i>Nezara viridula</i> (L.) .....	5
Green stink bug <i>Chinavia hilaris</i> (L.) .....	7
Brown stink bug <i>Euschistus servus</i> (Say) .....	9
Secondary economic species in the southeastern U.S. ....	10
Rice stink bug <i>Oebalus pugnax</i> (F.) .....	10
Redshouldered stink bug <i>Thyanta custator custator</i> (F.) .....	11
Redbanded stink bug <i>Piezodorus guildini</i> (Westwood) .....	11
Brown marmorated stink bug <i>Halyomorpha halys</i> Stål .....	12
Conspere stink bug <i>Euschistus conspersus</i> Uhler .....	13
Pest status and damage to plants .....	13
Sampling methods .....	15
Direct methods .....	15
Beat cloth .....	15
Sweep net .....	16
Pheromone trapping .....	17
Indirect methods .....	18
Management of stink bugs .....	20

Table of Contents (Continued)

	Page
Definition of Integrated Pest Management .....	20
Chemical control .....	21
Host plant resistance .....	25
Biological control .....	26
Cultural practices .....	30
Spatial patterns of stink bugs .....	33
Tracking insects .....	34
Research objectives .....	36
References cited .....	37
II. SPATIAL AND TEMPORAL DYNAMICS OF STINK BUGS (HEMIPTERA: PENTATOMIDAE) IN SOUTHEASTERN FARMSCAPES .....	56
Introduction .....	56
Materials and methods .....	60
Results .....	66
Discussion .....	73
References cited .....	95
III. BORDER APPLICATIONS OF INSECTICIDE TO CONTROL STINK BUGS (HEMIPTERA: PENTATOMIDAE) IN COTTON .....	102
Introduction .....	102
Materials and methods .....	104
Results .....	109
Discussion .....	113
References cited .....	129
IV. HARMONIC RADAR TAGGING FOR TRACKING MOVEMENT OF <i>Nezara viridula</i> (HEMIPTERA: PENTATOMIDAE) .....	134
Introduction .....	134
Materials and methods .....	136
Results .....	143
Discussion .....	144
References cited .....	153
V. HOST PREFERENCE OF THE PARASITOID <i>Trichopoda pennipes</i> (DIPTERA: TACHINIDAE) WITH <i>Euschistus servus</i> AND <i>Nezara</i> <i>viridula</i> (HEMIPTERA PENTATOMIDAE) .....	157
Introduction .....	157

Table of Contents (Continued)

	Page
Materials and methods .....	159
Results.....	161
Discussion.....	162
References cited.....	168
VI. OVIPOSITION BY <i>Cylindromyia euchenor</i> (WALKER) (DIPTERA: TACHINIDAE) IN THE BROWN STINK BUG, <i>Euschistus servus</i> (SAY) (HEMIPTERA: PENTATOMIDAE).....	171
References cited.....	178
VII. CONCLUSIONS.....	180
APPENDICES .....	188
A: SAS Code for Chapter II.....	189
B: SAS Code for Chapter III .....	208
C: SAS Code for Chapter IV .....	216
D: Letter of permission for Chapter V.....	228
E: Letter of permission for Chapter VI.....	229

## LIST OF TABLES

Table	Page
2.1	Numbers of grid sampling points and sample dates per farmscape and year with yearly densities of stink bug adults and nymphs per 50 sweeps ( $\pm$ SEM) for field sampling in Lee and Barnwell Counties, SC, and Tift County, GA from 2009 to 2011 ..... 81
2.2	Dispersion indices for stink bug adults in selected crops from Lee and Barnwell Counties, SC, and Tift County, GA ..... 82
2.3	Dispersion indices for stink bug nymphs in selected crops from Lee and Barnwell Counties, SC, and Tift County, GA ..... 85
2.4	SADIE summary data analyses for year-end total stink bug dispersion indices across all crops by location for 2009-2011 ..... 87
2.5	Statistical comparisons of stink bug densities at different distances from field borders and adjacent crop combinations in Lee and Barnwell County, SC, and Tift County, GA ..... 89
3.1	Summary of fields by location and treatment. Fields with ID numbers listed twice received both border and whole-field applications at different weeks of bloom ..... 119
3.2	ANOVA statistics for insecticide treatment, location within fields, week of bloom (WOB), and interactions on mean numbers of stink bugs and percentages of injury ..... 120
3.3	Summary data for SADIE analyses for year-end total stink bug densities and associated boll injury across all treatments ..... 121
3.4	SADIE summary data analyses for selected weekly stink bug dispersion indices and associated boll injury (at least one significant $I_a$ or $X$ ) across all treatments ..... 123
4.1	Statistical comparisons of <i>N. viridula</i> gender and harmonic radar tag attachment on distance travelled in mobility trials ..... 148
4.2	Number of stink bugs tagged and recovered per habitat and release date, with minimum, maximum, and average distances (males and females combined) moved (m) in 1 and 24 hours ..... 149

## LIST OF FIGURES

Figure	Page
2.1	Average densities for selected stink bug species ( $\pm$ SEM) and daily SADIE indices of dispersion over time in mixed crop farmscapes in Lee County, SC, and Tift County, GA ..... 91
2.2	Average densities for selected stink bug species ( $\pm$ SEM) and daily SADIE indices of dispersion over time in mixed crop farmscapes in Tift County, GA, and Barnwell County, SC ..... 92
2.3	Spatial interpolations of SADIE local aggregation indices for <i>E. servus</i> adults and nymphs over time in mixed crop farmscapes in Lee County, SC ..... 93
2.4	Spatial interpolations of SADIE local aggregation indices for selected species of stink bug adults and nymphs over time in mixed crop farmscapes in Barnwell County, SC ..... 94
3.1	Effects of A) week of bloom and B) location within fields on average numbers of stink bugs ( $\pm$ SEM) collected with sweep nets in cotton. Effects of C) week of bloom and D) insecticide treatment on average percentage of boll injury ( $\pm$ SEM). Effects of E) the interaction between week of bloom and insecticide treatment, and F) location within fields on average percentage of boll injury in cotton ..... 125
3.2	Mean boll injury ( $\pm$ SEM) for each field per WOB and treatment ..... 126
3.3	Spatial interpolations of A) boll injury (averaged over sample dates), B) SADIE local aggregation indices for boll injury (averaged over sample dates), C) SADIE local aggregation indices for stink bugs (all species summed over sample dates) and D) SADIE indices of association between stink bugs and boll damage ..... 127
3.4	Spatial interpolations of boll injury by week of bloom for A) Untreated field #1 in Bamberg County, SC 2007, B) Field #19 receiving whole-field insecticide applications in Barnwell County, SC 2007, and C) Field #12 receiving border-spray applications of insecticides in Florence County, SC, in 2009 ..... 128
4.1	Construction of harmonic radar tag for stink bug tracking ..... 150

List of Figures (Continued)

Figure		Page
4.2	Use of foam clamp for attachment of harmonic radar tags on stink bugs.....	151
4.3	Average distance ( $\pm$ SEM) covered by male and female <i>N. viridula</i> flying (m) and walking (cm) in laboratory mobility tests.....	152
5.1	Average numbers of eggs ( $\pm$ SEM) laid per host by <i>Trichopoda pennipes</i> in no-choice (same species, same gender) trials. ....	166
5.2	Effect of gender and species ( <i>Nezara viridula</i> and <i>Euschistus servus</i> ) on average numbers of eggs ( $\pm$ SEM) laid per host by <i>Trichopoda pennipes</i> in choice trials.....	167
6.1	Sequence of oviposition by <i>Cylindromyia euchenor</i> in <i>Euschistus servus</i> in a male/female choice test.....	177

## PREFACE

Chapter I of this dissertation is a literature review, presenting an introduction to stink bugs and their importance as pests in the southeastern United States. A short taxonomic overview and summary of stink bug biology is provided, with detailed descriptions of stink bugs of economic importance and summaries of various control and sampling methods.

Chapters II through VI detail my dissertation research. These sections have been adapted from manuscripts which have been published or submitted for publication in scientific journals in the course of my program of study. Chapter II will be submitted to *Journal of Insect Science* as “G. L. Pilkay, F. P. F. Reay-Jones, M. D. Toews, J. K. Greene, and W. C. Bridges. 2013. Spatial and temporal dynamics of stink bugs (Hemiptera: Pentatomidae) in southeastern farmscapes.” Chapter III will be submitted to *Journal of Insect Science* as “G. L. Pilkay, F. P. F. Reay-Jones, J. K. Greene, M. D. Toews, and W. C. Bridges. 2013. Border applications of insecticide to control stink bugs (Hemiptera: Pentatomidae) in cotton.” Chapter IV is Technical Contribution No. 6128 of the Clemson University Experiment Station and has been submitted to *Environmental Entomology* as “G. L. Pilkay, F. P. F. Reay-Jones, and J. K. Greene. 2013. Harmonic radar tagging for tracking movement of *Nezara viridula* (Hemiptera: Pentatomidae).” Chapter V is Technical Contribution No. 6122 of the Clemson University Experiment Station and has been published by *Journal of Entomological Science* as “G. L. Pilkay, F. P. F. Reay-Jones, and J. K. Greene. 2013. Host preference of the parasitoid *Trichopoda pennipes* (Diptera: Tachinidae) with *Euschistus servus* and *Nezara viridula* (Hemiptera:

Pentatomidae)“, copyrighted by Journal of Entomological Science and used with permission. Chapter VI is Technical Contribution No. 6095 of the Clemson University Experiment Station and has been published as a note, “G. L. Pilkay, F. P. F. Reay-Jones, and J. K. Greene. 2013. Oviposition by *Cylindromyia euchenor* (Walker) (Diptera: Tachinidae) in the brown stink bug, *Euschistus servus* (Say) (Hemiptera: Pentatomidae). Proceedings of the Entomological Society of Washington, 115”, copyrighted by the Entomological Society of Washington and used with permission.

Voucher specimens of *Trichopoda pennipes*, *Cylindromyia euchenor*, *Nezara viridula*, *Chinavia hilaris*, and *Euschistus servus* have been deposited in the Clemson University Arthropod Collection.



## CHAPTER I

### INTRODUCTION

#### General introduction to stink bugs

##### Taxonomy

Stink bugs belong to the order Hemiptera, the true bugs. Hemiptera, with 100,428 described species as of 2009 (Footitt and Adler 2009), with more still being discovered, are distinguished by the presence of piercing and sucking mouthparts and wings that are thin and membranous for much of the length, but are thick and hard at the base. The impression is given that the insects have only half-length wings, hence the name of the order. The stink bug family Pentatomidae has 4,123 described species (Panizzi and Schaefer 2000) and gets its scientific name from the five-sided shieldlike body. Also prominent are the three-segmented antennae with prominent flagellomeres, and the triangular scutellum on the thorax (Triplehorn and Johnson 2005). While present on almost all insects, the scutellum is particularly large and often brightly colored in stink bugs. Although some stink bugs are predatory, most are plant-feeders (Panizzi and Schaefer 2000).

Over two hundred species of stink bugs exist in North America (Triplehorn and Johnson 2005) but only a handful are of economic importance to agriculture in the southeastern U. S. Three species including the green stink bug, *Chinavia hilaris* (Say), the brown stink bug, *Euschistus servus* (Say), and the southern green stink bug, *Nezara viridula* (L.) are recognized as major pests in southeastern cotton, *Gossypium* sp. (Barbour et al. 1988, Greene et al. 1999, 2001), soybean, *Glycine max* (L.) (Jones and

Sullivan 1982, Jackai et al. 1990), corn, *Zea mays* (L.) (Negron and Riley 1987), and wheat, *Triticum aestivum* L. (Viator et al. 1983). More recently, the brown marmorated stink bug, *Halyomorpha halys* Stål, and the redbanded stink bug, *Piezodorus guildinii* (W.), invaded the United States (Nielsen and Hamilton 2009, Baur et al. 2010). Although most stink bug pests follow the general life cycle described below, each species has unique variations.

### Biology overview

Sexually mature females of all pentatomid species produce eggs, and the hemimetabolous life cycle contains five nymphal stadia before the adult stage. Stink bugs disperse rapidly from overwintering sites, with movements up to 1,000 meters per day documented in *N. viridula* (Kiritani and Sasaba 1969), until mating is accomplished, though movements are usually more localized (Kiritani and Sasaba 1969). Stink bug eggs, usually barrel-shaped with patterns or crownlike ornamentation, are laid in rows or groups, glued firmly to each other and to the surface (Triplehorn and Johnson 2005). The plants on which mating takes place are not always the same as those plants on which oviposition occurs (Kiritani and Kimura 1965). Hatching takes five days to three weeks, depending on temperature. Although exact timing varies with species and environmental factors, nymphal development, and diapause in adults, are generally controlled by day length and temperature. In Japan, diapause in *N. viridula* was induced in the laboratory at 25°C, with 10:14 (L:D) h lighting conditions to simulate the beginning of autumn (Musolin and Numata 2003). After the induction of overwintering, with gradual

decreases in temperature to 20°C and 15°C with five days of acclimation, *N. viridula* came out of diapause in response to longer days and higher temperatures in early spring (Musolin and Numata 2004). Stink bugs attaining adulthood earlier in the season, with more time to prepare for diapause, have a significantly higher fecundity after overwintering than those that matured late in autumn (Musolin and Numata 2004). Temperatures required for proper development for stink bugs can vary widely between and within species. With temperatures held at 20°C, developmental periods for *N. viridula*, the most studied of the major pest species, were shorter under 10:14 (L:D) h and 16:8 (L:D) h than under intermediate photoperiods. At 25°C, however, developmental time was slightly shorter under intermediate than short- and long-day conditions (Musolin and Numata 2003). The researchers assumed this photoperiod-mediated growth acceleration takes place in autumn when day-length is short, preparing the stink bugs for overwintering as adults. Nymphal development is unlikely to be affected by day-length under hot summer conditions (Musolin and Numata 2003). *Nezara viridula* has several genetic types, three of which were evaluated in Brazil at different temperatures (Vivan and Panizzi 2005). Although increased temperature and longer photoperiods generally accelerated nymphal development to adulthood, genetic variation in cold tolerance and the influence of photoperiod on development was found. Reproductive potential in *N. viridula* did not depend on diapause, and egg production remained unaffected by photoperiod, which the researchers related to the rapid spread of *N. viridula* to diverse parts of the world (Musolin et al. 2007). Development of *H. halys* was completed

between 17 and 33°C, with eggs hatching at 15°C when photoperiod was held at a constant 16:8 (L:D) h cycle (Nielsen et al. 2008).

Stink bug first instars usually are non-feeding, and stay in the area where oviposition occurred. During this non-feeding state, nymphs often remain together, probably for mutual physical and chemical defense (Lockwood 1986). Feeding begins after the first molt. By the third molt, nymphs usually no longer congregate. Nymphs usually feed on fresh, more watery and nutrient-rich portions of the plant, such as growing shoots, with fruits and seeds being attacked in some species (McPherson 1982, Nielsen and Hamilton 2009). Many stink bug species use a sequence of hosts throughout the season as plants develop, mature and senesce (Jones and Sullivan 1982, Tillman 2008a, 2010a). Adults emerging from overwintering sites lay eggs on cultivated and wild hosts. Among crop hosts, wheat is often the first available in the southeastern United States. Eggs are laid on wheat, and subsequent generations can disperse to corn after the wheat harvest (Blinka 2008, Reising 2011). In the southeastern United States, later generations are prone to attack cotton, soybean, and peanut, *Arachis hypogaea* L., though stink bug damage in peanut is not economically important (Jones and Sullivan 1982, Tillman 2008a, 2010a). However, peanut has been shown to be a reproductive host (Tillman 2008a). Stink bugs dispersal from peanut to cotton has been noted (Tillman et al. 2009).

## Stink bug species of economic importance

### Primary economic species in the southeastern U.S.

- Southern green stink bug, *Nezara viridula* (L.): As a worldwide agricultural pest, the southern green stink bug *N. viridula* is one of the best-studied and described species of Pentatomidae. *Nezara viridula* is highly prolific, completing as many as six generations in warm southern areas in the United States (Todd 1989). Adults are green, 12 mm long, and semi-migratory. Male *N. viridula* can be smaller than females (DeWitt and Armbrust 1973). Communication for mating uses pheromones and substrate-borne vibrations (Ryan and Walter 1992) and a strong attraction to pheromones has been successfully used in trapping this species (Tillman et al. 2010). Aggregation pheromones from males of *N. viridula* are also cross-attractive to *C. hilaris* and several egg and adult parasitoids (Buschman and Whitcomb 1980, Colazza et. al. 1999). Nymphs are gregarious until the fourth instar (Panizzi 1980, Lockwood and Story 1986). Developmental times depend on temperature, usually taking 35 days from oviposition to hatching (Todd 1989).

Kiritani and Kimura (1965) recorded 145 species in 32 plant families as hosts, and more recent studies have continued to find additional hosts (Todd and Herzog 1980). *Nezara viridula* is one of the major pest species in cotton in South Carolina and Georgia, with 14% and 22% of stink bug species collected with the beat cloth and sweep net methods, respectively, in field studies in 2007 and 2008 (Reay-Jones et al. 2009). Fifth instars of *N. viridula*, confined individually in a cage for seven days with a 13-d old boll, reduced yield by 59% compared with unexposed bolls (Greene et al. 1999). Populations

can build up on wheat before moving to other hosts (Blinka 2008). In South Carolina, a sequence of seasonal plant hosts encompassed both wild and cultivated plants (Jones and Sullivan 1981, 1982). Emergence of *N. viridula* occurred in March, with first-generation nymphs appearing by April (Jones and Sullivan 1982). *Nezara viridula*, after overwintering in leaf litter, behind bark, or on weeds and crotches of trees in deciduous woodland in South Carolina, mates and oviposits in small-headed grains and grasses, spring vegetables, corn, tobacco, *Nicotiana* spp., and clover, *Trifolium* spp., completing the first generation on these hosts (Jones and Sullivan 1981). A second generation, while still present on earlier hosts, also can attack tomatoes, *Solanum lycopersicum* L., and peanut as hosts become available (Todd 1989). The number of available wild weed hosts increases as the weather warms and plants emerge from dormancy. By late May to early June, nymphs were common on wild radish, *Raphanus raphanistrum* L., pepperweed, *Lepidium* ssp., and occasionally kudzu, *Pueraria lobata* (Willd.), and black cherry, *Prunus serotina* Erhart. Adults and fifth instars can be found on wheat from March to June (Reay-Jones 2010). From July through October, cowpea, *Vigna unguiculata* (L.), can host large numbers of adults and nymphs. Coffee senna, *Cassia occidentalis* L., and showy crotalaria, *Crotalaria spectabilis* Roth, were also common hosts for adults and fifth instars from late July to frost. Early-instar nymphs were rarely observed on coffee senna, though adults frequently mated on this plant (Jones and Sullivan 1982). Soybean is a particularly attractive third-generation host in all countries where cultivation occurs (Todd 1989). *Nezara viridula* in the fourth and fifth generations can oviposit and develop to adults entirely on soybean in Brazil, Japan, and North America (Panizzi and Schaefer

2000). Soybean is a major food source for *N. viridula* (Jones and Sullivan 1982, Todd 1989, Herbert and Toews 2012) and the overwintering generation often forms in soybean (Jones and Sullivan 1982). *Nezara viridula* is also a frequent pest of cotton (Greene et al. 1999, Greene et al. 2001a, Reay-Jones et al. 2010a, Reay-Jones et al. 2010b), and can transmit the bacterial pathogen, *Pantoea agglomerans*, which causes damage ranging from stained lint and seeds to boll loss (Ragsdale et al. 1979, Medrano et al. 2007). In Japan, *N. viridula* feeds on barley, *Hordeum vulgare* L., wheat, and radishes, but moves to oviposit on rice, *Oryza sativa* (L.), potatoes, *Solanum tuberosum* L., and soybean if available (Kiritani et al. 1965). *Nezara viridula* feeds on several wild hosts as winter approaches, such as castor beans, *Phaseolus vulgaris* L., but reproduction on these hosts has not been documented. *Nezara viridula* reproduces on many wild legumes such as beggar weed, *Desmodium tortuosum* (Swartz), and *Crotalaria* ssp. (Jones and Sullivan 1982). Tillman (2008a) documented all life stages of *N. viridula* on Georgia peanut.

- Green stink bug, *Chinavia hilaris* (Say): The green stink bug, *C. hilaris*, has a range covering most of North America, from Quebec through Florida, and on both coasts (McPherson 1982). Adult body length is between 13-18 mm, influenced by available food sources. Eggs are attached to the underside of leaves, with 24 or more eggs per oviposition (Panizzi and Schaefer 2000). Durations of 7, 8.9, 7.9, 8.9, and 12.8 days have been documented for each of the five nymphal stages, reared on soybean (Miner 1966).

*Chinavia hilaris* is highly polyphagous, feeding more frequently in trees and shrubs than *N. viridula* (McPherson 1982). *Chinavia hilaris* can also feed on green beans, *Phaseolus vulgaris* L., soybean and cotton, as well as other legumes in the southern

United States (Miner 1966). It is one of the more common pests in southeastern cotton (Greene et al. 1999, 2000). *Chinavia hilaris* causes ‘cat facing’ damage in peaches, *Prunus persica* (L.), and nectarines *Prunus persica* var. *nucipersica* (L.) in Georgia, though it is unclear as to how much damage it truly is responsible for in orchards, as other true bugs cause similar damage (Barbour et al. 1990).

In South Carolina, *C. hilaris* first appeared in black cherry after emerging from overwintering sites (Jones and Sullivan 1982). Within two weeks of the first nymphs appearing, densities were at a seasonal peak (Jones and Sullivan 1982). Elderberry, *Sambucus canadensis* L., was colonized in mid June, and a peak in nymphal population again occurred in only four weeks. Black cherry and elderberry produced a complete generation of the stink bugs. By July, *C. hilaris* can infest soybean fields, where feeding and ovipositing takes place (Herbert and Toews 2012). Peak populations of immature stink bugs can occur in September and October. Coffee senna can also support large populations of *C. hilaris*, and this plant may serve as a reservoir as adults move to new hosts. Other wild trees supporting breeding populations of *C. hilaris* in July and August are mimosa, *Mimosa* ssp., sumac, *Rhus copallina* L., and to a lesser extent, privet, *Ligustrum vulgare* L., redbud, *Cercis canadensis* L., and honey locust, *Gleditsia triacanthos* L. (Jones and Sullivan 1982). Cowpea and butterbean, *Phaseolus lunatus* L., support breeding populations in summer, but are superseded as hosts when soybean became more attractive to breeding adults. In 2008, *C. hilaris* was the most common Pentatomid collected by the beat cloth method in South Carolina and Georgia cotton,



comprising 69% of all species collected (Reay-Jones et al. 2009). Tillman et al. (2008b) described all life stages of *C. hilaris* occurring on peanut in Georgia.

- Brown stink bug, *Euschistus servus* (Say): The brown stink bug, *E. servus*, is found as far north as Quebec in North America, and is a serious pest in the South (Rolston and Kendrick 1961, McPherson 1982). According to McPherson (1982), two subspecies of *E. servus* exist, with *E. s. servus* (Say) being the most important in the Southeast. This species ranges from California to the Atlantic coast. Adult body length is 12-14 mm. Like many stink bugs, it can infest wheat (Reay-Jones 2010), but also commonly colonizes sowthistle, *Sonchus oleraceus* (L.), peppergrass, *Lepidium virginicum* (L.), and vetch, *Vicia* spp., in the southeastern United States (Jones and Sullivan 1982). Economic damage from *E. servus* has been documented in soybean (Daugherty 1967), corn (Sedlacek and Townsend 1988, Ni et al. 2010), cotton (Barbour et al. 1988), alfalfa, *Medicago sativa* L., (Russell 1952), sorghum, *Sorghum bicolor* (L), (Hall and Teetes 1981), tobacco (Reich 1991) and pecans, *Carya illinoensis*, (Wangenh.), where it causes fruit drop (Dutcher and Todd 1983). *Euschistus servus* has been documented at all life stages in Georgia peanut (Tillman 2008a, 2008b). *Euschistus servus* can emerge as early as March in the Southeast. By the time wheat is harvested and *E. servus* moves to corn, it has already completed the F1 generation, typically completing two generations a year (McPherson and Mohlenbrock 1976, Herbert and Toews 2011). Eggs are white and ‘kettle shaped’, smaller than those of *C. hilaris* (Munyanesa and McPherson 1994). This species has been shown to make up 18% of specimens collected

in southeastern cotton fields in South Carolina and Georgia, and 36-37% of specimens collected in wheat (Reay-Jones 2010, Reay-Jones et al. 2010b).

#### Secondary economic species in the southeastern U.S.

- Rice stink bug, *Oebalus pugnax* (F.): Ranging across eastern and central North America, and the northern Gulf Coast of Mexico (McPherson 1982), *O. pugnax* is 8-12 mm long as an adult (Panizzi and Schaefer 2000). After emergence and mating, eggs are deposited on the underside of host plant leaves. Red spots on the egg become very prominent before hatching in less than two weeks (McPherson and McPherson 2000). Overwintering occurs as adults. *Oebalus pugnax* prefers grass hosts (Daugherty and Foster 1966). In wheat in South Carolina, peak populations occur at the milk stage (Reay-Jones 2010). *Oebalus pugnax* also causes damage to wheat, oats, *Avena sativa* L., barley, rye, *Secale cereale* L. grain sorghum, corn, and forage grass (Hall and Teetes 1981, Hall and Teetes 1982, Harper et al. 1993). Both nymphs and adults feed on the flowering and milk stages in grasses, leading to empty grains. *Oebalus pugnax* transmits yeast-spot disease in rice, causing 'pecky rice' conditions that result in reduced grain value (Hall and Teetes 1982). The percentage peck was lower in panicles fed on in the hard dough stage than during heading, milk or soft dough stages (Espino and Way 2007a). No significant differences were found in percentage of 'peck' produced by *O. pugnax* in rice panicles attacked during heading, milk, soft dough or hard dough stages (Espino and Way 2007b). Feeding during the rice heading stage causes a loss of yield (Espino and Way 2008). Nymphal survival rates have been studied by Naresh and Smith

(1983) who noted longevity on rice and sorghum was greater than on its most common wild host, vasey grass, *Paspalum urvillei* Steudel.

- Redshouldered stink bug, *Thyanta custator custator* (F.): *Thyanta custator* is a minor pest, with a range restricted to the Gulf and Atlantic coast states in the United States. Populations increase in the early dough stage of wheat, but the species is primarily a pest of developing soybean across its range (Panizzi and Slansky 1985). *Thyanta custator* was once suggested as ranging across North America (Barber 1911) but this has been disputed by research that suggests *T. custator* was being improperly combined with other species in its genus (Ruckes 1957). *Thyanta custator* is linked with a yeast-spot infection in soybean crops (Daugherty 1967). Originally thought to feed on a range of hosts, only one consistent wild host, haw, *Viburnum* spp. has been reported after clarifying the species range (Panizzi 1985). However, in a later study eggs and early instar nymphs were found on wild legume hemp sesbania, *Sesbania emerus* (Aublet) (Panizzi and Schaefer 2000). In the laboratory, *T. custator* has been reared on soybean and peanut. *Thyanta custator* has been found in winter wheat in Georgia (Buntin and Greene 2004) and in South Carolina, making up 7% and 3% of stink bugs collected on wheat in 2008 and 2009, respectively (Reay-Jones 2010). Although development and breeding can take place on soybean, a study in Florida found fully mature soybean seeds were not an adequate food for this species (Panizzi and Slansky 1985).

- Redbanded stink bug, *Piezodorus guildini* (Westwood): *Piezodorus guildini* has been documented as a serious pest in soybean crops in Brazil (Panizzi and Smith 1977) and has recently become the most damaging pest on soybean in Louisiana (Baur et al.

2010). This species occurs in the United States from Texas to Georgia. It occurs in South Carolina but populations typically do not reach economic levels. *Piezodorus guildini* has not been recorded on many wild hosts other than rattlepod, *Crotalaria* spp., in Brazil (Panizzi and Smith 1977). In fields and laboratory settings, *P. guildini* has completed its life cycle on soybean, ovipositing after emergence from overwintering sites, and reaching full adulthood in roughly forty days depending on temperature (Panizzi and Smith 1977). In soybean, eggs are laid on the pods and the stems of the plants, as opposed to the leaves.

- Brown marmorated stink bug, *Halyomorpha halys* Stål: *Halyomorpha halys*, introduced to the United States from Asia, is an important pest of tree crops (Nielsen and Hamilton 2009), as well as peas, *Pisum sativum* L., cucumber, *Cucumis sativus* L., and various bean crops including soybean (Panizzi 2000) with over 300 hosts in its native range (Nielsen et al. 2009). First detected in Allentown, PA, in 1996 (Hoebeke and Carter 2003), *H. halys* is now found in the mid-Atlantic States, and isolated populations have been detected in Mississippi, Ohio, Oregon, and California as of 2009 (Nielsen and Hamilton 2009). It is a nuisance pest in houses (Kobayashi and Kimura 1969) as it invades structures in large numbers to overwinter. In the United States, *H. halys* is univoltine with peak abundance from July to September. Like *C. hilaris*, oviposition can occur in trees and shrubs with foxglove, *Paulownia tomentosa* Thunb., being the first host recorded with eggs in June, later spreading to other trees and shrubs, with every plant surveyed in the area of Allentown, PA found to host *H. halys*, suggesting a very wide host range in North America. In the area sampled, *H. halys* was always found in

greater numbers than native stink bug species (Nielsen et al. 2009). Studies of the effects of temperature on development found *H. halys* completing its life cycle in a wide range of temperature conditions (Nielsen et al. 2008), suggesting that this invasive stink bug has the potential to become a major pest in the United States.

- Conspersed stink bug, *Euschistus conspersus* Uhler: *Euschistus conspersus*, ranging across the western part of North America, is primarily a pest of alfalfa, preferring it to other crops until harvest, when it spreads to cotton and sorghum (Toscano and Stern 1976a). In western North American cotton production, particularly in California, large populations of *E. conspersus* are linked to decreased crop yields and low seed weight, as well as transmission of yeast-based infections on the west coast (Toscano and Stern 1976b). It is the most common species of stink bug in tomatoes in California (Zalom et al. 1996) with 97% of all species collected being *E. conspersus* and population densities of 9.5 adult and nymph stink bugs per meter of row in drop-cloth sampling.

#### Pest status and damage to plants

In the United States, the eradication of the boll weevil and widespread adoption of transgenic cotton cultivars expressing toxins from *Bacillus thuringiensis* (Bt) that control the heliothine complex have led to a decrease in the application of broad-spectrum insecticides on cotton (Greene et al. 1999, Bundy and McPherson 2000). This reduction in pesticide usage has allowed stink bugs, once considered secondary pests in cotton, to expand their damage in cotton in recent years (Greene and Turnipseed 1996, Greene et al. 1999, 2001). As Bt cultivars become more widespread world-wide, documented stink bug

damage on cotton has increased in the United States, South America, and Asia (Greene and Turnipseed 1996, Panizzi and Schaefer 2000).

Crops are damaged by both the mechanical action of feeding and by pathogens, either as opportunistic infections in the wound, or by transmitted bacterial and fungal organisms carried by the stink bugs (Ragsdale et al. 1979, Barbour et al. 1990, Medrano et al. 2007). In grain and legume crops, as well as kernels and beans, quality and value can be lowered (Hall and Teetes 1982), or entire heads or fruiting bodies lost (Jackai et al. 1990, Espino and Way 2008). In tree crops, fruit drop or damage can result, such as pecan losses or peach ‘catfacing’ (Panizzi and Schaefer 2000). Stink bugs also can be a pest in corn (Negron and Riley 1987), particularly adjacent to wheat (Blinka 2008). Low kernel weights, loss of kernel yield, and abortion of small ears may result from stink bug feeding with severity dependant on the corn’s growth stage (Ni et al. 2010). Feeding on corn seedlings deforms or stunts the developing whorls, trapping new leaves inside the older ones, and causing what growers refer to as “Buggy-whip” damage (Buntin 2010).

Crop losses caused by stink bug damage in cotton were estimated at \$60 million in the United States in 2001 (Williams 2002), \$10.2 million in 2007 (Williams 2008), and \$31 million in 2008 (Williams 2009). Stink bugs infested 6.5 million ha of cotton in 2006 and destroyed an estimated 151,347 bales across the United States. Damage was particularly severe in the southeastern United States, with losses of 51,607, 25,000, and 20,488 bales in North Carolina, South Carolina, and Georgia, respectively (Williams 2007). In addition, significant yield losses from this pest complex are frequent in soybean (McPherson and McPherson 2000); in Georgia alone, losses can reach \$13 million

(Douce and McPherson 1991). Because of the widespread pest status of stink bugs across multiple crops in the United States, and particularly in the Southeast, alternative management strategies must be developed to reduce yield loss and also to reduce the use of pyrethroid or organophosphahate insecticides currently applied.

### Sampling methods

Effective sampling methods are critical to integrated pest management (IPM) programs. Sampling of insects can have two distinct objectives: (1) to determine if a pest population has reached an economic threshold, a level at which the pest is capable of causing enough damage to warrant the expense of using a control tactic, and (2) to estimate a pest population density as close as possible to the true mean of the population (Sokal and Rohlf 1969, Ruesink 1980). Properly developed sequential sampling plans and techniques can reduce the costs of sampling by 40-60% (Wilson 1994) without an increase in average error rates. Adequate sampling can help to reduce the chance of a grower either applying an insecticide when it is not needed or not applying an insecticide when it is needed.

#### Direct methods for stink bug sampling

##### Beat cloth

The beat cloth (or shake sheet or drop cloth) typically consists of a white cloth, or a light colored metal or plastic sheet. Size can vary with the crop or area to be sampled. This cloth can be used in any crop where the sheet can fit under the canopy and is

particularly useful when a plant may be too large to sample by other means, such as with trees and shrubs or large bushy plants (Kogan 1980, Pedigo and Rice 2009). For example, in cotton, after the beat cloth is placed on the ground adjacent cotton plants to be sampled, typically between rows, the plants are vigorously shaken over the cloth to dislodge stink bugs, which then fall onto the cloth for enumeration (Reay-Jones et al. 2009). The beat-cloth method was noted as being the fastest direct sampling method of those surveyed in cotton by Knutson et al. (2008), but had low relative capture efficiency, as small insects either were not dislodged to drop onto the cloth, or escaped before being counted by the surveyor. The beat-cloth method can damage plants and be ineffective in sampling pests that fly or jump, such as grasshoppers and some plant bugs, though it is very effective against insects that drop to the ground when disturbed, such as stink bugs. (Kogan 1980, Pedigo and Rice 2009).

### Sweep net

A net can be used to sweep rows of a crop to collect insects in any crop that will not be seriously damaged by the motion of the net (Kogan 1980, Outward et al. 2008, Pedigo and Rice 2009). The net should be swung in a regular pendulum-like motion, perpendicular to the row and through the top of the row canopy (Knutson et al. 2008). In cotton, the sweep net was found to generally be less time-efficient than the drop-cloth method, and only recovered on average 10-12% of the targeted insects when evaluated in Texas (Knutson et al. 2008). The net was reported to not consistently dislodge the insects. Count data generated using sweep-net sampling, however, was generally more reliable



than drop cloth sampling as the net prevented insects from escaping before being totaled (Knutson et al. 2008). At lower densities, however, the sweep net was also found to have greater cost-reliability than the beat cloth at thresholds of two bugs per 3.7 m in cotton (Reay-Jones et al. 2009). In soybean, the sweep net is commonly used for insect sampling as it can capture more insects per man-hour than any other method without increased cost for equipment (Ruesink and Kogan 1975, Pedigo and Rice 2009). When using a sweep net, it is important that the style of sweeping be standardized and kept constant between users, or considerable variation in results can occur (Kogan 1980, Pedigo and Rice 2009).

#### Pheromone trapping

Pheromone trapping has been used successfully with many species of stink bugs. Aggregation pheromones have been shown to attract both genders of adults and late instars of the same species (Mitchell and Mau 1971, Harris and Todd 1980). Pheromones also can be cross-attractive to different species. For example, *N. viridula* aggregation pheromone is cross attractive to *C. hilaris*, and several egg and adult parasitoids (Buschman and Whitcomb 1980, Colazza et. al 1999) while *E. servus* aggregation pheromone is cross-attractive to the oriental stink bug, *Plautia stali* Scott (Tillman et al. 2010). Synthetically produced pheromones for traps are available commercially, with the most common being *trans*-(*Z*)-(1*S*,2*R*,4*S*)-epoxybisabolene and *cis*-(*Z*)-1*R*,2*S*,4*S*)-epoxybisabolene in a 3:1 ratio, based on *N. viridula* (Aldrich et al. 1987, Baker et al.1987) and methyl (2*E*,4*Z*)-decadienoate (Aldrich et al. 1991), which is used for the various *Euschistus* species. Pheromone traps can be made in a number of forms (Mizell

and Edders 1995, Ohlendorf 1999) but the most common in the southeastern United States consists of a trap top containing the pheromone bait placed on a yellow pyramidal base attached to a post approximately 1.3 meters in height (Mitchell and Mau 1971, Mizell and Edders 1995). The traps are baited, and a cattle ear tag containing impregnated insecticide can optionally be placed inside the trap to kill insects entering (Cottrell 2001). The completed pyramid trap is placed wherever sampling is desired. Pheromone traps are vulnerable to the weather, as the chemical baits lose effectiveness over time, and the pheromone plume drifts in the wind, changing the direction of attractiveness (Pedigo and Rice 2009). Additionally, even with the insecticidal ear tag, stink bugs may escape the trap top (Leskey and Hogmire 2005). Pheromone traps are best used as a means of determining the presence or absence of a pest species, or relative abundance and potential damage, rather than a measure of mean density (Pedigo and Rice 2009). Pheromone traps offer a considerable savings in time and effort for simple detections over continuous manual sampling (Parajulee et al. 1998)

#### Indirect methods for stink bug sampling

A widely used sampling method in southeastern cotton is the collection of cotton bolls ( $\approx 2.5$  cm in diameter; Willrich et al. 2004) for internal injury assessment caused by stink bug feeding (Greene et al. 2001a, Reay-Jones et al. 2010a). This method does not directly measure the population of insects in the field; rather it is used as an indirect measure of insect abundance through observed feeding injury to bolls. The most precise estimates of stink bug damage in developing cotton bolls are obtained by dissection,

checking for signs of internal feeding damage, such as internal wall warts or stained cotton lint (Greene et al. 1999, Bundy et al. 2000). Boll sampling is important as it can be ten times more sensitive than sweep net or beat cloth (Toews et al. 2008). The permanent nature of boll injury relative to the temporal presence of stink bugs on a cotton plant, or the plant portion being sampled with a beat cloth, emphasizes the additional value of using injury as a measure of stink bug presence (Reay-Jones et al. 2010b). However, this method is perceived as requiring more time and effort than many scouts and growers are willing to invest, resulting in the use of the external evidence of stink bug damage on the bolls as an estimate. However, external observations are not as accurate as dissection in determining the percentage of injury in the field. If relying on the examination of external lesions as an alternate sampling procedure, 20 bolls per sample are required for accurate management decisions, whereas 10 bolls per sampling site is adequate when using dissection (Toews et al. 2009).

Indirect measurements or damage indices are also used in other crops. Damage thresholds measuring the number of corn seedlings with twisted leaves caused by stink bug feeding are used as populations of stink bugs are difficult to scout in seedling corn (Buntin 2010). In soybean, stink bug injury is measured as a percentage of seeds damaged (Todd and Herzog 1980). Evaluating seed damage requires collected pods to be opened and seeds to be removed and examined in much the same way damage is evaluated using cotton boll dissection (Todd and Herzog 1980).

## Management of stink bugs

### Definition of Integrated Pest Management

After the widespread adoption of synthetic fertilizers, mechanized agriculture, irrigation, and high yielding varieties of crops, control of pest insects for many years relied exclusively on frequent insecticide applications (Atkins 1978). The overuse of insecticides can lead to environmental issues and development of resistance. Insects have shown a remarkable ability to develop resistance to insecticides, with over 500 species resistant to one or multiple insecticides (Georghiou and Lagunes-Tejeda 1991). Insecticides are expensive and time consuming to apply, and can have unintended disruptive effects on the ecosystem. However, chemical insecticides remain the most effective and rapid pest control and prevention method available.

Integrated Pest Management (IPM) is a comprehensive approach to pest management, which attempts to use all suitable control methods in as compatible a manner as possible to prevent pest populations from reaching economic thresholds. This approach merges cultural, biological, and chemical control tactics, among others (Kogan 1998). Michael Atkins (1978) defined IPM as “the combination of as many suitable control methods as is practical into an ecologically harmonized system, designed to maintain pest populations at levels below those which would cause economically significant losses to agriculture and forestry or endanger human health”. Several pest control tactics are combined (‘integrated’), thereby reducing selection pressure from any one given tactic.

## Chemical control

Many insecticides have been used in the past, with chlorinated hydrocarbons, organophosphates, and organochlorophosphate insecticides being the most common (Jackai et al. 1990, Javahery 1990). Today in the United States, pyrethroid and organophosphate insecticides are used to reduce infestations and damage in multiple crops (Greene et al. 2001a, 2010; Layton 2002; Greene 2013a, 2013b). Recently, neonicotinoids and chitin inhibitors have been considered as alternative chemical treatments (Kamminga et al. 2009).

In conjunction with this increase in pesticide use targeting sucking insects, insecticide resistance in stink bugs has been documented as a growing threat, with stink bug populations in areas as diverse as Brazil to Mississippi and Kansas already demonstrating decreased sensitivity to broad categories of insecticides (Sosa-Gomez et al. 2001, Baur et al. 2010). Glass-vial bioassays indicated susceptibility to insecticides differs among species (Snodgrass et al. 2005), with organophosphates being less effective than pyrethroids for *C. hilaris* and *N. viridula*. *Euschistus servus* has been documented as tolerant to commonly used pyrethroids (Emfinger et al. 2001, Greene et al. 2001b, Willrich et al. 2003), though acephate, dicrotophos, and high rates of the pyrethroids bifenthrin, cypermethrin, cyfluthrin, *z*-cypermethrin, and cyhalothrin all successfully produced significant adult mortality (Greene et al. 2001b, Willrich et al. 2003). Alternating classes of insecticides over time can help to reduce the development of resistance (Meyer 2001, Willrich et al. 2003), and is best used as a bridge measure, while other more sustainable pest management tactics are developed. Green bean dip bioassays

showed *C. hilaris* as highly susceptible to pyrethroids and most organophosphates and neonicotinoids (Kamminga et al. 2009). The neonicotinoids dinotefuran and clothianidin were toxic to *C. hilaris*, and thiamethoxam and acetamiprid were toxic to *E. servus* (Kamminga et al. 2009). Field trials in soybean showed the neonicotinoids dinotefuran, imidacloprid, and thiamethoxam were efficacious at controlling stink bugs and had comparable performance to organophosphates and pyrethroids, indicating neonicotinoid insecticides as an alternative to growers for managing stink bugs in integrated pest management programs (Kamminga et al. 2009). The amount of insecticides used could be reduced while increasing effectiveness through the addition of sodium chloride, which increased the time stink bugs touch food before feeding, thus increasing contact with the insecticide (Niva and Panizzi 1996). Natural insecticide extracts from plants, such as neem seed, *Azadirachta indica* (L.), can decrease the scarring caused by stink bug feeding on pecan nuts (Seymour et al. 1995).

When using chemical control measures, careful monitoring of population densities is important to assess the need for applications. According to the Clemson University Extension Pest Management Handbook (Greene 2013a), scouting in cotton should begin when small bolls appear. Recent research suggests thresholds in cotton for stink bugs should vary with plant phenology. This involves using a more aggressive threshold (10% boll damage) from the third to the fifth week of bloom, due to the increased susceptibility to economic damage at this stage (Bachelier et al. 2007, Greene et al. 2008, 2009, Greene 2013a). Organophosphate insecticide such as dicotophos should

be used in fields with infestations predominated by *E. servus*, which is more tolerant of pyrethroid insecticides (Greene et al. 2001b, Willrich et al. 2003).

Soybean thresholds for insecticide application vary with row width (Greene 2013b) and are based on mean densities of insects sampled. Using beat-cloth methods, the recommended number of bugs detected per row-foot which should trigger spraying is one bug per 0.34 m for 0.97 m rows, 0.8 bugs for 0.76 m, 0.5 for 0.53 m, 0.03 for 0.36 m, and 0.02 for 0.18 m row spacing (Greene et al. 2013b). Thresholds with sweep net sampling are not as well defined, but one to two stink bugs per 10 sweeps is the current recommended level.

As populations of stink bugs in corn are difficult to scout in the seedling stage, damage thresholds are used instead, with insecticide applications recommended at roughly 10% damage. Insecticides used at planting, though effective on many pests, have not been shown to be effective on stink bugs, though systemic seed treatments of clothianidin and thiamethoxam will suppress damage at the 0.25 mg a.i. per seed rate, with the 0.50 mg a.i. per seed rate required for good control (Buntin 2010). If stink bugs damage developing ears before silking, the ears can be deformed into a curved shape. These ears fail to develop properly and are more susceptible to infection by corn smut fungus (Buntin 2010). Guidelines developed for corn production in Georgia suggest that in later stages of plant development, stink bug density thresholds can be used. At the elongation (vegetative tassel) stage, one stink bug per two plants present is sufficient to cause economic damage (Buntin 2010). During the pollination to blister stages, stink bugs can damage individual kernels by feeding through the husk, and control is warranted

if populations reach one bug per plant at this stage. In corn, pyrethroid insecticides are recommended for most stink bug species. High rates of bifenthrin can provide about 75-90% control of *E. servus* (Buntin 2010).

An additional way to delay insecticide resistance beyond varying classes of insecticide used is to reduce the amount of active ingredient applied to fields by only treating defined areas of fields. The border-spray concept was suggested following analyses which demonstrated boll damage generally starting along cotton field edges in South Carolina and Georgia (Toews and Shurley 2009, Reeves et al. 2010). Within a week or two of initial infestation, additional damage was evident in interior portions of the field, with colonization of these areas likely originating from the edges. Therefore, treating field borders upon first signs of damage could replace treatment of the entire field or at least forestall whole-field treatments. Border treatments have been used successfully in the control of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and the green peach aphid, *Myzus persicae* (Sulzer), in potato fields (Blom et al. 2002, Carroll and Radcliffe 2008), and the codling moth, *Cydia pomonella* (L.), apple maggot, *Rhagoletis pomonella* (Walsh), and plum curculio, *Conotrachelus nenuphar* (Herbst), in apple, *Malus domestica* Borkh, orchards (Chouinard et al. 1992, Trimble and Solymar 1997). Pesticide savings would be important from the perspective of reducing release of active ingredients, decreasing time to make applications, and conserving natural enemies by not treating the entire field. Additional savings to the grower are obtained through reduction of fuel and labor costs required to apply the insecticides.



### Host plant resistance

Transgenic Bt technology, used to control lepidopterans in agriculture, has been widely adopted in cotton, with most of the crop in South Carolina and Georgia carrying Bt genes (Williams 2008, Toews and Shurley 2009). Bt toxins have no effect on stink bugs, however, and the development of host resistance to manage sucking insects has concentrated on mechanical resistance to feeding. Because softer plant parts are generally the most vulnerable, and older, harder growth is largely ignored, toughening the exterior should lead to less damage (Follett et al. 2009). Toughness may be of secondary consideration to other factors in stink bug resistance, as studies of damage by *N. viridula* on macadamia nut, *Macadamia* spp., in Hawaii demonstrated a thicker-hulled cultivar having more damage than a cultivar with thinner hulls and, presumably, less protected nuts (Follett et al. 2009). The adoption of soybean cultivars with resistance to stink bug injury has been hampered by lower yields in the resistant cultivars as opposed to the nonresistant cultivar (Jackai et al. 1990). Four soybean breeding lines with either Hutcheson × IAC-100 or IAC-100 × 'V71-370' in their genealogy have been identified as possible breeding material for stink bug resistance, but are still limited due to lower yields and later maturity. Additionally, seed sizes in modern resistant cultivars are smaller than average (Boethel 1999, McPherson et al. 2007).

Insect resistance genes have been identified in corn. Corn lines were screened for resistance by assessing the percentage of kernels damaged by *E. servus* and *N. viridula*. Lines C3S1B73-5b and C3S1B73-4 were resistant to stink bugs, with nonresistant corn lines suffering an average of 8% damage, and some resistant hybrids sustaining less than

2% kernel damage (Ni et al. 2007). When phenotypic traits such as corn ear size, husk extension, and husk tightness were examined, husk extension and coverage seemed to be the most important traits minimizing stink bug damage (Ni et al. 2007).

### Biological control

A complex of parasitoids and predators is known to impact stink bug populations in the United States. Many species of predators have been observed to prey on stink bugs in laboratory trials, though feeding results in laboratory trials cannot be used as evidence of wild food preferences without further evidence, owing to the lack of comparative selections of available prey (Miller 1956). Associating species of predators with stink bugs is difficult, complicating implementation of classical biological control programs involving predators (Miller 1956). Birds are frequent predators of stink bug nymphs and adults, as well as eggs in some cases. Miller (1956) noted that the stomachs of thirty-three bird species in North America were confirmed to contain stink bug nymphs, adults, and egg remains. The sage sparrow, *Amphispiza nevadensis* (Ridgeway), was noteworthy for actively seeking stink bugs. Reptiles did not seem to be major predators in his survey, and lizards were observed rejecting stink bugs after biting. Spiders were noted to accept stink bugs as prey, but in most cases were simple opportunistic predators, taking anything which happened to be trapped in their webs.

Stink bug adults have been observed being taken by dragonflies and consumed by mantids on the plants (Miller 1956). The big-eyed bug, *Geocoris punctipes* (Say), and spined soldier bug, *Podisus maculiventris* (Say), feed on many insects, including nymphs

and adults of stink bugs (Tillman et al. 2004, Tillman 2008b). *Podisus maculiventris* is known to feed on *E. servus* in corn, cotton, soybean, and peanut. Red imported fire ants, *Solenopsis invicta* Buren, can significantly reduce stink bug populations (Krispyn and Todd 1982) and have been reported as major predators in soybean in Louisiana on all stink bug life stages, especially eggs and early instar nymphs (Starn 1978).

*Trichopoda pennipes* (F.) (Diptera: Tachinidae), an endoparasitoid of stink bug adults and late-instar nymphs, is one of the most-studied natural enemies of *N. viridula* in the United States (Worthley 1924, Todd and Lewis 1976, Buschman and Witcomb 1980, Panizzi and Slansky 1985, Jones 1988). *Trichopoda pennipes* is native to North and South America and has been introduced to Hawaii, Australia, several Pacific islands, South Africa, Europe, and Israel for control of *N. viridula* (Jones 1988, Colazza et al. 1996, Freidberg et al. 2011). Adults of *T. pennipes* have a bright orange abdomen and black head, thorax, legs, and wings. The species is often referred to as the feather-legged fly because it has a fringe of short black hairs on the tibia. Females are distinguished by the black tip of the abdomen (Dietrick and van den Bosch 1957). Each female parasitoid lays on average 100 eggs, which are typically placed singly on the body of a large nymph or adult stink bug (Pickett et al. 1996). Eggs are oval, white or gray, and can occur on almost any part of the stink bug. When many eggs are laid on the same stink bug, only one successful larva will survive in each host. The first instar hatches from the egg and bores directly into the host stink bug, feeding on the body fluids of the host for about two weeks. On reaching the third instar, the larva exits the host between the posterior abdominal segments. After emergence, the larva burrows approximately three cm into the

soil to pupate. Emergence of adult flies occurs in two weeks. Adult parasitoids immediately begin to look for food in the form of nectar (Pickett et al. 1996). Three generations may occur per year, depending on climate, with the last generation of the season remaining as second instar larvae, overwintering inside the body of the host stink bug until the next emergence cycle. Adult flies emerge in late spring or early summer shortly after stink bugs emerge (Dietrick and van den Bosh 1957). Adult parasitism rates by *T. pennipes* seem to vary with host species. In a 1975 study in South Carolina, parasitism of *N. viridula* ranged from 37-65% in Blackville, 3-13% in Barnwell, 0-25% in Williams, and 43-82% in Dale during four six-week sampling periods. Overall parasitism of *C. hilaris* ranged from 0-54%. *Trichopoda pennipes* has been documented as using the squash bug *Anasa tristis* (DeGeer) (Worthley 1924), and *P. guildinii* (Panizzi and Slansky 1985) as hosts. Limited data are available on parasitism rates in other stink bug species.

At high populations of parasitoids relative to host bugs, superparasitization has been documented in *T. pennipes* with multiple larvae entering and attempting to develop in the same host. Superparasitization has been shown to decrease the parasitoid survival rate to only 38 to 78% (Shahjahtan 1968). The percentage of emerging larvae decreased as the larval density per stink bug increased, with 64-78% emergence at two-five larvae per bug, and 38% emergence when seven larvae attempted to develop in a host. The host's fecundity was shown to be about 59% of unparasitized host rates, but egg fertility remained unaffected (Shahjahan 1968).

A possible relationship between plant hosts and parasitism rates has been recorded (Jones et al. 1996). Prior to entering overwintering sites on cassius, *Cassia occidentalis* L., 53% of adult *N. viridula* were recorded with *T. pennipes* eggs during October and November. Adult parasitism rates were 38% on cowpea, *Vigna unguiculata* (L.) Walpers, and 57% on crotolaria, *Crotalaria spectabilis* Roth, out of 1,030 samples. When overwintering, 22.3% of 112 adult *N. viridula* had at least one *T. pennipes* egg attached to the cuticle (Jones et al. 1996).

Other less common species of adult parasitoids exist in the southeastern United States, with surveys from Florida, Kentucky, Louisiana, North Carolina, and South Carolina yielding 16 species of parasitoids gathered from 14 species of Pentatomidae (Jones et al. 1996). Tachinid flies of the genus *Cylindromyia* have been documented in South Carolina (Eger and Ables 1981), but host preferences and behavior in this genus has not been studied.

Egg parasitoids, particularly *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), are common natural enemies of stink bugs (Colazza et al. 1999, Koppel et al. 2009). *Trissolcus basalis* has been used successfully in an inundative release program in Brazil, maintaining populations of stink bugs below economic levels in soybean (Panizzi and Schaefer 2000). In Brazil, several species of *Trissolcus* were examined for use as biological control agents (Laumann et al. 2008). *Trissolcus* were found to have a type III functional response with an increase in the parasitism rate as host densities increased, as opposed to a type II response where the parasitism rate decreases as host densities increases. Several other species of egg parasitoids have been associated with

stink bugs, including 14 different species documented from 12 stink bugs species in South Carolina (Jones et al. 1996). *Telenomus podisi* Ashmed has been shown to have a strong affinity for the eggs of *C. hilaris* as a host (Javahery 1990). In another study, stink bugs were reduced by 54% in a trap crop of early-maturing soybeans and by 58% in the main soybean crop after inundative releases of the egg parasitoid *T. basalis* that included inoculative placement of parasitized *N. viridula* egg masses (Correa-Ferreira and Moscardi 1995). In Virginia, egg parasitism rates varied within different crops, with the highest egg parasitism rates found in vegetable crops such as collard, *Brassica oleracea* L. with *E. servus* eggs hosting the highest percentages of hymenopteran parasitoids (Koppel et al. 2009). Egg parasitism was documented as having a significant impact on stink bugs in corn in Georgia with eggs of *N. viridula* and *E. servus* both being attacked by a complex of parasitoids. Also in Georgia, egg parasitoids were recorded to switch preferred hosts, depending on the proportion of available host species (Tillman 2010b).

### Cultural practices

Tillage can have a substantial impact on arthropod diversity and abundance. Minimizing or removing soil disturbance under conservation tillage practices profoundly modifies the agroecosystem (All and Musick 1986). The degree of disturbance varies with the type of tillage. Seedbed preparation can range in depth from plow tillage to surface tillage to no tillage. In addition, double cropping is popular in areas of the southeastern United States and often involves conservation tillage; i.e., planting a winter crop followed by a no-till late spring/summer crop. In soybean studies in Florida, pre-

plant tillage had little effect on the population densities of *N. viridula* (Funderburk et al. 1990). Densities of *N. viridula* increased in soybean under conservation tillage, but no significantly greater damage to the seedlings or yield was recorded (Lema et al. 1980). Reduced tillage in a sorghum-cotton rotation was shown not to impact populations of natural enemies but had inconsistent effects on *O. pugnax* and associated injury. In sorghum-cotton plots with minimum tillage, *O. pugnax* densities were 52 per 10 heads of sorghum sampled, while sorghum-cotton plots with conventional tillage demonstrated densities of 41 per 10 sampled heads bugs (Chilcutt and Matocha 2007). The opposite was true in plots with continuous sorghum plantings, suggesting that tillage interacts with crop rotations when impacting stink bugs (Chilcutt and Matocha 2007).

Taking into account the seasonal movements of pests within and between crops, avoiding adjacent placement of crops in ways that do not support consecutive stink bug hosting may offer a way to decrease populations and prevent economic damage (Toews and Shurley 2009, Reeves et al. 2010). In trials in Georgia, cotton quality was most affected when located adjacent to soybean or peanut. The proximity of corn did not seem to significantly impact crop quality (Toews and Shurley 2009). Studies in South Carolina support this observation, finding populations of stink bugs to be higher in cotton adjoining peanut and soybean fields than corn, cotton, or woodlands (Reeves et al. 2010). Eliminating wild hosts has been suggested as a possible control tactic, though wild field borders, considered as wild reservoir, were thought to have little overall impact (Panizzi 1997, Outward et al. 2008). Earlier planting times for crops have been suggested to avoid

the emergence window of stink bugs coinciding with the vulnerable periods of plant development (McPherson et al. 1988, Bundy and McPherson 2000).

Trap cropping has been attempted with varying levels of success in the United States and Brazil. Early-maturing soybean cultivars were used as a trap crop to attract large numbers of stink bugs in Arkansas, which were then treated with insecticides (Smith et al. 2009). Although this offered a decrease in stink bug populations in the short term in the main soybean crop, the populations rebounded to damaging levels later in the season. Stink bugs were believed to have not come from the trap crop, but had instead originated from other sources in the surrounding area (Smith et al. 2009). Another study found soybean trap cropping to be effective if insecticides were used while the population of stink bugs was predominantly developing nymphs (McPherson and Newsom 1984). If applications of insecticide were delayed, or if the stink bugs reached adulthood, the trap crop increased the populations of stink bugs in the surrounding areas. Combining trap crop practices with biological control has been attempted in Brazil, with inoculative releases of the egg parasitoid *T. basalis* in early-maturing soybean (Correa-Ferreira and Moscardi 1995). Populations of *N. viridula* were maintained below economic levels after tags containing 5,000 *T. basalis* eggs were released at one tag per hectare.

In Georgia, sorghum has been studied as a possible trap crop along the interface of a corn-cotton farmscape (Tillman 2006). In cotton with adjoining sorghum trap crops, populations of *N. viridula* never reached economic thresholds (Tillman 2006). When this system was used adjacent to a peanut-cotton farmscape, *N. viridula* again dispersed to the sorghum. Using an economic threshold of one *N. viridula* captured per 1.82 m of row in



cotton, insecticide applications were required 1.4 times in control plots and 0.2 times in plots adjacent to sorghum (Tillman 2006). Parasitism by *T. pennipes* was higher in the sorghum trap crop than in surrounding cotton (Tillman 2006).

### Spatial patterns of stink bugs

The development of appropriate sampling and management plans for insects depends on the understanding of the spatial patterns of the pest to be controlled. Two complementary approaches to analyzing spatial data are commonly used. One relies on estimates of mean and variance of insect densities from multiple samples to determine various indices of aggregation (Lloyd 1967, Taylor 1984, Southwood and Henderson 2000). The other method using two or three dimensional statistics account for the spatial location of samples (Leibold et al. 1993). Spatially referenced sampling data have been used to describe distributions of *E. servus* and *N. viridula* in peanut-cotton farmscapes in Georgia (Tillman et al. 2009) and stink bugs and boll injury in cotton fields (Reay-Jones et al. 2010b). Among studies documenting the spatial patterns of stink bugs, *N. viridula* was shown to have a clumped distribution in soybean fields in the United States (Todd and Herzog 1980). In Japan, the aggregated spatial patterns of male *N. viridula* in rice fields were found to be partially caused by their attraction to females (Nakasuji et al. 1965). Aggregation is also caused through ovipositional behavior of females laying eggs in masses (Hokyo and Kiritani 1962), with limited nymphal dispersion occurring until later instars (Kiritani et al. 1965, Todd 1989).

Several studies have shown greater stink bug abundance concentrated along the border of fields in wheat, cotton, and tomatoes (Zalom et al. 1996, Reay-Jones 2010, Reay-Jones et al. 2010b, Reeves et al. 2010). Within fields, aggregation may be due to pheromones and substrate-borne vibrational signaling (Harris and Todd 1980, Tillman et al. 2010, Lampson et al. 2010, Lampson et al. 2013). Movement and dispersion of stink bugs is strongly dependent on the size and nature of surrounding crops and vegetation, with dispersion patterns generally following the fruiting cycle of crops. Stink bugs have been documented dispersing from corn and peanut to cotton (Tillman 2008a) and edge-feeding and corresponding boll damage has been recorded as more common in cotton fields adjacent to peanut and soybean than other crops (Smith et al. 2009, Reeves et al. 2010). Additionally, southeastern farmscapes are typically characterized by a mosaic of relatively small field sizes of wheat, corn, cotton, peanuts, and soybean, providing stink bugs with a suitable host sequence throughout the season (Tillman et al. 2009, Toews and Shurley 2009, Smith et al. 2009, Reay-Jones et al. 2010b, Reeves et al. 2010, Herbert and Toews 2011, 2012). As such, practices implemented at a farmscape level in one crop such as insecticide applications, trap cropping, or harvesting, can potentially have an impact on stink bug densities and injury in other crops.

### Tracking insects

Studies addressing spatial distributions of insects in agricultural landscapes do not address actual movements of individuals. Movement of populations is inferred to occur over time using sampling methods such as sweep nets, drop cloths, and pheromone traps

(Knight and Croft 1987, Fitt et al. 1989, Gage et al. 1990, Hoffman et al. 1992, Byers 1993, Reay-Jones et al. 2009, Reay-Jones 2012). Methods of marking, releasing, and recapturing, used to sample the same organisms over time, cause disturbance of biological processes by the acts of marking and capturing which undoubtedly impact behavior (Jolly 1965, Begon 1979, Nichols 1992). Various tracking methods (Wallin and Ekblom 1988, Riecken and Raths 1996) have been used to study movement, migration, and behavior of organisms. Among such methods, harmonic radar, a form of radio tracking, has allowed detailed study of the ecology of snakes (Engelstoft et al. 1999), snails (Lövei et al. 1997) and insects such as bees (Carreck and Poppy 1996, Osborne et al. 1999), moths (Riley et al.1998), and carabid beetles (O’Neal et al.2004).

Harmonic radar operates by transmitting a directional radar signal to the radar tag, consisting of an antenna (also called an aerial) and a Schottky barrier diode. The diode, powered by the radar signal, requires no other power source to generate a responding transmission (Colpitts and Boiteau 2004). The receiving unit generates audible tones at different intensities when receiving this response to allow the searching individual to localize the tag. These techniques have been proposed to study the movement of stink bugs, assuming the sturdy physical structure and relatively large size of the insect would allow a radar tag of suitable size to be designed and carried to monitor movement within a field. Development of a radar tag which would permit observations of stink bug movement among wild and cultivated host plants could help to develop more efficient scouting and treatment programs.

### Research objectives

In light of previous research and existing needs, the objectives of this work are to:

- (1) Assess temporal and spatial dynamics of stink bugs at the spatial scale of the farmscape.
- (2) Evaluate in-field border applications of insecticide to mitigate whole field stink bug infestations in cotton.
- (3) Determine the feasibility of harmonic-radar tracking to monitor the movements of stink bugs in farmscapes.
- (4) Determine host preference of the parasitoid *T. pennipes* with *N. viridula* and *E. servus*.
- (5) Describe the oviposition by the parasitoid *C. euchenor* in *E. servus*.

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## CHAPTER II

### SPATIAL AND TEMPORAL DYNAMICS OF STINK BUGS (HEMIPTERA: PENTATOMIDAE) IN SOUTHEASTERN FARMSCAPES

#### Introduction

The widespread adoption of transgenic cultivars of cotton, *Gossypium hirsutum* (L.), expressing *Bacillus thuringiensis* (Bt) toxins to control the heliothine complex and the eradication of the boll weevil, *Anthonomus grandis grandis* Boheman, have decreased the need for the application of broad-spectrum insecticides on cotton in the southeastern United States (Greene et al. 1999, Bundy and McPherson 2000). This reduction in pesticide use has allowed stink bugs to greatly expand their damage on cotton (Greene et al. 1999, 2001). As Bt cultivars have become more widespread, documented stink bug damage on cotton has increased in the Asia, South America, and the United States (Greene et al. 1999, Panizzi and Schaefer 2000, Zeng et al. 2009). Crop losses in cotton caused by stink bugs were estimated at \$31 million in 2008 (Williams 2009). Significant yield losses from this pest complex are frequent in soybean, *Glycine max* (L.), with up to \$60 million in losses annually in the United States (McPherson and McPherson 2000). Stink bugs also can be a serious pest in corn, *Zea mays* (L.) (Negron and Riley 1987, Ni et al. 2010). In the southeastern United States, populations can develop on peanut, *Arachis hypogaea* (L.), though stink bugs are not an economically important pest in this crop (Tillman 2008a, Tillman 2008b).

Phytophagous stink bugs extract fluids from plant tissues with piercing and sucking mouthparts (McPherson and McPherson 2000). Crops are damaged by the



mechanical and chemical action of stink bug feeding, with loss of turgor pressure and injection of digestive enzymes. Pathogens, either as opportunistic infections in the wound or by transmitted bacterial and fungal organisms carried by the stink bug, also contribute to feeding damage (Ragsdale et al. 1979, Barbour et al. 1990, Medrano et al. 2007). Feeding damage on a developing cotton boll ranges from stained lint and damaged seeds to pathogen-induced boll rot or boll abortion (Ragsdale et al. 1979, Barbour et al. 1990, Medrano et al. 2007). Stink bugs also transmit yeast-spot disease in soybean (Daugherty 1967). In grain and legume crops, kernels and beans can have their quality and value lowered, or entire heads or fruiting bodies can be lost (Hall and Teetes 1982, Espino and Way 2008). In corn, low kernel weights, loss of kernel yield, and abortion of small ears can result from stink bug feeding, with severity dependent on the growth stage (Ni et al. 2010). Edible plant parts may become distasteful as a result of stink bug feeding, with a bitter taste or pithy texture (Callahan et al. 1960). Alternative management strategies must be developed to reduce yield loss and the use of broad-spectrum pyrethroid or organophosphate insecticides currently applied.

The predominant pest species of phytophagous stink bugs in the region are the green stink bug, *Chinavia hilaris* (Say), the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euschistus servus* (Say). As polyphagous pests, the movement of stink bugs within patches of agricultural and wild hosts in proximity to one another (farmscapes) is closely linked to crop phenology and the availability of suitable food sources (Jones and Sullivan 1982, Ehler 2000b, Tillman et al 2009). Southeastern farmscapes are typically characterized by a mosaic of relatively small fields of cotton,

soybean, corn, wheat, *Triticum aestivum* (L.), and peanut, providing stink bugs with a sequence of available and suitable hosts throughout the season (Jones and Sullivan 1982, Toews and Shurley 2009, Tillman et al 2009, Reay-Jones et al. 2010, Reeves et al. 2010). Understanding the spatial and temporal dynamics of stink bugs within fields and farmscapes can assist in developing management strategies based on pest insect colonization of crops.

Among studies documenting the spatial patterns of stink bugs, *N. viridula* was shown to have a clumped distribution in soybean fields in the United States (Todd and Herzog 1980). In Japan, the aggregated spatial patterns of male *N. viridula* in rice fields is partially caused by their attraction to females (Nakasuji et al. 1965). Aggregation is also caused through ovipositional behavior of females laying eggs in masses (Hokyo and Kiritani 1962), with limited nymphal dispersion occurring until later instars (Kiritani et al. 1965). Aggregated patterns have been recorded for the consperse stink bug, *Euschistus conspersus* (Uhler), in tomato, *Solanum lycopersicum* (L.), with greater numbers along the edge of the field (Zalom et al. 1996). Other studies in agroecosystems in the southeastern United States have demonstrated greater stink bug abundance along field borders in wheat (Reay-Jones 2010) and cotton (Reay-Jones et al. 2010, Reeves et al. 2010). Stink bug invasion has been suggested to occur from alternate hosts surrounding fields, while within-field aggregation may be partially due to pheromones (Harris and Todd 1980, Tillman et al. 2010).

Patterns of dispersal and associated host crop colonization also have been described, with *N. viridula* dispersing from corn and peanut to cotton (Tillman 2006).

Peanut hosts all life stages and acts as a reproductive host (Tillman 2008a). *Euschistus servus* and *C. hilaris* disperse from wheat to corn (Blinka 2008). Abundance of stink bugs and associated boll damage are more common in cotton fields adjacent to peanut and soybean than other crops (Smith et al. 2009, Reeves et al. 2010).

One-dimensional spatial statistics can use the mean and variance of stink bug densities to describe sampling distributions, but such methods do not take sampling locations into account (Southwood and Henderson 2000). Two- and three-dimensional statistical methods use the spatial location of samples to allow for interpolation of population densities in unsampled areas (Leibhold et al. 1993). Spatial analysis by Distance IndicEs (SADIE) red-blue methodology (Perry et al. 1999) identifies high population clusters or low-density gaps represented by graphical displays of red and blue. This method has been used to describe distributions of *E. servus* and *N. viridula* in peanut-cotton farmscapes in Georgia (Tillman et al. 2009), and stink bugs and associated boll injury in southeastern cotton fields (Reay-Jones et al. 2010). Stink bug emergence patterns have previously been monitored in six different habitats in South Carolina (Jones and Sullivan 1981) and seasonal abundance and population structures of *E. servus*, *C. hilaris*, and *N. viridula* have been studied in Georgia (Herbert and Toews 2011, 2012). Many of these previous studies were based on stink bug sampling data from a limited number of crops, from sampling experimental plots of relatively small scale, or without using the spatial location of samples for analysis. As such, ecological studies on stink bugs are needed to better quantify their dynamics, using georeferenced sampling points in commercial farmscapes on a larger scale. The aim of this study was to examine the

spatial and temporal dynamics of stink bugs in mixed-crop commercial farmscapes over multiple years in South Carolina and in Georgia.

### Materials and methods

Fields sampled. Stink bugs were sampled from 2009 to 2011 within three farmscapes, each consisting of portions of commercial farms with fields of wheat double-cropped with soybean, full-season soybean, cotton, peanut, and corn, as well as uncultivated fallow areas. Farmscapes were located in Lee County, SC, Barnwell County, SC, and Tift County, GA. Additionally, watermelon, *Citrullus lanatus* (Thunb.), and grain sorghum, *Sorghum bicolor* (L.), were present in Tift County. The total field area sampled was 163.5 ha in Lee County, SC, 166.6 ha in Barnwell County, SC, and 208.5 ha in Tift County, GA. At the beginning of the project, GPS coordinates of all cultivated and non-cultivated field sampling points were recorded and maps created using geographical information systems (GIS) software ArcView 9.2 (ESRI 2006).

In 2009, insecticide applications were made with *beta*-cyfluthrin in Lee County (0.017 kg [AI]/ha) to cotton on 15 July, to full-season soybean on 27 July, and to double-crop soybean on 24 August. Applications were limited in 2010 to full-season soybean on 28 July and in 2011 to cotton on 20 July and to full-season soybean on 12 August. In Barnwell County, *beta*-cyfluthrin applications (0.07 [AI]/ha) were made to cotton on 1 July 2009, and in 2010 to cotton, peanut, and soybean on 27 July, and in 2011 to peanut on 20 July, and to cotton on 3 August. In Tift County, in 2009 the grass borders between fields received an insecticide application on 20 July, and were mown on 31 August and 2

October. In 2010, the borders were mown on 8 July and 22 July. No data were available for insecticide applications in 2010 and 2011, or for mowing the field borders for 2011.

Stink bug sampling. A sampling plan following an overall regular grid (150 m) across each farmscape was used in all crops. Fallow areas located in the grid in Barnwell and Tift Counties were sampled in addition to crops. The number of sampling dates (9-28) and points (45-73) varied with the year and county (Table 1). Sampling points were marked with 2-m fiber glass flags. GPS coordinates of all sampling points were recorded. Two sub-samples of 25 sweeps (net diameter = 38 cm) were made for each crop per sample point with the exception of corn, where two sub-samples of 25 plants were visually examined. Adults and nymphs of each stink bug species were recorded from each sample. Phenological stages were recorded for each crop, using vegetative and reproductive stages for corn and soybean, week of bloom (WOB) for cotton, and the Zadoks scale (Zadoks et al. 1974) growth stage for wheat. Fallow, peanut, grain sorghum, and watermelon stages were not recorded.

To determine the effect of distance from field edges on densities, stink bugs in Lee and Barnwell Counties were also sampled along transects at 0 (first two rows of the crop), 5, 10, and 25 m from field edges. In Tift County, transects were placed in the grass border adjacent to the field and at 5, 10, and 25 meters into the fields on each side of the border. Numbers of transects varied with years and farmscapes from two to three per field. Sampling was conducted as previously described.

Data analyses. Counts of adults and nymphs of the three primary pest species found in the two 25-sweep subsamples were summed at each sampling point prior to

analysis. Aggregation indexes were determined using the variance-mean ratio ( $I_D = s^2/x$ ) where  $s^2$  is the sample variance and  $x$  is the sample mean (Southwood and Henderson 2000) for individual and combined species for both life stages for weekly and cumulative annual counts for each farmscape. Departure from a ratio equal to one was tested by  $\chi^2 = s^2(n-1)/\bar{x}$  with  $n - 1$  degrees of freedom, where  $n$  is the number of samples (Southwood and Henderson 2000). Coefficients of Taylor's power law and Iwao's patchiness regression for each species and farmscape per year were calculated in SigmaPlot (2006). Taylor's power law relates mean density to variance by the equation  $s^2 = ax^b$  (Taylor 1961, 1984) where  $s^2$  is the variance,  $x$  is the mean of the sample, and  $a$  and  $b$  are Taylor's coefficients, with a nonlinear regression used rather than log-log transformation to avoid overestimation of variances at low densities (Wilson 1985, 1994). Iwao's patchiness regression is defined as  $\dot{x} = \alpha + \beta x$ , where  $\dot{x}$  is the mean crowding index calculated by  $\dot{x} = (s^2/x + 1)$  (Lloyd 1967),  $\alpha$  is the index of basic contagion, and  $\beta$  is the density contagiousness coefficient. Slopes of Taylor's power law and Iwao's patchiness regression were compared with a value of one, using t-tests [ $t = (\text{slope} - 1) / (\text{SE of slope})$ ], with  $n - 2$  df and a probability level of  $P = 0.05$  (Zar 1999).

The SADIE red-blue methodology of Perry et al. (1999) was used to identify clusters of high-density counts or gaps of low-density counts, using weekly and seasonal totals for each crop, stink bug species, and life stage. A local clustering index was assigned to each sample point, with either a positive cluster index ( $\bar{v}_i$ ) for counts above the mean or a negative gap index ( $\bar{v}_j$ ) for counts below the mean. Randomness is indicated by  $\bar{v}_i = -\bar{v}_j = 1$ . Nonrandomness was quantified by comparing the observed

patterns with random rearrangements across the sampling area. The overall index of dispersion ( $I_a$ ) can indicate an aggregated ( $>1$ ), random ( $=1$ ), or uniform distribution ( $<1$ ). The null hypothesis of spatial randomness was rejected for  $P < 0.025$  (aggregation) or  $P > 0.975$  (uniformity).

The SADIE association tool was used to determine spatial associations between adults and nymphs for each species and for total species for each farmscape and year. An overall index of association ( $X$ ) was determined between each paired dataset, with positive associations for  $X > 0$  ( $P < 0.025$ ) or negative associations for  $X < 0$  ( $P > 0.975$ ). Mean  $X$  was determined from the local spatial associations ( $X_k$ ) for each sampling point  $k$ . A positive association between two variables indicates a patch or gap for both variables, whereas a negative association indicates a patch of one variable and a gap of another (Perry 1997, 1998). Selected SADIE local aggregation indices were imported into the geographical information system software ArcView 9.2 (ESRI 2006), and the Inverse Distance Weighting (IDW) spatial statistical method was used to visualize stink bug aggregation patterns. Cell values in IDW are interpolated using a linear weighted combination of data points around each cell. SADIE was chosen over more traditional geostatistical methods, such as kriging, because it can be used to illustrate local variability in spatial distribution and association among datasets sharing the same sampling points (Perry et al. 2002).

The influence of distance from the edge of fields and the effect of adjacent crop plantings on stink bug densities were analyzed separately for each farmscape. The response variables were the total numbers of each primary pest species and all species

combined for each life stage averaged across sample dates. Not all crop and adjacent crop combinations occurred in all farmscapes. As such, crop and adjacent combinations were combined into a fixed ‘crop/adjacent crop’ effect. ‘Crop/adjacent crop’ effects in Lee County consisted of adjacent fields of corn/cotton, corn/wheat-double-crop soybean, cotton/soybean, cotton/woods, wheat/woods, corn/woods, wheat/cotton, and soybean/woods. Adjacent fields in Barnwell County consisted of cotton/corn, cotton/fallow, cotton/soybean, corn/fallow, soybean/fallow, and soybean/peanut. In Tift County, where transects were separated by grass borders, adjacent fields consisted of cotton/pines, cotton/pecan, cotton/soybean, cotton/sorghum, and cotton/watermelon. The borders were considered to be adjacent to both fields on each side and were combined into a single value. Because only certain fields were used in certain years, an effect combining the two into ‘field/year’ was created. The treatment design of the study was a two-factor factorial of distance from edge and ‘crop/adjacent crop’ combinations. The experiment design of the study was a split plot with subsampling. The whole plot factor was ‘crop/adjacent crop’ arranged in a completely randomized design with ‘field/year’ as replicates. The sub-plot factor was distance arranged in a randomized complete block design with ‘field/year’ as blocks. The two to three transects within each field were subsamples. A linear model was developed including distance, ‘crop/adjacent crop’, and their interaction as fixed effects, and ‘year/field’ within ‘crop/adjacent crop’ (i.e., whole plot error or error<sub>A</sub>), interaction of distance with ‘year/field’ (i.e., subplot error or error<sub>B</sub>), and residual error (i.e., subsampling error or error<sub>C</sub>) as random effects:

$$Y_{ijkl} = u + FY_i + C_j + FY(C)_{ij} + D_k + C*D_{ik} + D*FY(C)_{ijk} + T(D*FY(C))_{ijkl}$$



where  $Y_{ijkl}$  is the response variable in ‘year/field’  $i$ , ‘crop/adjacent crop’  $j$ , distance  $k$ , and transect  $l$ ;  $u$  is the overall mean of the response;  $FY_i$  is the effect of ‘year/field’  $i$ ;  $C_j$  is the effect of crop  $j$ ;  $FY(C)_{ij}$  is the effect of ‘year/field’  $i$  within ‘crop/adjacent crop’  $j$  (error<sub>A</sub>);  $D_k$  is the effect of distance  $k$ ;  $C*D^*_{ik}$  is the interaction effect of ‘crop/adjacent crop’  $j$  and distance  $k$ ;  $D*FY(C)_{ijk}$  is the interaction effect of distance  $k$  and ‘year/field’  $i$  within ‘crop/adjacent crop’  $j$  (error<sub>B</sub>); and  $T(D*FY(C))_{ijkl}$  is the effect of transect  $l$  within distance  $k$  and ‘year/field’  $i$  within ‘crop/adjacent crop’  $j$  (error<sub>C</sub>).

PROC GLIMMIX (SAS Institute 2008) was chosen to estimate and test model terms, as the experiment design of the study involved multiple random effects at different levels, resulting in a split-plot design that required correction for the random effects and appropriate error terms for the level of the split plot. Examination of the count data, using Proc FREQ determined that the data followed a normal distribution. As such, no transformations or link functions in GLIMMIX were necessary. Count data for corn/woods and wheat/cotton adjacent crops were insufficient for analysis in Lee County and were omitted from the model. Significance for model terms was determined using a probability level of 95% ( $P < 0.05$ ). Degrees of freedom were calculated using the Kenward-Roger degrees of freedom approximation (Kenward and Roger 1997). Because distance is a continuous variable and crop and adjacent crop combinations were considered a single effect, contrast statements were used to evaluate the impact of distance and crop/adjacent effects over traditional pairwise comparisons. As different treatment combinations were present in each farmscape, contrast coefficients were manually assigned as needed.

## Results

Across all years and farmscapes, *E. servus* was the most abundant stink bug (64.7% of all species and life stages), with *N. viridula* (12.9%) and *C. hilaris* (11.0%) found in lower numbers. Densities of adults and nymphs varied with year and farmscape (Table 1). The rice stink bug, *Oebalus pugnax* (F.), was the most numerous non-economic species, comprising 11.4% of total adults and nymphs of all species, and was found predominantly in wheat, with 84.2% of *O. pugnax* samples found in 2009. The red shouldered stink bug, *Thyanta custator* (F.), the dusky stink bug, *Euschistus tristigmus* (Say), and the spined soldier bug, *Podisus maculiventris* (Say), were <1% of total captures.

Indices of dispersion are presented only for farmscapes by crop and year where adequate data were available (Table 2). No populations in watermelon or sorghum were sufficient for analysis. For adults, the variance to mean ratio was significantly greater than one in 43 of 56 indices (76.8%), indicating aggregated distributions (Table 2). *Euschistus servus* was aggregated 39.5% of the time. Aggregated distributions for *N. viridula* (11.6%) and *C. hilaris* (9.3%) occurred less often, with the remaining 16.4% for the combined totals of adults of all species. Most aggregated distributions were found in soybean (32.6%), with cotton (20.9%), corn (18.6%), fallow (4.7%) and peanut (2.3%) showing lower percentages. Adult stink bugs had aggregated distributions in 9.3% of farmscape-date combinations in both wheat and double-cropped soybean. Lee County held 39.3% of aggregated distributions for adults, with 19.6% in Barnwell County, and 16.1% in Tift County (Table 2). Nymphs were aggregated in 27 of 30 distributions

(90.0%), with 33.3% for *E. servus*, 14.8% for *N. viridula*, 3.7% for *C. hilaris*, and the remaining 38.6% of aggregated distributions found for the combined totals of adults of all species. Nymphs were most often aggregated in soybean (48.1%), with fewer aggregated distributions in fallow (18.5%), wheat (14.8%), double-cropped soybean (11.1%), cotton (3.7%) and peanut (3.7%). The majority of significant indices for nymphs were found in Lee County (53.3%), with 6.7% in Barnwell County and 30.0% in Tift County (Table 3).

Data were sufficient for analysis using Taylor's power law and Iwao's patchiness regression in 86 year-farmscape-crop combinations (56 for adult stink bugs and 30 for nymphs) (Tables 2 and 3). Slopes for Taylor's power law were significantly ( $P < 0.05$ ) different from one, indicating a nonrandom distribution, for adults in 22 of 56 year-farmscape-crop combinations (39.3%) in Lee (19.6%), Barnwell (5.4%), and Tift (14.3%) counties (Table 2), whereas slopes were significantly different from one for nymphs in 10 of 30 regressions (33.3%) for Lee (26.6%), Barnwell (3.3%), and Tift (3.3%) (Table 3). For nymphs, slopes of Taylor's power law were generally  $>1$ , indicating aggregated distributions with the exception of two in Lee County in 2009, where nymphs of all species combined in cotton and *N. viridula* nymphs in double-cropped soybean had uniform distributions. The density contagiousness coefficient  $\beta$  of Iwao's patchiness regression was significantly different from one in 20 of 56 cases (35.7%) for adults in Lee (19.6%), Barnwell (1.8%), and Tift Counties (14.3%) (Table 2), and six of 30 cases (20.0%) for nymphs in Lee County (13.3%), with 3.3% each in Barnwell and Tift Counties (Table 3). All significant density contagiousness coefficients indicated

aggregation, with the exception of one for nymphs of *E. servus* in wheat in Lee County in 2010, which indicated a uniform distribution.

SADIE aggregation indices for year-end summary data were significant in 11 of 60 analyses (18.3%) for the three main pest species and all species summed with six (54.5%) significant indices for adults and five (45.5%) for nymphs (Table 4). All significant year-end SADIE indices indicated aggregated distributions in adults and nymphs, and all 22 significant associations between adults and nymphs were positive out of 28 paired datasets (Table 4). SADIE also was used in 608 separate sample datasets for weekly totals separated by species for all three farmscapes and years. Of those, 258 adult and 127 nymph datasets contained captures at two or more points, permitting analysis. Adults and nymphs had significant patches or gaps in 11.2% and 22.8% of analyzed weekly datasets, respectively (Figs. 1 and 2). Adults and nymphs of *E. servus* (41.4% and 48.3%, respectively) and *C. hilaris* (10.3%, 6.9%) had more significant indices than *N. viridula* (3.4% and 3.4%). The majority of significant indices were from combined totals of adults (44.9%) and nymphs (41.4%). All significant SADIE indices indicated aggregation with the exception of uniform distributions for adult *N. viridula* in Lee County on 22 March 2009, *E. servus* nymphs in Lee County on 12 May 2009, and adult *E. servus* in Tift County on 22 July 2010. The SADIE association tool detected significant associations between adult and nymphal stink bugs in 80.0% of 96 paired weekly datasets, with 20.8% of all significant associations for *E. servus*, 3.1% for *C. hilaris*, and 4.2% for *N. viridula*. Associations between the combined total adults and nymphs represented 71.9% of the significant associations. All associations of adults and

nymphs were positive with the exceptions of a limited number in 2009, with *E. servus* on 19 May, *N. viridula* on 12 July, and all species combined on 19 May and 24 August negatively associated in Lee County, and 31 August in Tift County where *E. servus* adults and nymphs were negatively associated. The limited number of negative associations suggested significant clusters of adults and nymphs were generally found in the same area of the farmscape.

IDW interpolation maps for weekly SADIE aggregation indices are presented for *E. servus* adults and nymphs in Lee County in 2009 (Fig. 3), and *C. hilaris* adults and nymphs in Barnwell County in 2009 (Fig. 4). Indices for *N. viridula* were insufficient for interpolation. In Lee County in 2009, *E. servus* nymphs were aggregated in five weeks of sampling, as opposed to three for adults (Fig. 3). Peak populations in corn were rarely above 0.5 stink bugs in 50 sweeps, and clustering was not observed. Soybean and cotton adjacent to one another demonstrated adult clustering in late July (Figs. 1 and 3). In Barnwell County in 2009, *C. hilaris* demonstrated significant adult clustering in fields of cotton and soybean adjacent to one another on 1 July 2009 (Figs. 2 and 4).

Distance from field edges did not have a significant effect on stink bug densities in any farmscape sampled ( $P > 0.05$ ) (Table 5). Contrast statements for crop/adjacent effects were assigned to better quantify estimated increases in average captures in significant crop/adjacent combinations compared to all other possible crop/adjacent combinations, so that for any hypothetical sample 'x', samples in significant crop/adjacent combinations would be estimated to be 'x + estimate' higher on average than in any other crop/adjacent combination in the farmscape. In Lee County,

crop/adjacent effects were significant ( $P < 0.05$ ), with higher estimated captures in crop/adjacent combinations with full-season soybean or woods (consisting of cotton fields adjacent to soybean fields, cotton fields adjacent to woods, soybean fields adjacent to cotton fields, wheat fields adjacent to woods, and soybean fields adjacent to woods) than in any other combination. Average stink bug density in these crop/adjacent combinations was estimated to be  $0.20 \pm 0.07$  [SEM] ( $t = 2.74$ ;  $df = 13.2$ ;  $P = 0.0167$ ) higher for adults of *E. servus*, *N. viridula* ( $0.11 \pm 0.03$ ;  $t = 3.64$ ;  $df = 11.78$ ;  $P = 0.0035$ ), and combined adults of all species ( $0.46 \pm 0.11$ ;  $t = 4.04$ ;  $df = 13.83$ ;  $P = 0.0012$ ) than in any other crop/adjacent combination in Lee County. Nymphs of *E. servus* ( $1.04 \pm 0.28$ ;  $t = 3.70$ ;  $df = 12.11$ ;  $P = 0.0030$ ), *C. hilaris* ( $0.57 \pm 0.08$ ;  $t = 7.14$ ;  $df = 12.76$ ;  $P < 0.0001$ ), and combined nymphs of all species ( $1.83 \pm 0.31$ ;  $t = 5.88$ ;  $df = 12.38$ ;  $P = < 0.0001$ ) demonstrated crop/adjacent effects limited to soybean and cotton fields adjacent to each other. Adults of *C. hilaris* and nymphs of *N. viridula* were not significantly influenced by crop/adjacent effects ( $P > 0.05$ ). Barnwell County, lacking woods transects, still exhibited higher estimated densities on soybean fields adjacent to cotton or peanut fields and cotton fields adjacent to soybean or peanut fields for *C. hilaris* adults ( $0.31 \pm 0.03$ ;  $t = 10.53$ ;  $df = 41$ ;  $P = < 0.0001$ ), as well as adults ( $0.46 \pm 0.08$ ;  $t = 6.09$ ;  $df = 25.66$ ;  $P = < 0.0001$ ) and nymphs ( $0.03 \pm 0.01$ ;  $t = 2.98$ ;  $df = 164$ ;  $P = < 0.0033$ ) of all species combined. In Tift County, no crop/adjacent effect of any crop combination influenced stink bug densities for any species. Interactions between crop/adjacent effects and distance were detected in Lee County for *E. servus* adults, adults and nymphs of *N. viridula*, nymphs of *C. hilaris*, and the combined nymphs of all species (Table 5).

However, post-hoc analyses using contrast statements and mean separation tests could not clearly separate any trends.

In wheat (present only in Lee County), stink bugs were first detected in early April, with *E. servus* sampled when flag leaves were visible (Zadoks stage 37). Numbers of adult *E. servus* increased rapidly as wheat entered the boot stage, with adult *N. viridula* first collected in wheat during heading (stage 50). Nymphs of either species were not collected in wheat in any year before the dough stage (Fig. 1). *Chinavia hilaris* was not collected in wheat. The numbers of stink bugs collected decreased as the grain dried.

Stink bugs were next detected in corn, with *E. servus* found in the V10 stage in Lee and Barnwell Counties. *Chinavia hilaris* was also found for the first time in Barnwell County in corn at V10 in 2009 (Fig. 2), though this species was not found in corn in Lee County in any year, and corn was not available for sampling in Tift County (Figs. 1 and 2). *Nezara viridula* was not found in corn in Lee County in 2009 and 2011, though 0.1 insects per 50 plants sampled were found at V10 in 2010, with no *N. viridula* were found in this crop in later sampling. Densities of *E. servus* in corn peaked during the milk stage, corresponding with peak densities in wheat in Lee County (Fig. 1). In Barnwell County, where wheat was not present, adult *E. servus* were found in corn, with a maximum density of 1.5 insects per 50 sweeps in the milk stage, with densities decreasing as the corn matured (data not shown due to low densities).

Stink bugs were first detected in cotton during squaring, and all three species were found in stage V10 to R1 soybean at approximately the same time in all locations where both crops were present. The first adult population peaks in cotton for *E. servus* and *N.*

*viridula* occurred in the first WOB in all locations, with *C. hilaris* also peaking in the first WOB in Tift County in 2010 (data not shown). Peak populations for all three species in soybean did not occur until R4 for adults, with a sharp increase in nymphs for all species in soybean in R6, typically three to four weeks after adult peaks (Figs. 1 and 2). Double-cropped soybean, planted after wheat had been harvested, also had adult peaks around R4, with a nymphal peak at R6. *Chinavia hilaris* was rarely sampled in peanut, while densities of *E. servus* in peanut were also low whenever found in this crop. *Nezara viridula* was not found in peanut (Figs. 1 and 2).

Examining densities of adults and nymphs by species at all sample farmscapes, *E. servus* showed two distinct peaks (one in wheat and one in soybean), with nymphal peaks following adult peaks by approximately two to four weeks (Figs. 1 and 2). A single peak of *E. servus* occurred in 2011 in mid-summer in Lee County. No clear pattern could be detected for *E. servus* in Tift County, likely due to low densities (Fig. 2). In Tift County in 2011, where sampling was not undertaken until August, a limited number (0.02 per 50 sweeps) of nymphs were found in the first WOB in cotton in the early fall, as cotton had been replanted due to severe drought.

*Chinavia hilaris*, found in lower numbers than *E. servus*, showed two adult peaks in Barnwell County in 2009, with one occurring in corn at R1 and soybean at V10, concurrently, and the other occurring at R4 in soybean, with low numbers of nymphs in both cases (Fig. 2). Adult peaks in full-season soybean and peanut were recorded two weeks after the applications of insecticide treatments (Fig. 2). Two *C. hilaris* adult peaks were recorded at R1 and R6 and one nymph peak at R6 in Lee County in soybean in 2009



(data not shown). Densities were low for *C. hilaris* in 2010 and 2011, with only single adult and nymph peaks each year occurring at R6 in soybean and in the eighth WOB in cotton in Lee County. In Tift County in 2010, *C. hilaris* had two nymph peaks in the sixth WOB in cotton and early fall in fallow, though counts were insufficient for analysis in 2009 and 2011 (data not shown).

Data for *N. viridula* are shown only for Tift County in 2009 due to low densities and no clear patterns in all years and farmscapes (Fig. 1). Nymphs of *N. viridula* increased in wheat in Lee County in 2009 at stage 87, following a smaller adult peak during stage 73. One isolated peak of nymphs in cotton in 2009, with no adult peak preceding it, occurred in the first WOB one week after the cotton was sprayed with insecticide. A peak of adults was found in cotton in 2010, in the sixth WOB, five weeks after the field received the only insecticide application of the year. A peak in nymphs at the first WOB in cotton was also recorded in Tift County in 2009, though densities never increased beyond 0.03 insects per 50 sweeps in cotton samples. (Fig. 1).

### Discussion

Insect densities among and within fields varied in different farmscapes (Table 1) and in different crops (Figs. 1 and 2). Densities of the three primary pest species were 1.3-fold higher in 2009 than in 2010 and 2011 combined. In 2011, drought conditions were widespread across the southeastern United States. Low stink bug densities (Table 1) in the sampling grid limited the application of SADIE, with most analyses indicating randomness (Figs. 3 and 4). The lack of responses to insecticide applications by *E. servus*

(Fig. 1) might be due to this species being tolerant to pyrethroids (Emfinger et al. 2001, Willrich et al. 2003). No organophosphate insecticides were applied. However, low densities likely impaired our ability to observe pesticide responses. Densities of *C. hilaris* and *N. viridula* were insufficient to determine insecticide responses.

Slopes of Taylor's power law were greater than one in 31% of analyses, indicating a clumped distribution for adult and nymph stink bugs. Fit of Iwao's patchiness regression also generally indicated clumped distributions for adult and nymph stink bugs ( $\beta > 1$ ) when  $\beta$  was significantly different from one, but distributions were random in 64% of analyses, supporting the results of Taylor's power law. The majority of significant slopes for both regressions were found in areas of highest densities, such as in Lee County or in soybean.

Although SADIE and IDW of local aggregation indices previously have been used to describe the spatial dynamics of stink bugs (Tillman et al. 2009, Reay-Jones et al. 2010), this study is the first to attempt to use these techniques to quantify the spatial and temporal dispersal of adults and nymphs of multiple species across multiple years and farmscapes in different states. SADIE detected fewer aggregations than the variance-to-mean ratio, Taylor's power law, or Iwao's patchiness regression, with 82% of SADIE analyses indicating randomness. Many arthropod species, including stink bugs, are spatially aggregated (Taylor et al. 1978, Reay-Jones et al. 2009, Reay-Jones et al. 2010, Reay-Jones 2012). This aggregation can vary with species and life stage. Based on SADIE, nymphs were slightly more frequently aggregated than adults. Nymphs do not disperse from the ovipositional site until maturation to late instars (Kiritani et al. 1965),

as illustrated by the high counts in soybean in Lee County, with up to 16 nymphs in a single sample. However, Thomas et al. (2001) noted SADIE is not a sensitive method using smaller datasets. Xu and Madden (2004) also suggested the number of clusters and positions influence the index more than the cluster sizes. In our study, SADIE analyses sometimes used datasets with numbers as low as three stink bugs per field. Increased captures, either by increasing the size of the areas sampled, or increasing the number of points in a field sampled might have increased the aggregations and associations detected by SADIE. Despite these limitations, clusters of *E. servus*, often along soybean interfaces, could be clearly detected on 21 July 2009 by interpolation of SADIE indices in Lee County (Fig. 3). Aggregation along the edge of soybean fields also was demonstrated by *C. hilaris* in Barnwell County on 1 July 2009 (Fig. 4).

*Euschistus servus* is bivoltine in Arkansas, Georgia, Illinois, and Virginia (Woodside 1946, Rolston and Kendrick 1961, Munyaneza and McPherson 1994, Herbert and Toews 2011). Though insecticide applications hindered our ability to detect clear generation peaks, our study supports bivoltinism in *E. servus*. Two distinct populations of nymphs were recorded for *E. servus* in Lee County in 2009 and 2010, with two also recorded in Barnwell County in 2010, with the first generation of nymphs in wheat, and the last pre-diapause generation in soybean. The low numbers of *E. servus* nymphs collected in Barnwell County might have been inadequate to determine peaks in 2009 and 2011 and in Lee County in 2011. Although data from Tift County demonstrated late-season peaks in all years, no early-season peak was observed. As no wheat was available for sampling in Tift County, sampling started in late June in 2009 and 2010, and

sampling was delayed until mid-August in 2011. Sampling in Tift County might have missed the early-season peak, which was recorded in other studies from the same area in early May (Herbert and Toews 2011).

Densities of *C. hilaris* in corn have been recorded as low when found at all (Tillman 2010), consistent with our observations in Lee and Barnwell Counties in all three years where none were detected in corn. Cotton and soybean both hosted adults later in the season, and the largest nymph peaks in Lee County in all years were in soybean in R6, likely the last generation before diapause. Early generations of *C. hilaris* develop on nonagricultural hosts (Herbert and Toews 2012), moving into cotton and soybean later in the season. Several tree species have been recorded hosting the first generation in South Carolina (Jones and Sullivan 1981, 1982). *Chinavia hilaris* might be bivoltine in South Carolina and Georgia, but early-season populations would have had to develop away from our sampling points (all in agronomic crops), remaining undetected until second-generation adults moved into the farmscape. More research remains to be done to clarify the overwintering behavior and life cycle of *C. hilaris*.

The plants on which *N. viridula* mate are not always the same as those plants on which oviposition occurs (Kiritani et al. 1965). The irregular nymphal peaks across multiple crops (Fig. 1) suggest multivoltinism in *N. viridula*, a view supported by Jones and Sullivan (1981, 1982) and Herbert and Toews (2012), but the low densities and use of insecticide hindered our ability to accurately determine multivoltinism.

Stink bugs disperse following the fruiting cycle of crops, with densities decreasing in other crops as plant senescence begins (Jones and Sullivan 1982). The

diversity of crops and relatively small size of fields in the Southeast leads to colonization of patches within a farmscape. Our results confirm peak populations occurring sequentially in order of crop fruiting (Figs. 1 and 2). No stink bugs were recorded in grain sorghum in Tift County. Grain sorghum is a highly desirable host and a potential trap crop when used in conjunction with cotton (Tillman 2006). However, only two to four sampling points were located in sorghum per year. The lack of captures might be due to the low numbers of sample points in addition to low densities.

The predictable sequence of host usage in our study and others could allow for insecticide applications timed to target aggregations of stink bugs, possibly in the nondispersing nymphal stage in soybean and cotton, resulting in lower costs of application. Stink bugs are rarely economic pests of wheat, but a reproductive peak can occur on wheat before moving to corn crops. Insecticide treatments are sometimes warranted in corn adjacent to wheat (Reisig 2011). The predictable peak populations of stink bugs during squaring and the first WOB in cotton might permit more efficient usage of insecticides by timing an application to occur early in egg hatching or during the early nymphal instars, as suggested by Herbert and Toews (2012), preventing the peak populations of nymphs from developing. In all species, soybean hosted the largest numbers of stink bugs, with cotton fields adjacent to soybean fields often hosting peak populations on the same day (Figs. 3 and 4). More research is required into the dispersion of stink bugs within and among fields. At present, stink bug movements are inferred through sampling over time and cannot be directly observed. Harmonic radar tracking, which uses radio signals to locate and follow small animals and insects, is being

investigated as a means to monitor stink bug dispersal in the farmscape (Pilkay et al. 2013a). Radar tracking would possibly permit the observations of movement in real time.

The proximity of small fields of different crops in the southeastern United States typically does not permit placing crops at distances adequate to avoid stink bug dispersion to sequential hosts. *Nezara viridula* has been recorded dispersing up to 1,000 meters per day (Kiritani and Sasaba 1969). The lack of impact of distance from field edge on stink bug densities in our study likely results from the low densities of stink bugs overall. Despite this, higher densities in fields adjacent to full-season soybean or woods compared to any crop adjacent to corn or double-crop soybean fields were clear in Lee County, possibly due to woods providing stink bugs with non-agricultural hosts and shelter in inclement weather or for overwintering. Full-season soybean had the highest stink bug densities out of all sampled crops in our study. Barnwell County, lacking transects adjacent to wood, still exhibited higher stink bug densities in cotton fields adjacent to soybean or peanut and soybean fields adjacent cotton or peanut fields than in any other crop/adjacent combination. Our results support past studies in Georgia and South Carolina where stink bug densities were higher in cotton adjacent to peanut and soybean fields than in cotton adjacent to corn or other cotton fields (Toews and Shurley 2009, Reeves et al 2010). In Tift County, where fields were separated by grass borders that were regularly treated with insecticides and mowed, contrast statements found no difference in densities between fields adjacent to soybean and fields bordering other crops for any species (Table 5). The treatment of borders separating crops influenced stink bug dispersion between fields in the farmscape. Stink bug densities could

potentially be reduced by planting to minimize cotton-soybean interfaces where possible and applying insecticides in grass between fields, so long as care was taken to use a product labeled for both adjacent crops.

Among cultural practices used to manage stink bugs, early-maturing soybean cultivars have been used as a trap crop to attract large numbers of stink bugs of multiple species in Arkansas, which were then treated with insecticides (Smith et al. 2009). Although this offered a decrease in stink bug densities in the short term in the main soybean crop, densities rebounded to damaging levels later in the season. Stink bugs were believed to have not come from the trap crop but, instead, had originated from other soybean crops in the surrounding area (Smith et al. 2009). Another study found soybean trap cropping as working well if insecticides were used while the stink bugs were still immature (McPherson and Newsom 1984). If applications of insecticide were delayed, or if the stink bugs reached adulthood, the trap crop increased the populations of stink bugs in the surrounding areas. Combining trap crop practices with biological control has been attempted by growing sorghum along the interface of a corn-cotton farmscape, similar to the concept of a disruptive border. In cotton with adjoining sorghum trap crops, *N. viridula* densities never reached economic thresholds (Tillman 2006). When this system was used adjacent to a peanut-cotton farmscape, *N. viridula* dispersed to the sorghum. Parasitism by the adult parasitoid *Trichopoda pennipes* (F.) (Diptera: Tachinidae) was higher in sorghum trap crop plots than surrounding cotton (Tillman 2006).

The application of integrated pest management relies on an understanding of pest ecology. This study confirms the patterns of spatial and temporal variation in seasonal

dispersion and host usage by multiple pest species of stink bugs across multiple locations over time in the southeastern United States. In a farmscape with grass borders between crops, no crop/adjacent effects were found, despite the crops being within flying range. Prior studies have suggested altering crop planting dates to avoid damage from bivoltine species (Herbert and Toews 2012), though such strategies were noted to be ineffective against multivoltine species. Our stink bug complex must be examined with the varying life cycles of multiple species in mind. Further investigations into increasing the influence of natural enemies must take our stink bug species complex into account, as not all native parasitoids in farmscapes target all stink bug species equally (Worthley 1924, Jones et al. 1996, Pilkay et al. 2013b, 2013c). A focus on stink bug ecology could help to mitigate the impact of the stink bug complex in multiple crops in the southeastern United States.



Table 2.1. Numbers of grid sampling points and sample dates per farmscape and year with yearly densities of stink bug adults and nymphs per 50 sweeps ( $\pm$  SEM) for field sampling in Lee and Barnwell Counties, SC, and Tift County, GA from 2009 to 2011.

Year	Species	Barnwell County			Lee County			Tift County		
		Sample points/dates	Adult	Nymph	Sample points/dates	Adult	Nymph	Sample points/dates	Adult	Nymph
2009	<i>E. servus</i>	51/9	1.25 $\pm$ 0.21	0.10 $\pm$ 0.02	53/28	6.36 $\pm$ 0.87	5.92 $\pm$ 0.80	73/16	1.21 $\pm$ 0.19	0.62 $\pm$ 0.19
	<i>C. hilaris</i>	51/9	0.98 $\pm$ 0.34	0.14 $\pm$ 0.06	53/28	1.00 $\pm$ 0.20	0.79 $\pm$ 0.19	73/16	0.10 $\pm$ 0.04	0.01 $\pm$ 0.01
	<i>N. viridula</i>	51/9	0.16 $\pm$ 0.09	0.08 $\pm$ 0.06	53/28	1.58 $\pm$ 0.31	3.75 $\pm$ 0.63	73/16	0.34 $\pm$ 0.10	0.21 $\pm$ 0.08
	All	51/9	2.39 $\pm$ 0.46	0.24 $\pm$ 0.10	53/28	8.94 $\pm$ 1.04	10.47 $\pm$ 1.21	73/16	1.64 $\pm$ 0.22	0.84 $\pm$ 0.22
2010	<i>E. servus</i>	51/12	0.59 $\pm$ 0.16	0.12 $\pm$ 0.05	52/24	2.02 $\pm$ 0.35	2.09 $\pm$ 0.48	73/14	0.70 $\pm$ 0.11	0.53 $\pm$ 0.17
	<i>C. hilaris</i>	51/12	0.33 $\pm$ 0.11	0.22 $\pm$ 0.13	52/24	0.36 $\pm$ 0.14	0.19 $\pm$ 0.09	73/14	0.30 $\pm$ 0.07	0.15 $\pm$ 0.08
	<i>N. viridula</i>	51/12	0.02 $\pm$ 0.02	0.08 $\pm$ 0.05	52/24	0.34 $\pm$ 0.12	0.28 $\pm$ 0.12	73/14	0.15 $\pm$ 0.05	0.05 $\pm$ 0.03
	All	51/12	0.94 $\pm$ 0.23	0.41 $\pm$ 0.16	52/24	2.72 $\pm$ 0.51	2.57 $\pm$ 0.58	73/14	1.15 $\pm$ 0.15	0.74 $\pm$ 0.21
2011	<i>E. servus</i>	45/17	0.71 $\pm$ 0.31	0.09 $\pm$ 0.02	53/21	4.00 $\pm$ 1.08	3.09 $\pm$ 1.13	73/11	0.30 $\pm$ 0.08	0.37 $\pm$ 0.13
	<i>C. hilaris</i>	45/17	0.09 $\pm$ 0.04	0.04 $\pm$ 0.03	53/21	0.30 $\pm$ 0.10	1.13 $\pm$ 0.45	73/11	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01
	<i>N. viridula</i>	45/17	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	53/21	0.00 $\pm$ 0.00	0.02 $\pm$ 0.02	73/11	0.00 $\pm$ 0.00	0.01 $\pm$ 0.01
	All	45/17	0.80 $\pm$ 0.31	0.07 $\pm$ 0.04	53/21	4.30 $\pm$ 1.08	4.25 $\pm$ 1.56	73/11	0.32 $\pm$ 0.08	0.40 $\pm$ 0.14

Table 2.2. Dispersion indices for stink bug adults in selected crops from Lee and Barnwell Counties, SC, and Tift County, GA. Locations and crops with insufficient samples for analysis have been omitted. Double-crop soybean are indicated by ‘DSB’.

Location	Year	Crop	Species	Taylor's power law				Iwao's regression				I <sub>D</sub>
				<i>a</i>	<i>b</i>	R <sup>2</sup>	<i>t</i> -value for slope = 1	$\alpha$	$\beta$	R <sup>2</sup>	<i>t</i> -value for slope = 1	
Barnwell	2009	Corn	<i>E. servus</i>	1.386	1.239	0.980	1.759	-0.061	1.448	0.866	1.363	1.371 <sup>b</sup>
Barnwell	2009	Corn	All	1.163	1.001	0.997	0.018	0.020	1.185	0.930	0.988	1.487 <sup>b</sup>
Barnwell	2009	Cotton	<i>E. servus</i>	1.566	0.681	0.709	-0.797	0.351	3.026	0.192	0.578	2.267 <sup>b</sup>
Barnwell	2009	Cotton	All	1.566	0.681	0.709	-0.797	0.351	3.026	0.192	0.578	2.267 <sup>b</sup>
Barnwell	2009	Soybean	<i>E. servus</i>	0.771	0.773	0.757	-0.757	0.089	1.000	0.093	0.000	0.917
Barnwell	2009	Soybean	<i>C. hilaris</i>	92.073	4.501	0.674	1.874	-0.360	8.596	0.370	1.791	3.159 <sup>b</sup>
Barnwell	2009	Soybean	All	4.724	1.773	0.615	0.932	-0.991	6.327	0.504	2.245	2.842 <sup>b</sup>
Barnwell	2010	Cotton	<i>E. servus</i>	3.506	1.491	0.976	3.186 <sup>a</sup>	-0.162	3.940	0.591	1.554	1.154 <sup>b</sup>
Barnwell	2010	Cotton	All	1.672	1.126	0.976	1.297	-0.004	2.958	0.647	2.831 <sup>a</sup>	1.346 <sup>b</sup>
Barnwell	2010	Soybean	All	0.954	0.557	0.689	-1.125	0.464	0.608	0.052	-0.624	1.247
Barnwell	2011	Corn	<i>E. servus</i>	1.251	3.681	0.814	3.023 <sup>a</sup>	-0.406	2.677	0.667	2.173	2.083 <sup>b</sup>
Barnwell	2011	Corn	All	1.251	3.681	0.814	3.023 <sup>a</sup>	-0.406	2.677	0.667	2.173	2.084 <sup>b</sup>
Barnwell	2011	Cotton	<i>E. servus</i>	7.092	1.926	0.980	2.555	-0.063	2.670	0.581	1.353	1.210 <sup>b</sup>
Barnwell	2011	Cotton	All	3.080	1.479	0.966	2.174	-0.156	3.271	0.589	1.818	1.123
Lee	2009	Cotton	<i>E. servus</i>	1.141	1.108	0.999	4.750 <sup>a</sup>	-0.051	1.174	0.975	2.155	1.429 <sup>b</sup>
Lee	2009	Cotton	All	1.122	1.319	0.975	4.132 <sup>a</sup>	-0.117	1.044	0.746	0.216	1.001
Lee	2009	Soybean	<i>E. servus</i>	2.248	1.322	0.560	0.750	-0.259	2.740	0.479	2.110	1.936 <sup>b</sup>
Lee	2009	Soybean	<i>N. viridula</i>	1.947	1.101	0.783	0.250	-0.245	4.931	0.300	1.877	1.639 <sup>b</sup>

Location	Year	Crop	Species	Taylor's power law				Iwao's regression				I <sub>D</sub>
				<i>a</i>	<i>b</i>	R <sup>2</sup>	<i>t</i> -value for slope = 1	<i>a</i>	β	R <sup>2</sup>	<i>t</i> -value for slope = 1	
Lee	2009	Soybean	<i>C. hilaris</i>	0.720	0.677	0.830	-1.707	0.103	1.121	0.171	0.170	1.216 <sup>b</sup>
Lee	2009	Soybean	All	2.379	1.750	0.655	1.597	-0.317	2.663	0.523	2.266 <sup>a</sup>	2.354 <sup>b</sup>
Lee	2009	Wheat	<i>E. servus</i>	1.927	2.010	0.997	14.064 <sup>a</sup>	-0.455	2.680	0.960	8.671 <sup>a</sup>	5.099 <sup>b</sup>
Lee	2009	Wheat	<i>N. viridula</i>	2.301	0.981	0.630	-0.035	-0.255	6.503	0.234	1.552	2.364 <sup>b</sup>
Lee	2009	Wheat	All	1.530	2.179	0.985	7.371 <sup>a</sup>	-0.226	2.457	0.881	4.730 <sup>a</sup>	4.869 <sup>b</sup>
Lee	2009	DSB	<i>E. servus</i>	7.467	2.241	0.815	2.057	-0.326	3.677	0.487	2.841 <sup>a</sup>	1.303 <sup>b</sup>
Lee	2009	DSB	<i>N. viridula</i>	1.756	0.987	0.987	-0.182	-0.011	2.507	0.831	3.184 <sup>a</sup>	2.017 <sup>b</sup>
Lee	2009	DSB	<i>C. hilaris</i>	7.151	1.708	0.975	4.252 <sup>a</sup>	-0.413	9.266	0.911	9.611 <sup>a</sup>	1.899 <sup>b</sup>
Lee	2009	DSB	All	1.913	1.131	0.917	1.028	-0.104	2.291	0.763	3.518 <sup>a</sup>	1.908 <sup>b</sup>
Lee	2010	Corn	<i>E. servus</i>	26.174	3.863	0.960	4.335 <sup>a</sup>	-0.135	2.118	0.632	1.934	1.381 <sup>b</sup>
Lee	2010	Corn	All	12.701	3.230	0.987	10.216 <sup>a</sup>	-0.321	3.401	0.664	2.672 <sup>a</sup>	1.767 <sup>b</sup>
Lee	2010	Soybean	<i>E. servus</i>	0.980	0.719	0.402	-0.648	0.035	1.392	0.162	0.440	1.265 <sup>b</sup>
Lee	2010	Soybean	<i>C. hilaris</i>	1.846	1.318	0.997	6.094 <sup>a</sup>	0.265	1.534	0.906	1.341	2.246 <sup>b</sup>
Lee	2010	Soybean	All	2.022	0.776	0.596	-0.828	0.205	1.790	0.424	1.241	2.222 <sup>b</sup>
Lee	2010	Wheat	All	2.255	2.955	0.972	6.498 <sup>a</sup>	-1.073	3.693	0.740	4.005 <sup>a</sup>	2.224 <sup>b</sup>
Lee	2011	Corn	<i>E. servus</i>	0.016	7.449	0.998	18.622 <sup>a</sup>	-0.561	5.293	0.855	4.158 <sup>a</sup>	12.201 <sup>b</sup>
Lee	2011	Corn	All	0.016	7.449	0.998	18.622 <sup>a</sup>	-0.561	5.293	0.855	4.158 <sup>a</sup>	12.201 <sup>b</sup>
Lee	2011	Cotton	<i>E. servus</i>	1.605	1.183	0.897	0.571	-0.095	2.485	0.167	1.021	1.113
Lee	2011	Cotton	All	1.219	0.960	0.631	-0.088	-0.038	3.363	0.092	0.756	1.314 <sup>b</sup>

Location	Year	Crop	Species	Taylor's power law				Iwao's regression				I <sub>D</sub>
				<i>a</i>	<i>b</i>	R <sup>2</sup>	<i>t</i> -value for slope = 1	$\alpha$	$\beta$	R <sup>2</sup>	<i>t</i> -value for slope = 1	
Lee	2011	Soybean	<i>E. servus</i>	1.545	0.779	0.670	-0.605	0.497	1.121	0.157	0.179	1.904 <sup>b</sup>
Lee	2011	Soybean	All	1.649	0.645	0.762	-1.776	0.538	1.113	0.297	0.224	2.117 <sup>b</sup>
Tift	2009	Cotton	<i>E. servus</i>	1.126	1.066	0.981	0.972	-0.050	1.233	0.535	0.603	1.047
Tift	2009	Cotton	All	0.828	0.900	0.936	-0.901	0.073	0.629	0.029	-0.331	1.074
Tift	2009	Fallow	<i>E. servus</i>	1.760	1.226	0.993	3.167	0.086	1.668	0.637	1.299	1.794 <sup>b</sup>
Tift	2009	Fallow	All	2.004	1.223	0.995	3.783 <sup>a</sup>	0.132	1.923	0.724	1.906	1.988 <sup>b</sup>
Tift	2009	Soybean	<i>E. servus</i>	3.223	1.239	0.667	0.386	-0.492	5.717	0.392	1.845	2.548 <sup>b</sup>
Tift	2009	Soybean	<i>N. viridula</i>	1.708	0.994	0.768	-0.014	-0.175	4.135	0.220	1.229	1.753 <sup>b</sup>
Tift	2009	Soybean	All	2.311	1.002	0.861	0.010	-0.026	2.992	0.570	2.204 <sup>a</sup>	2.482 <sup>b</sup>
Tift	2010	Cotton	<i>E. servus</i>	1.169	1.040	0.959	0.328	-0.040	1.984	0.375	1.133	1.087
Tift	2010	Cotton	<i>N. viridula</i>	0.593	0.775	0.873	-1.348	0.170	1.510	0.007	0.068	1.211 <sup>b</sup>
Tift	2010	Cotton	<i>C. hilaris</i>	1.056	1.008	0.954	0.060	-0.026	1.671	0.173	0.530	1.062
Tift	2010	Cotton	All	1.477	1.063	0.983	0.810	0.032	2.427	0.721	2.676 <sup>a</sup>	1.399 <sup>b</sup>
Tift	2010	Fallow	<i>E. servus</i>	3.543	1.451	0.995	4.676 <sup>a</sup>	-0.226	5.623	0.918	4.656 <sup>a</sup>	1.240 <sup>b</sup>
Tift	2010	Fallow	All	4.262	1.553	0.996	6.598 <sup>a</sup>	-0.326	6.163	0.899	4.579 <sup>a</sup>	1.196
Tift	2010	Peanut	<i>E. servus</i>	2.331	1.438	0.997	10.985 <sup>a</sup>	-0.214	2.783	0.933	6.306 <sup>a</sup>	1.398 <sup>b</sup>
Tift	2010	Peanut	All	2.293	1.418	0.996	10.377 <sup>a</sup>	-0.195	2.733	0.930	6.480 <sup>a</sup>	1.359
Tift	2011	Fallow	<i>E. servus</i>	2.212	1.374	0.991	4.220 <sup>a</sup>	1.145	1.333	0.592	-3.650 <sup>a</sup>	1.099
Tift	2011	Fallow	All	2.212	1.374	0.991	4.220 <sup>a</sup>	1.145	1.333	0.592	-3.650 <sup>a</sup>	1.069

I<sub>D</sub> = Overall index of dispersion, aggregated (>1), random (1) or uniform (<1).

<sup>a</sup>  $P < 0.05$ .

<sup>b</sup>  $\chi^2$  test indicated significant difference from 1 ( $P < 0.05$ ).

Table 2.3. Dispersion indices for stink bug nymphs in selected crops from Lee and Barnwell Counties, SC, and Tift County, GA. Locations and crops with insufficient samples for calculation have been omitted. Double-crop soybean are indicated by ‘DSB’.

Location	Year	Crop	Species	Taylor's power law				Iwao's regression				I <sub>D</sub>
				<i>a</i>	<i>b</i>	R <sup>2</sup>	<i>t</i> -value for slope = 1	$\alpha$	$\beta$	R <sup>2</sup>	<i>t</i> -value for slope = 1	
Barnwell	2009	Soybean	All	1.499	1.044	0.926	0.282	-0.030	3.424	0.342	1.353	1.339 <sup>b</sup>
Barnwell	2010	Soybean	All	3.734	2.391	0.995	8.652 <sup>a</sup>	-1.733	5.533	0.943	7.519 <sup>a</sup>	3.489 <sup>b</sup>
Lee	2009	Cotton	All	0.615	0.326	0.510	-2.319 <sup>a</sup>	0.540	0.294	0.020	-0.749	1.478
Lee	2009	Soybean	<i>E. servus</i>	4.799	1.064	0.595	0.091	0.293	3.076	0.411	1.915	4.631 <sup>b</sup>
Lee	2009	Soybean	<i>N. viridula</i>	3.958	0.780	0.932	-1.742	2.746	1.168	0.112	0.107	4.823 <sup>b</sup>
Lee	2009	Soybean	<i>C. hilaris</i>	2.174	0.851	0.702	-0.389	0.174	3.988	0.216	1.154	2.691 <sup>b</sup>
Lee	2009	Soybean	All	12.925	0.482	0.683	-1.762	3.906	1.966	0.248	0.835	8.437 <sup>b</sup>
Lee	2009	Wheat	<i>E. servus</i>	2.653	1.260	0.991	3.013	0.976	1.467	0.817	1.405	5.646 <sup>b</sup>
Lee	2009	Wheat	<i>N. viridula</i>	4.163	0.868	0.994	-2.431 <sup>a</sup>	0.452	2.311	0.776	1.887	5.218 <sup>b</sup>
Lee	2009	Wheat	All	3.367	1.005	0.998	0.181	1.313	1.218	0.911	1.187	7.118 <sup>b</sup>
Lee	2009	DSB	<i>E. servus</i>	1.840	8.317	0.998	10.498 <sup>a</sup>	-0.358	2.468	0.956	5.163 <sup>a</sup>	2.781 <sup>b</sup>
Lee	2009	DSB	<i>N. viridula</i>	3.066	1.495	0.999	6.972 <sup>a</sup>	0.088	2.591	0.935	4.612 <sup>a</sup>	5.135 <sup>b</sup>
Lee	2009	DSB	All	1.171	1.945	0.998	11.779 <sup>a</sup>	0.123	1.741	0.937	3.707 <sup>a</sup>	5.433 <sup>b</sup>
Lee	2010	Soybean	<i>E. servus</i>	0.000	21.453	0.988	8.174 <sup>a</sup>	-0.223	2.735	0.641	1.955	5.274 <sup>b</sup>
Lee	2010	Soybean	<i>N. viridula</i>	4.755	1.554	0.978	2.829 <sup>a</sup>	0.399	3.946	0.347	1.837	2.073 <sup>b</sup>
Lee	2010	Soybean	All	4.670	0.906	0.617	-0.139	0.096	2.336	0.614	1.669	5.709 <sup>b</sup>
Lee	2010	Wheat	<i>E. servus</i>	1.399	0.000	0.244	0.005	6.684	-2.863	0.413	-2.462 <sup>a</sup>	2.180
Lee	2010	Wheat	All	0.757	1.649	0.963	2.948 <sup>a</sup>	-0.196	1.156	0.904	0.924	2.179 <sup>b</sup>

Location	Year	Crop	Species	Taylor's power law				Iwao's regression				I <sub>D</sub>
				<i>a</i>	<i>b</i>	R <sup>2</sup>	<i>t</i> -value for slope = 1	<i>a</i>	β	R <sup>2</sup>	<i>t</i> -value for slope = 1	
Lee	2011	Soybean	<i>E. servus</i>	12.767	0.733	0.460	-0.354	1.020	1.989	0.456	1.158	11.413 <sup>b</sup>
Lee	2011	Soybean	All	20.965	0.452	0.559	-1.863	3.700	1.233	0.472	0.409	13.871 <sup>b</sup>
Tift	2009	Fallow	<i>E. servus</i>	2.439	0.944	0.957	-0.448	0.980	2.359	0.200	0.586	2.714 <sup>b</sup>
Tift	2009	Fallow	All	2.498	1.024	0.946	0.149	0.560	3.000	0.286	0.926	2.520 <sup>b</sup>
Tift	2009	Soybean	<i>E. servus</i>	2.786	1.427	0.930	0.613	-0.230	3.530	0.739	2.290	2.324 <sup>b</sup>
Tift	2009	Soybean	All	1.882	0.614	0.890	-1.833	0.762	2.411	0.174	0.742	2.691 <sup>b</sup>
Tift	2010	Cotton	All	240.718	2.882	0.996	10.926 <sup>a</sup>	-1.047	34.257	0.840	5.273 <sup>a</sup>	2.524 <sup>b</sup>
Tift	2010	Fallow	<i>E. servus</i>	2.223	1.476	0.832	0.728	-0.092	2.081	0.460	0.831	1.240 <sup>b</sup>
Tift	2010	Fallow	All	2.647	1.694	0.913	1.196	-0.069	1.822	0.657	1.082	1.215 <sup>b</sup>
Tift	2010	Peanut	All	1.341	0.885	0.989	-1.939	0.438	1.150	0.244	0.110	1.755 <sup>b</sup>
Tift	2011	Fallow	<i>E. servus</i>	1.558	1.092	0.945	0.627	-0.032	2.558	0.546	1.335	1.333 <sup>b</sup>
Tift	2011	Fallow	All	1.516	1.133	0.949	0.769	-0.051	2.143	0.598	1.301	1.254

I<sub>D</sub> = Overall index of dispersion, aggregated (>1), random (1) or uniform (<1).

<sup>a</sup>  $P < 0.05$ .

<sup>b</sup>  $\chi^2$  test indicated significant difference from 1 ( $P < 0.05$ ).

Table 2.4. SADIE summary data analyses for year-end total stink bug dispersion indices across all crops by location for 2009-2011.

Location	Year	Species	Adult		Nymph		Association	
			$I_a$	$P_a$	$I_a$	$P_a$	$X$	P(X)
Barnwell	2009	<i>E. servus</i>	2.022 <sup>a</sup>	0.0002	--	--	--	--
		<i>C. hilaris</i>	1.195	0.1421	1.037	0.3476	0.464 <sup>b</sup>	0.0089
		<i>N. viridula</i>	1.058	0.3204	1.418	0.0273	0.367 <sup>b</sup>	0.0151
		All	1.825 <sup>a</sup>	0.0007	1.156	0.1820	0.598 <sup>b</sup>	<0.0001
Barnwell	2010	<i>E. servus</i>	1.175	0.1584	1.116	0.2247	0.485 <sup>b</sup>	0.0010
		<i>C. hilaris</i>	1.180	0.1537	1.023	0.3866	0.326 <sup>b</sup>	0.0230
		<i>N. viridula</i>	--	--	1.118	0.2306	--	--
		All	1.309	0.0754	0.958	0.5247	0.324 <sup>b</sup>	0.0187
Barnwell	2011	<i>E. servus</i>	0.987	0.4597	--	--	--	--
		<i>C. hilaris</i>	0.887	0.6906	1.312	0.0618	0.434 <sup>b</sup>	0.0110
		<i>N. viridula</i>	--	--	--	--	--	--
		All	1.054	0.3377	1.167	0.1798	0.090	0.3061
Lee	2009	<i>E. servus</i>	1.249	0.0984	1.427	0.0255	0.569 <sup>b</sup>	<0.0001
		<i>C. hilaris</i>	0.885	0.7178	1.103	0.2423	0.293	0.0328
		<i>N. viridula</i>	1.310	0.0603	1.357	0.0402	0.535 <sup>b</sup>	<0.0001
		All	1.361	0.0385	1.472 <sup>a</sup>	0.0146	0.706 <sup>b</sup>	<0.0001
Lee	2010	<i>E. servus</i>	1.289	0.0670	2.016 <sup>a</sup>	0.0002	0.579 <sup>b</sup>	<0.0001
		<i>C. hilaris</i>	1.667 <sup>a</sup>	0.0017	1.477 <sup>a</sup>	0.0132	0.491 <sup>b</sup>	0.0006
		<i>N. viridula</i>	1.024	0.3752	1.151	0.1766	0.439 <sup>b</sup>	0.0046
		All	1.389	0.0275	2.058 <sup>a</sup>	0.0002	0.617 <sup>b</sup>	<0.0001

Location	Year	Species	Adult		Nymph		Association	
			$I_a$	$P_a$	$I_a$	$P_a$	$X$	P(X)
Lee	2011	<i>E. servus</i>	1.852 <sup>a</sup>	0.0003	1.041	0.3472	0.360 <sup>b</sup>	0.0141
		<i>C. hilaris</i>	0.813	0.8966	1.115	0.2199	0.413 <sup>b</sup>	0.0020
		<i>N. viridula</i>	--	--	--	--	--	--
		All	1.835 <sup>a</sup>	0.0003	1.070	0.2961	0.384 <sup>b</sup>	0.0061
Tift	2009	<i>E. servus</i>	1.292	0.0771	1.259	0.0897	0.291 <sup>b</sup>	0.0069
		<i>C. hilaris</i>	0.853	0.7783	--	--	--	--
		<i>N. viridula</i>	1.524 <sup>a</sup>	0.0144	1.368	0.0474	0.582 <sup>b</sup>	<0.0001
		All	1.441	0.0258	1.435	0.0282	0.398 <sup>b</sup>	<0.0001
Tift	2010	<i>E. servus</i>	1.119	0.2226	1.030	0.3617	0.118	0.1826
		<i>C. hilaris</i>	1.124	0.2041	0.899	0.6524	-0.028	0.5934
		<i>N. viridula</i>	1.008	0.4153	1.139	0.1966	0.123	0.1888
		All	1.207	0.1354	1.104	0.2517	0.165	0.0874
Tift	2011	<i>E. servus</i>	1.049	0.3270	1.499 <sup>a</sup>	0.0188	0.359 <sup>b</sup>	0.0031
		<i>C. hilaris</i>	--	--	--	--	--	--
		<i>N. viridula</i>	--	--	--	--	--	--
		All	1.103	0.2422	1.425	0.0288	0.407 <sup>b</sup>	0.0009

$I_a$  = Overall index of dispersion indicating aggregated (>1), random (1) or uniform (<1) pattern. <sup>a</sup>Significance in aggregation determined by  $\alpha = 0.05$  ( $P < 0.025$  or  $P > 0.975$ ).

$P_a$  = p-value for null hypothesis of spatial randomness.

$X$  = Overall index of aggregation between each paired dataset. <sup>b</sup>Significance in association is positive for  $X > 0$  ( $P < 0.025$ ) or negative for  $X < 0$  ( $P > 0.975$ ).

Missing data represented by ‘-’ indicate that insect counts were insufficient to generate aggregation indices.



Table 2.5. Statistical comparisons of stink bug densities at different distances from field borders and adjacent crop combinations in Lee and Barnwell County, SC, and Tift County, GA.

Location	Effect	<i>Euschistus servus</i>						<i>Nezara viridula</i>					
		Adult			Nymph			Adult			Nymph		
		DF	F	P	DF	F	P	DF	F	P	DF	F	P
Lee	Crop/Adjacent	8, 11.45	0.62	0.7495	8, 11.42	3.84	0.0198	8, 11.41	2.84	0.0538	8, 11.45	0.62	0.7495
	Distance	3, 5.10	0.14	0.9305	3, 5.28	0.20	0.8898	3, 4.52	1.10	0.4364	3, 5.10	0.14	0.9305
	Interaction	24, 155.70	2.14	0.0030	24, 161.90	0.82	0.7090	24, 115.90	1.69	0.0358	24, 155.70	2.14	0.0030
Barnwell	Crop/Adjacent	10, 18.37	0.87	0.5719	10, 7.98	1.50	0.2895	10, 19.38	0.62	0.7767	10, 164.00	1.08	0.3809
	Distance	3, 123	0.15	0.9317	3, 3.54	0.11	0.9514	3, 123.00	0.48	0.6990	3, 164.00	1.24	0.2971
	Interaction	30, 123	0.45	0.9930	30, 67.40	0.71	0.8532	30, 123.00	0.70	0.8742	30, 164.00	1.08	0.3676
Tift	Crop/Adjacent	11, 1.56	0.17	0.9967	11, 17.33	0.11	0.9996	11, 16.41	0.58	0.8195	11, 12.82	0.75	0.6813
	Distance	2, 3.27	0.51	0.6410	2, 74.59	1.59	0.2102	2, 2.30	0.62	0.6098	2, 3.27	0.04	0.9589
	Interaction	22, 147.30	0.39	0.9939	22, 74.59	0.69	0.8372	22, 64.30	1.14	0.3294	22, 106.00	0.44	0.9856

Location	Effect	<i>Chinavia hilaris</i>						All species					
		Adult			Nymph			Adult			Nymph		
		DF	F	P	DF	F	P	DF	F	P	DF	F	P
Lee	Crop/Adjacent	8, 11.54	2.24	0.1037	8, 11.27	9.78	0.0004	8, 13.14	4.29	0.0098	8, 11.43	7.88	0.0011
	Distance	3, 5.55	0.93	0.4865	3, 5.57	0.41	0.7508	3, 6.26	1.60	0.2811	3, 5.56	0.03	0.9937
	Interaction	24, 162.60	0.82	0.7124	24, 166.40	2.65	0.0002	24, 161.60	1.50	0.0733	24, 164.20	2.39	0.0007
Barnwell	Crop/Adjacent	10, 41.00	15.56	<0.0001	10, 20.48	1.11	0.4004	10, 19.11	4.40	0.0027	10, 164.00	2.89	0.0024
	Distance	3, 123.00	0.32	0.8122	3, 153.10	0.56	0.6455	3, 123.00	0.21	0.8923	3, 164.00	1.18	0.3178
	Interaction	30, 123.00	0.91	0.6026	30, 153.10	0.54	0.9751	30, 123.00	0.51	0.9826	30, 164.00	1.11	0.3327
Tift	Crop/Adjacent	11, 14.95	0.74	0.6919	11, 5.31	0.05	1.0000	11, 11.50	0.18	0.9956	11, 16.96	0.13	0.9994
	Distance	2, 7.70	1.24	0.3401	2, 1.00	0.00	1.0000	2, 3.94	0.75	0.5288	2, 54.50	1.30	0.2809
	Interaction	22, 178.10	1.43	0.1044	22, 1.00	0.00	1.0000	22, 150.20	0.54	0.9544	22, 54.50	0.50	0.9602

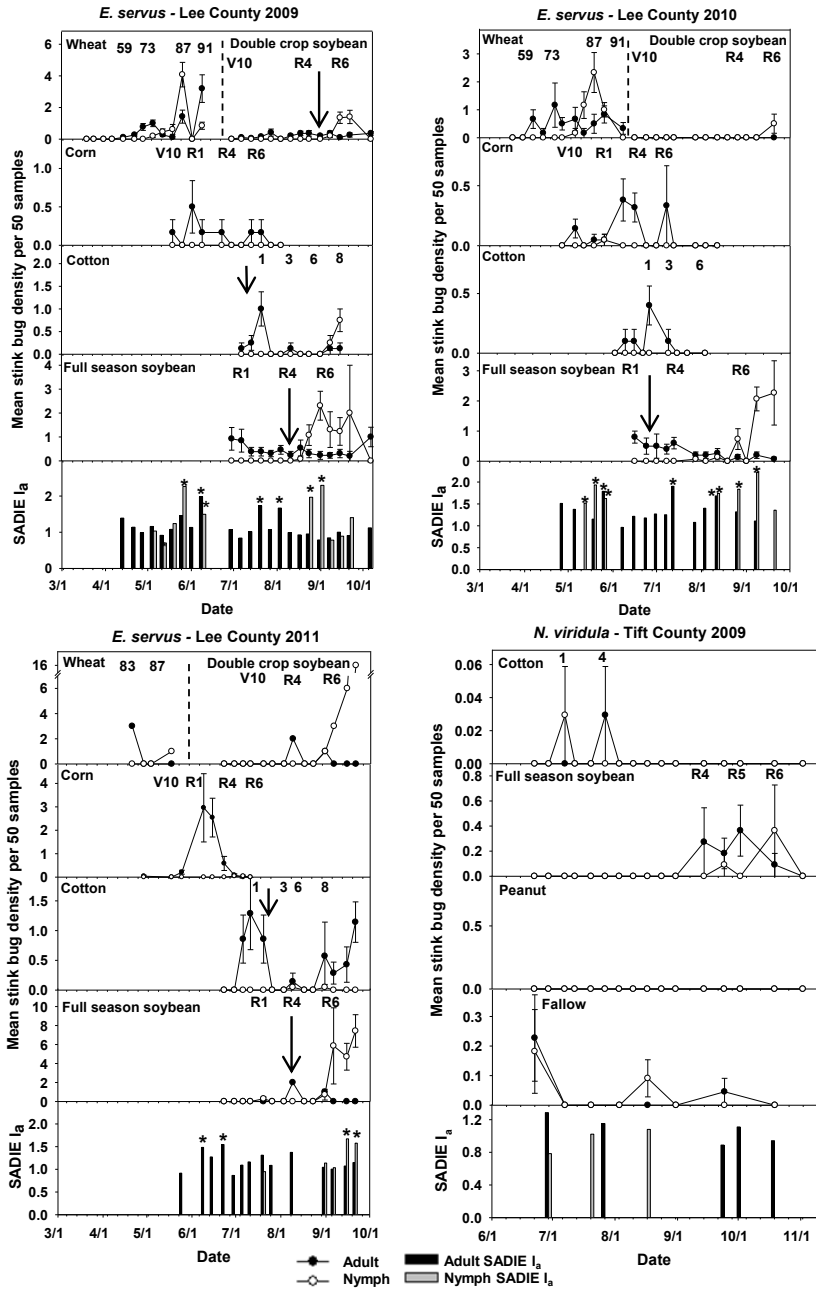


Fig. 2.1. Average densities for selected stink bug species ( $\pm$  SEM) and daily SADIE indices of dispersion over time in mixed crop farmscapes in Lee County, SC, and Tift County, GA. Arrows indicate dates sprayed. Crop phenology indicated by vegetative (V) and reproductive stages (R) in soybean and corn. Cotton stages are indicated by week of bloom. Wheat stages follow Zadoks scale. Peanut remained reproductive throughout the sampling periods.

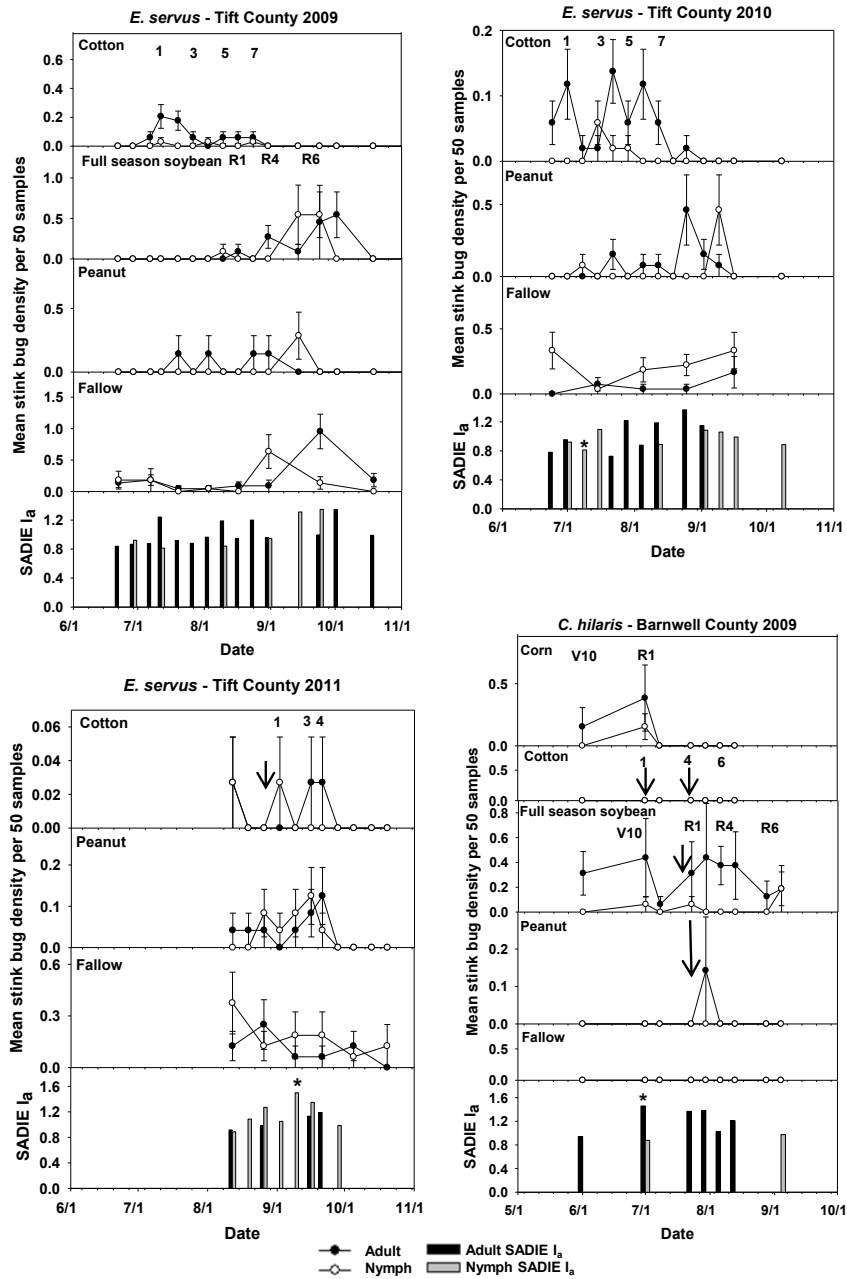


Fig. 2.2. Average densities ( $\pm$  SEM) and daily SADIE indices of dispersion for selected stink bug species over time in mixed crop farmscapes in Tift County, GA and Barnwell County, SC. Crop phenology indicated by vegetative (V) and reproductive stages (R) in soybean and corn. Cotton stages are indicated by week of bloom. Peanut remained reproductive throughout the sampling periods.

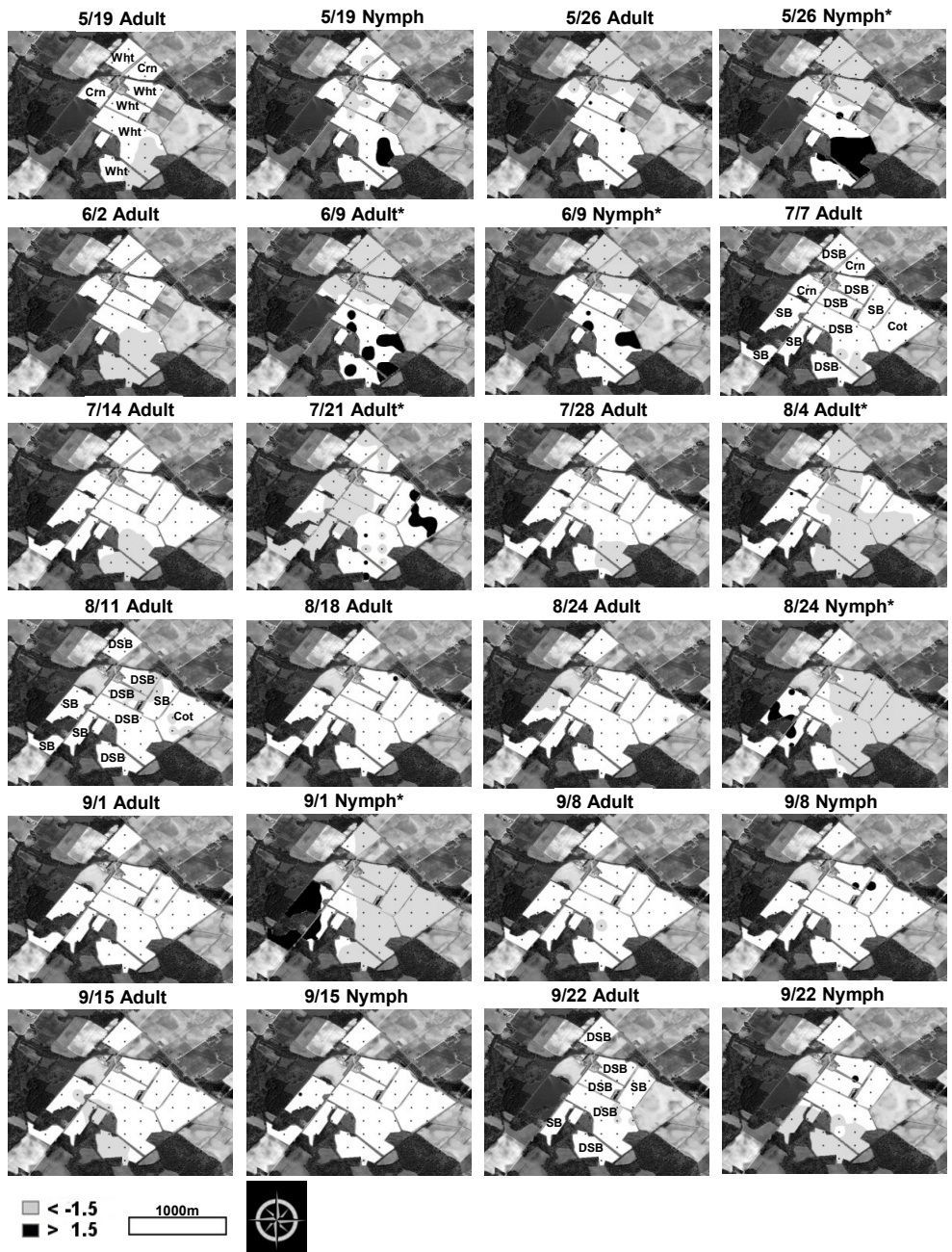


Fig. 2.3. Spatial interpolations of SADIE local aggregation indices for *E. servus* adults and nymphs over time in mixed crop farmscapes in Lee County, SC. Missing dates indicate insect counts were insufficient to generate local aggregation indices. Asterisks next to dates indicate significant ( $P < 0.025$ ) aggregations. “Wht”, “Crm”, “Cot”, “SB” and “DSB” indicate wheat, corn, cotton, soybean, and double-crop soybean, respectively.

Barnwell County *C. hilaris* 2009

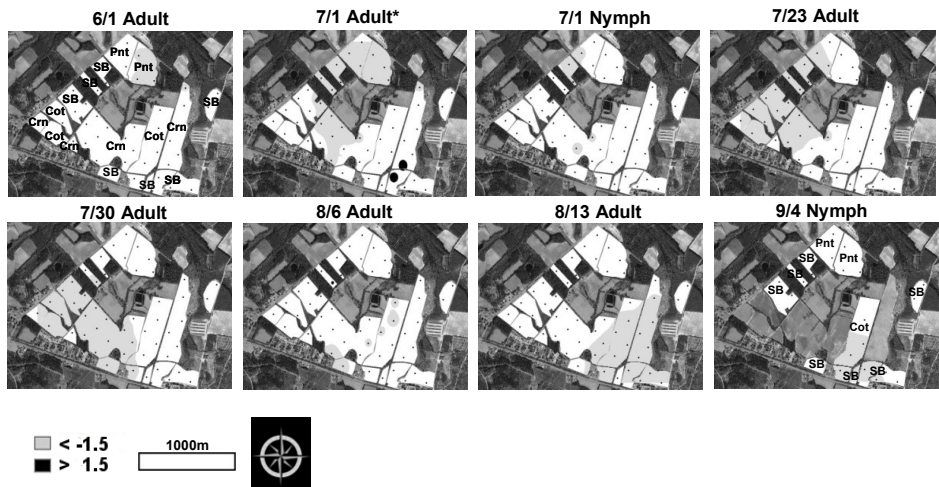


Fig. 2.4. Spatial interpolations of SADIE local aggregation indices for selected species of stink bug adults and nymphs over time in mixed crop farmscapes in Barnwell County, SC. Missing dates indicate insect counts were insufficient to generate local aggregation indices. Asterisks next to dates indicate significant ( $P < 0.025$ ) aggregations. “Crn”, “Cot”, “SB”, “Pnt”, “WM” and “F” indicate corn, cotton, soybean, peanut, watermelon, and fallow, respectively.

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## CHAPTER III

### BORDER APPLICATIONS OF INSECTICIDE TO CONTROL STINK BUGS

#### (HEMIPTERA: PENTATOMIDAE) IN COTTON

##### Introduction

Successful eradication of the boll weevil, *Anthonomus grandis grandis* Boheman, and widespread adoption of transgenic cultivars of cotton, *Gossypium hirsutum* (L.) expressing *Bacillus thuringiensis* (Bt) toxins to control the heliothine complex have led to a decrease in the application of broad-spectrum insecticides on cotton in the southeastern United States (Greene et al. 1999, Bundy and McPherson 2000). Reductions in insecticide usage have allowed stink bugs, once considered secondary pests, to become primary pests of cotton production (Greene et al. 1999, 2001). As Bt cultivars have become more widespread worldwide, increasing stink bug damage to cotton has been documented in Asia, South America, and the United States (Greene et al. 1999, Panizzi and Schaefer 2000, Zeng et al. 2009). The predominant pest species of phytophagous stink bugs in cotton in the southeastern United States are the green stink bug, *Chinavia hilaris* (Say), the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euschistus servus* (Say) (McPherson and McPherson 2000).

Cotton is damaged both by the mechanical and enzymatic action of stink bug feeding and by pathogen introduction, either as opportunistic infections in the wound or by direct transmission of bacterial and fungal organisms (Ragsdale et al. 1979, Barbour et al. 1990, Medrano et al. 2007). Stink bug feeding injury to the developing cotton boll ranges from stained lint and damaged seeds to pathogen-induced boll rot and aborted

bolls (Wene and Sheets 1964, Barbour et al. 1990, Medrano et al. 2009). Stink bug management relies on scouting and judicious use of insecticides when thresholds are reached. Scouting consists of either collection of immature bolls for internal injury assessment or the use of sweep nets and beat cloths to directly sample stink bugs (Greene et al. 2001, Reay-Jones et al. 2009, 2010a). Cooperative Extension in southeastern states recommend a dynamic treatment threshold of 50% boll injury in the first week of bloom (WOB), 30% for the second WOB, 10% for the third to fifth WOB, 20% for the sixth WOB, and 30% for the seventh WOB (Bachelier et al. 2007, Greene et al. 2008, 2009). The dynamic threshold has replaced older static thresholds of 20% boll injury, which over- or underestimate the need for insecticide applications. Because populations of susceptible bolls increase over the progression of the flowering period, applications of insecticide are more appropriately timed to prevent economic damage, using the dynamic threshold. Bolls that have accumulated 600 heat units beyond anthesis (24 d) are generally safe from stink bug injury (Willrich et al. 2004c).

Because of the widespread and increasing pest status of stink bugs on multiple crops in the United States, alternative management strategies must be developed to protect yields and reduce the frequent use of broad spectrum pyrethroid or organophosphosphate insecticides. The border spray concept was suggested following studies showing boll injury generally starting along cotton field edges in South Carolina and Georgia (Toews and Shurley 2009, Reeves et al. 2010). Within a week or two of initial infestation, additional injury was evident in interior portions of the field, with colonization likely originating from the edges. Therefore, treating field borders with

insecticide upon first signs of injury could mitigate the need to treat the entire field. Border treatments have been successfully used in the control of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), and the green peach aphid, *Myzus persicae* (Sulzer), in potato, *Solanum tuberosum* L. (Blom et al. 2002, Carroll and Radcliffe 2008), and the codling moth, *Cydia pomonella* (L.), apple maggot, *Rhagoletis pomonella* (Walsh), and plum curculio, *Conotrachelus nenuphar* (Herbst), in apple, *Malus domestica* Borkh (Trimble and Solymar 1997, Chouinard et al. 1992). Potential benefits from border applications include reduced quantity of active ingredient applied, reduced time to make applications, and increased conservation of natural enemies. Additional savings to the grower could be obtained through reduction of fuel required for applications. The objective of this study was to compare the impact on stink bugs and boll injury of border application of insecticides with whole-field application in cotton in South Carolina and in Georgia.

### Materials and methods

Trials were conducted in commercial and experiment station fields located in Georgia and South Carolina from 2007 to 2011. Fields were spatially mapped, using GIS mapping software (ArcView 9.2, ESRI 2006), then overlaid with a grid of sampling points at 0.4 ha intervals starting from the margins. Each sampling point was marked using a 2-m tall flag on a fiberglass pole with a unique identifying number and designated as 'interior' or 'exterior', with exterior sample points located on the outer edges of the field and interior sites entirely enclosed within the field. The GPS coordinates of all sampling



points were recorded. Starting at first bloom, weekly collections of soft quarter-sized bolls ( $\approx 2.5$  cm in diameter) for injury evaluation and sweep-net (diameter = 38 cm) samples for stink bugs were obtained at each sampling point. Numbers of bolls collected at each sampling point varied from 10 to 20 depending on field. Stink bug sampling consisted of two 25 sweep sub-samples, with each sweep covering two rows of cotton. Adults and nymphs of the major economic species (*N. viridula*, *C. hilaris*, and *E. servus*) were totaled for each sample prior to analysis. Bolls were returned to the laboratory for dissection. Internal injury to an individual boll (classified on a binomial scale) consisted of callus growths (warts) or stained lint (Greene et al. 1999, Bundy et al. 2000).

A total of 26 cotton fields were selected, with ten receiving border treatments, eight receiving whole-field treatments, and eight with no insecticide treatment (Table 1). Two of the border-treated fields in Barnwell County in 2009 received initial border treatments, but growers followed up with whole-field sprays in the fourth WOB. In Tift County in 2010, one field initially received border treatments followed by a whole-field treatment in the sixth WOB. For analysis, these fields were divided into pre- and post-whole-field application periods, with stink bug and boll-injury data analyzed with the appropriate group for the period.

In-field border treatments were triggered at first bloom, regardless of insect presence, except for field 17 in Florence County in 2011, which was treated in the second WOB. In South Carolina (six fields), tank-mixed applications of dicotophos (Bidrin 8) at 0.28 kg[AI]/ha + *lambda*-cyhalothrin (Karate 2.08) at 0.033 kg[AI]/ha were made using a small trailer mounted mist sprayer. In Georgia (four fields), border sprays were made

with 0.113 kg[AI]/ha Bidrin 8 + 0.056 kg[AI]/ha *beta*-cyfluthrin (Baythroid XL). The mist sprayer was pulled behind a pickup truck around the field, calibrated to deliver the correct amount of pesticide based on ground speed. The mist sprayer treated 12.1 to 24.4 m into the field depending on prevailing wind, providing an average coverage distance of 18.2 m. In field 17, the mist sprayer was used in the third WOB and a high-clearance tractor with a boom width of 19.3 m was used in the second, fourth, and fifth WOB. For this field, the full boom width was used to spray the longer sides of the field, and half the boom (7.7 m) was used to spray the ends of the field. Subsequent border applications were intended to be made weekly during first two to four weeks of bloom, though actual application numbers and times varied with field (Table 1). Fields receiving whole-field insecticide applications (five in South Carolina, three in Georgia) were treated with the same insecticides as in the border-treatments based on the widely accepted dynamic thresholds of 50, 30, 10, 10, 10, 20, and 30% for the first through seventh WOB (Bacheler et al. 2007, Greene et al. 2008, 2009). Some variation in insecticide applications occurred among the different growers in the commercial fields (Table 1).

Data analyses. The first to seventh WOB were analyzed for all fields. The response variables were the proportion of injured bolls and stink bug densities summed across species and life stages. A split-plot design with repeated measures was used with treatment (border, whole-field, and none) as the main-plot factor arranged in a completely randomized design with field as replicates. The sub-plot factor was location (interior and exterior) arranged in a randomized complete block design with field as the blocks. Week of bloom was the repeated measures factor. A linear model was developed with

treatment, interior or exterior location, week of bloom, and their interactions as fixed effects, and fields within treatments (i.e., whole plot error or error<sub>A</sub>), interaction of interior or exterior location within fields (i.e., subplot error or error<sub>B</sub>), and residual error with possible correlation (i.e., repeated measures error or error<sub>C</sub>) as random:

$$Y_{ijkl} = \mu + T_i + F(T)_{ij} + P_k + T^*P_{ik} + P^*F(T)_{ijk} + W_l + T^*W_{il} + P^*W_{kl} + T^*P^*W_{ikl} + W^*P^*F(T)_{ijkl}$$

where  $Y_{ijkl}$  is the response value in treatment  $i$ , field  $j$ , location  $k$ , and week  $l$ ;  $\mu$  is the overall mean of the response;  $T_i$  is the effect of treatment  $i$ ;  $F(T)_{ij}$  is the effect of field  $j$  within treatment  $i$  (error<sub>A</sub>);  $P_k$  is the effect of location  $k$ ;  $T^*P_{ik}$  is the interaction effect of treatment  $i$  and location  $k$ ;  $P^*F(T)_{ijk}$  is the interaction effect of location  $k$  and field  $j$  within treatment  $i$  (error<sub>B</sub>);  $W_l$  is the effect of week  $l$ ;  $T^*W_{il}$  is the interaction effect of treatment  $i$  and week  $l$ ;  $P^*W_{kl}$  is the interaction effect of location  $k$  and week  $l$ ;  $T^*P^*W_{ikl}$  is the interaction effect of treatment  $i$ , location  $k$ , and week  $l$ ; and  $W^*P^*F(T)_{ijkl}$  is the interaction effect of week  $l$ , location  $k$ , and field  $j$  within treatment  $i$  (error<sub>C</sub>). Model terms were tested using analysis of variance (ANOVA) in PROC GLIMMIX (SAS Institute 2008). PROC GLIMMIX was chosen to estimate the model terms as the experimental design of the study involved random effects such as fields within treatments and residual error with possible correlations. GLIMMIX allows for random effects and appropriate error terms for the level of the split plot. Residuals produced from the model were checked with PROC UNIVARIATE (SAS Institute 2008) and scatterplots to check assumptions. The Shapiro-Wilk test was used to assess normality, and Levene's test was used to check for equal variance. These procedures showed that average stink bug counts

and boll injury required no transformation. As such, a normal distribution was used with means separated using the Tukey-Kramer method to compensate for the unequal number of replications per treatment (Kramer 1956, Hayter 1984). Significance for model terms was determined using a probability level of 95% ( $P < 0.05$ ).

The Spatial Analysis by Distance Indices (SADIE) red-blue methodology of Perry et al. (1999) was used to analyze stink bug count data and percentage boll injury (converted to integer form) separately for each field, with sampling points expressed as absolute positions. SADIE analysis determines the minimum distance  $D$  needed to achieve regularity, which is the distance moved by counts in the observed sample to reach the most uniform distribution possible. A clustering index was assigned to every sampling point, with either a positive patch index ( $\bar{v}_i$ ) for sites above the mean for each field-sampling date combination, or a negative index value ( $\bar{v}_j$ ) for sites below the mean. Randomness was indicated by indices  $\bar{v}_i = -\bar{v}_j = 1$ . Nonrandomness was quantified by comparing the observed patterns with hypothetical random rearrangements across the sampling area. The overall index of dispersion ( $I_a$ ) can indicate an aggregated pattern ( $>1$ ), randomness ( $=1$ ), or a uniform distribution ( $<1$ ) in the field. Spatial randomness is rejected for  $P < 0.025$  (aggregation) or  $P > 0.975$  (uniformity).

The SADIE association tool was used to determine spatial associations between stink bug counts and the percentage of boll injury expressed as counts. An overall index of association ( $X$ ) was determined between each paired dataset, with positive associations for  $X > 0$  ( $P < 0.025$ ) or negative associations for  $X < 0$  ( $P > 0.975$ ). Mean  $X$  was determined from the local spatial associations ( $X_k$ ) generated by the association tool for

each sampling location  $k$ . At the local scale, a positive association between two variables indicates the presence of either a patch or a gap for both variables; a negative association indicates the presence of a patch for one variable and gap for the other variable at the same location (Perry 1997, 1998). Selected SADIE aggregation indices for stink bugs, boll injury, and association indices were imported into the geographical information system software ArcView 9.2 (ESRI 2006) and the Inverse Distance Weighting (IDW) method was used to create interpolation maps (one per treatment) for three selected fields. These fields were chosen for similar size and number of insecticide applications in the border and whole-field treatments.

### Results

Over five years of study, a total of 73,758 bolls and 1,293 stink bugs were collected, with 48.6% *E. servus*, 30.2% *C. hilaris*, 17.2% *N. viridula*, and 4.0% other stink bug species. Average stink bugs captured per 50 sweeps was  $0.3 \pm 0.03$  (SEM) (range of 0–20), and the average percentage boll injury was  $16.0 \pm 0.7$  (range of 0–100%) per sample. Stink bug densities were not significantly influenced by treatment ( $P > 0.05$ ), but were 1.6-fold greater on the exterior compared with the interior of fields averaged across treatments and WOB (Table 2 and Fig. 1B). Averaged across all treatments, numbers of stink bugs increased from the second to the fourth WOB, trending lower in the sixth but not significantly (Fig. 1A). Cotton boll injury was lowest across treatments in the first WOB, significantly increased until the fourth WOB, and remained consistently near 20% until the seventh WOB (Fig. 1C). No significant differences in boll injury were

detected between border and whole-field applications, but both had significantly lower injury than untreated fields (Figs. 1D and 2). Average boll injury was significantly lower in whole-field and border treated fields compared to untreated fields in the sixth WOB, though trends for greater injury in untreated fields were noted from the fourth WOB (Fig. 1E). Boll injury was 1.5-fold greater in the exterior of the fields than in the field interior (Fig. 1F). All three of the fields receiving both border and whole-field treatments exceeded thresholds at least once (Fig. 2). Fields receiving no insecticide applications exceeded the threshold 55% of the time. Fields receiving whole-field or border applications of insecticide exceeded thresholds 41 and 30% of the time, respectively.

Sufficient numbers of stink bugs for SADIE analyses were found in 25 of 29 (86.2%) year-end totals (Table 3). Only three year-end SADIE indices (12%) indicated significant aggregation ( $P < 0.025$ ) at the 5% error level, two in border application fields and one in a whole-field application field. Out of 29 average yearly boll injury indices, five (17%) indicated significant aggregation ( $P < 0.025$ ), with four in border-sprayed fields and one in a whole-field application field. Despite the low number of significant  $I_a$  indices, significant associations were found between stink bugs and boll injury averaged across sampling dates in nine out of 25 analyses (36%), with three in fields receiving border applications, four in fields receiving whole-field applications, and two in untreated fields.

SADIE aggregation indices also varied with week of bloom. Out of 157 weekly sample datasets for stink bug counts and boll injury by field, 105 stink bug (67%) and 134 boll injury (85%) datasets contained captures or injury at two or more sampling

points within a field, permitting analysis. Aggregation indices were significant for 11 stink bug datasets (10%) and 14 boll injury datasets (10%). In the fourth WOB, stink bugs in fields without treatments were aggregated only once. Stink bugs were aggregated three times in the first, third, and seventh WOB in the eight untreated fields in 49 sampled weeks (6.1%) (Table 4). Stink bugs and boll injury were significantly associated in three out of 39 (7.7%) paired weekly datasets in the third, sixth, and seventh WOB in untreated fields (Table 4). Stink bugs in fields receiving border treatments were significantly aggregated in four of 30 (13.3%) sample weeks, from the fourth (one significant index), fifth (two) and sixth (one) WOB, and injury was aggregated in the second (two), fourth (one) fifth (one) and sixth (one) WOB in 31 sampled weeks from seven fields (16.1%). Significant associations between stink bugs and boll injury were found in three out of 23 paired weekly datasets (13%) from border-treated fields in the fourth (one) and fifth (two) WOB (Table 4). In fields receiving whole-field insecticide applications, stink bugs were sampled in sufficient numbers in 6 out of 33 sample weeks, with uniform distributions (18.2%) from the second (one), third (one), and fourth (one) WOB and aggregated distributions in the fourth (two) and seventh (one) WOB. Boll injury in whole-field treated fields was significantly aggregated in four of 42 sample weeks in the third (one), sixth (one) and seventh (two) WOB.

Associations between stink bugs and boll injury were analyzed for 29 weeks; five (17.2%) were significant in the second (one) fifth (two) and sixth (two) WOB (Table 4). In fields receiving both whole-field and border treatments, only field 9 provided stink bug capture data. No stink bugs were found in samples from fields 10 and 11 in Cameron, SC.

Injury from those fields was significantly aggregated in just two of 12 sample weeks, both in the third WOB (Table 4).

Interpolation maps confirmed greater stink bug densities, boll injury, and associations primarily along the field exteriors (Fig. 3). The untreated field 1 in 2007 had year-end average injury of  $30.1 \pm 2.6\%$ , whereas field 19 in 2007 and field 12 in 2009, receiving whole-field and border treatments, respectively, had yearly average boll injury levels of  $23.9 \pm 1.5\%$  and  $20.9 \pm 2.4\%$  (Fig. 3). The SADIE indices of association between year-end stink bugs totals and mean injury for these three fields were significant only in the border-treated field (Fig. 3).

In the same three fields (Fig. 4), boll injury was detected in the second WOB for all three fields, peaking in the fifth WOB for the untreated field, the third WOB in the whole-field treated field, and the fifth WOB for the border-treated field. By the fourth WOB, all fields were above threshold. Injury in field 19 receiving whole-field treatments fell to 26.5% by the fifth WOB after insecticide applications but remained above threshold until the seventh WOB (Figs. 2 and 4). In field 12 receiving border applications, injury levels were above threshold in weeks 4 and 5, with a maximum injury level of 22.9%, dropping below threshold in the sixth WOB. Injury levels in the untreated field 1 reached 52.9% in the fifth WOB and remained consistently above threshold for the remainder of the season. Injury decreased in all fields in the sixth WOB, though higher levels were found in the untreated field in the seventh WOB than in either treated field.



## Discussion

To be considered effective, border applications of insecticide must provide substantially equivalent control of boll injury as applications to the whole field. Our study demonstrated border applications reducing stink bug injury in cotton as effectively as whole-field insecticide treatments (Table 2 and Fig. 1). The area of fields receiving border applications ranged from 12.9 to 30.6% of the total field area, resulting in an average of 77.3% savings in active ingredient alone over whole-field applications. Additional savings in fuel costs, manpower hours, and equipment maintenance expenditures likely further increase the value of border treatments to the grower.

The lack of significant effects of insecticide treatments on stink bug populations in this study is likely due to the mobility of stink bugs, as opposed to the semi-permanent nature of boll injury, which is one reason why growers and consultants prefer to use injury when scouting cotton (Reay-Jones et al. 2010a). Border and whole-field treatments decreased average boll injury, but did not greatly influence the general timing of injury peaks (Figs. 1, 2, and 4). The highest injury levels were recorded during the third through fifth WOB, when the greatest proportion of bolls susceptible to feeding injury occur in cotton (Bachelier et al. 2007, Greene et al. 2008, 2009, Greene 2013) saw the highest injury levels recorded (Fig. 1).

Many arthropod species, including stink bugs, exhibit spatial aggregation (Taylor et al. 1978, Reay-Jones et al. 2010b, Reay-Jones 2010). Significant SADIE indices based on stink bug counts indicated aggregation 73% of the time and a uniform distribution 27% of the time. Uniform distributions for weekly stink bug densities were limited to

fields receiving whole-field insecticide treatments, and 50% of aggregated distributions were detected in border-treated fields, implying that border-treatments had an influence on patterns of aggregation within fields. No significant uniform distributions for boll injury were detected. During the weeks where the maximum number of bolls are susceptible to stink bug damage (Wene and Sheets 1964, Barbour et al. 1990, Medrano et al. 2009), the fourth WOB contained 50% of the significant stink bug SADIE indices, with the third and fourth WOB together containing 50% of the significant boll injury SADIE indices across all treatments. The fifth WOB contained 38% of significant associations between stink bug counts and boll injury (Fig. 2), indicating that the weeks with the most bolls susceptible to stink bug injury also hosted the highest aggregations of injured bolls and associations between stink bugs and injury. All significant SADIE indices still totaled to only 10% of total indices. The limited numbers of significant SADIE aggregation indices for stink bugs are likely due to low counts, as Thomas et al. (2001) also noted SADIE as not being a sensitive method when applied to smaller datasets. Xu and Madden (2004) suggested the number of samples and their positions might influence the aggregation index more than their sizes. However, despite the average percentage of injured bolls collected being 5.3-fold greater than average stink bug densities, no difference in the percentage of significant SADIE indices was found between boll injury and stink bug densities. As 90% of SADIE indices indicated that distributions were not significantly different from random, insecticide treatments might have changed the level of severity of the damage, without overall changes in the location within the field where damage occurred (Fig. 4). A lack of location by treatment

interaction was found for stink bugs, though a strong trend was observed ( $P = 0.0556$ ) for the increase in boll injury in exterior portions of the field compared to interior portions to be more pronounced in untreated fields than in treated fields (Table 2).

Neither border nor whole-field treatments suppressed boll damage below the economic threshold in all cases (Table 2, Figs. 1 and 2), suggesting the insecticide applications did not provide complete control during peak susceptibility to injury, or more applications during these times were needed. The timing of whole-field applications did not always follow Extension recommended practices for applications of the insecticide. For example, field 20 in the whole-field treatment group received a single insecticide application in the sixth WOB despite being over threshold in the third and fourth weeks of bloom. Field 22 was treated with a whole-field application in the second WOB, but remained above threshold until an additional application in the sixth WOB. Increased insecticide applications during the third to fifth WOB, when more bolls were vulnerable to damage, might have reduced injury rates. In border-treated fields, field 11 received applications in the first and second WOB, yet remained over threshold until the sixth WOB when it received a whole-field treatment. Field 16 was treated in the first and second WOB, and received no further applications despite exceeding thresholds in the third, fourth, and fifth WOB due to weather problems (Table 1, Fig. 2). Despite the variations in numbers of treatments, the number of sample dates where fields with either whole-field or border treatments were over threshold, and the amounts by which threshold was exceeded, were still generally lower than fields receiving no treatment (Figs. 1 and 2).

Data from fields receiving both border and whole-field treatments suggested initial border-treatments in the first three weeks of bloom could be supplemented by a whole-field treatment in the fourth WOB, as in fields 9 and 10. Injury in field 9 dropped below threshold after the whole-field treatment. Field 10 remained a marginal 2% above threshold. Although these two example fields provide limited data, results suggest border applications of insecticide provided control during the early part of the season in these fields before a whole-field application was needed during the more damage-sensitive periods of crop growth.

The relationship between boll injury and yield loss is not linear (Willrich et al. 2004b). Although injury to young bolls can lead to boll dropping and complete loss, the result of damage to larger, older bolls can be highly variable in influence depending on the extent of physical damage (Willrich et al. 2004a, Willrich et al 2004b). *Nezara viridula* and *E. servus* can transmit the pathogenic boll-rot bacterium *Pantoea agglomerans* to cotton bolls (Medrano et al. 2007, 2011). *Chinavia hilaris* has been documented as transmitting yeast spot disease in soybean (Daugherty 1967), however, it has not been documented as transmitting boll rot-pathogens. Cotton yield drops with increased numbers of puncture wounds by *C. hilaris* (Barbour et al. 1990). Drought stress, as was present in Tift County in 2011, also can lead to boll abortion (Herbert and Toews 2012). Boll injury by stink bugs and cotton quality are most affected by surrounding crops when cotton fields are located adjacent to soybean or peanut, though proximity to corn does not significantly impact crop quality (Toews and Shurley 2009).

Further studies with yield measurements taken for all three treatments would provide useful information which could further clarify the protective value of border applications.

Parasitoids of stink bugs, such as *Trichopoda pennipes* (F.) (Diptera: Tachinidae), which parasitize adults, and *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae), which target eggs, can be important in reducing pest populations (Ehler 2000, Jones et al. 1996, Eger and Ables 1981). Parasitoids are more sensitive to insecticide applications than their stink bug hosts (Orr et al. 1989, Tillman 2011). Although not quantified here, border applications of insecticides might be less detrimental to parasitoids than whole-field applications. Conservation of natural enemies in untreated cotton has been linked to a decrease in secondary pest outbreaks of spider mites (Trombidiformes: Tetranychidae) and whiteflies (Hemiptera: Aleyrodidae) compared with outbreaks in cotton treated with broad-spectrum insecticides (Naranjo 2001). Fields receiving border treatments would preserve the interior of the field as an untreated refuge for natural enemies. Border treatments have also been suggested to reduce the potential for insecticide resistance in insect pests by reducing the amount of active ingredient applied to fields (Blom et al. 2002).

Information obtained from five years of field data indicate border applications of insecticide are useful in limiting boll injury by stink bugs in cotton as effectively as traditional whole-field insecticide applications. Boll injury in fields with border applications of insecticides was not significantly different from boll injury in fields with whole-field applications. It is important to reiterate that border treatments were automatically applied at the first WOB, regardless of pest pressure. The corresponding

amount of active ingredient required for border applications was 77.3% less than what would have been required for whole-field applications. Considerable cost savings in chemicals would be augmented with savings in fuel and man-hours to provide additional economic advantages to the grower. The potential benefits for improvement to IPM through conservation of natural enemies and the prevention of insecticide resistance require more investigation, but would provide additional incentives to growers to attempt border-treatments in cotton for stink bug control in the southeastern United States. Growers may wish to test the border spray concept with a high-clearance tractor, as this equipment is less influenced by wind and is more readily available to growers.

Table 3.1. Summary of fields by location and insecticide treatment. Fields with ID numbers listed twice received both border and whole-field applications at different weeks of bloom.

Field location	County, State	Year	Field	Treatment	WOB treated	Area (ha)	Area treated (%)
Bamberg	Bamberg, SC	2007	1	None	--	9.1	--
Barnwell	Barnwell, SC	2007	2	None	--	19.0	--
Tift	Tift, GA	2007	3	None	--	11.6	--
Bamberg	Bamberg, SC	2008	4	None	--	8.9	--
Barnwell	Barnwell, SC	2008	5	None	--	6.0	--
Barnwell	Barnwell, SC	2008	6	None	--	15.9	--
Tift	Tift, GA	2008	7	None	--	8.9	--
Tift	Tift, GA	2010	8	None	--	10.1	--
Cameron	Barnwell, SC	2009	9	Border	1,2,3	9.2	24.8
Cameron	Barnwell, SC	2009	9	Whole-field	4	9.2	100.0
Cameron	Barnwell, SC	2009	10	Border	1,2,3	17.2	18.7
Cameron	Barnwell, SC	2009	10	Whole-field	4	17.2	100.0
Tift	Tift, GA	2010	11	Border	1,2	36.1	13.0
Tift	Tift, GA	2010	11	Whole-field	6	36.1	100.0
Pee Dee	Florence, SC	2009	12	Border	1,3,4,5	7.8	30.1
Tift	Tift, GA	2009	13	Border	1,2	9.8	25.2
Cameron	Barnwell, SC	2010	14	Border	1,2,3,4	17.2	18.7
Pee Dee	Florence, SC	2010	15	Border	1,2,3,4,5	7.8	30.1
Tift	Tift, GA	2010	16	Border	1,2	31.7	13.2
Pee Dee	Florence, SC	2011	17	Border	2,3,4,5	7.8	26.9
Tift	Tift, GA	2011	18	Border	1,2	30.7	17.2
Barnwell	Barnwell, SC	2007	19	Whole-field	1,4,6,7	11.9	100.0
Lee	Lee, SC	2007	20	Whole-field	6	11.4	100.0
Barnwell	Barnwell, SC	2008	21	Whole-field	5,6	15.8	100.0
Lee	Lee, SC	2008	22	Whole-field	2	11.9	100.0
Tift	Tift, GA	2009	23	Whole-field	4	17.5	100.0
Tift	Tift, GA	2009	24	Whole-field	2,3,4	10.6	100.0
Blackville	Barnwell, SC	2010	25	Whole-field	1,2,5,6	17.5	100.0
Tift	Tift, GA	2011	26	Whole-field	2,4	28.3	100.0

Table 3.2. ANOVA statistics for influence of insecticide treatment, location within fields, week of bloom (WOB), and interactions on mean numbers of stink bugs and percentages of injury. Treatment had three levels (whole-field, border, none), location had two (interior, exterior) and week of bloom had seven.

Variable	Effect	DF	F-value	P-value
Injury	Treatment	2, 26	5.34	0.0114
	Location	1, 26	7.07	0.0132
	WOB	6, 200	18.74	<0.0001
	Location × Treatment	2, 26	3.24	0.0556
	WOB × Treatment	12, 200	5.06	<0.0001
	WOB × Location	6, 200	0.41	0.8731
	Treatment × WOB × Location	12, 200	0.68	0.7650
Stink bugs	Treatment	2, 26	2.01	0.1544
	Location	1, 26	6.31	0.0185
	WOB	6, 220	4.17	0.0005
	Location × Treatment	2, 26	0.83	0.4485
	WOB × Treatment	12, 220	1.24	0.2586
	WOB × Location	6, 220	1.07	0.3801
	Treatment × WOB × Location	12, 220	0.56	0.8761



Table 3.3. Summary data for SADIE analyses for year-end total stink bug densities and associated boll injury across all treatments.

Field location	Year	Field	Treatment	Total weeks sampled	Total sample points	Stink bug density				% Injury				Association	
						Mean	SE	$I_a$	$P_a$	Mean	SE	$I_a$	$P_a$	$X$	P(X)
Bamberg	2007	1	None	7	17	4.4	0.6	1.338	0.0959	30.1	2.6	1.089	0.3015	0.196	0.2294
Barnwell	2007	2	None	7	25	5.0	0.9	0.845	0.6893	27.6	1.8	1.028	0.3503	0.204	0.2147
Tift	2007	3	None	6	21	2.9	1.1	1.341	0.0506	23.2	2.3	1.251	0.1220	0.539 <sup>b</sup>	0.0185
Bamberg	2008	4	None	7	15	2.9	0.6	0.749	0.9521	18.3	1.9	0.938	0.5232	-0.058	0.5636
Barnwell	2008	5	None	5	11	4.8	1.3	0.986	0.4738	15.3	1.9	0.950	0.5494	0.670	0.0359
Barnwell	2008	6	None	7	28	3.5	0.7	1.263	0.0860	15.3	1.4	1.306	0.0592	0.672 <sup>b</sup>	0.0006
Tift	2008	7	None	3	23	0.5	0.1	1.444	0.0454	30.0	2.4	1.062	0.3064	0.058	0.4155
Tift	2010	8	None	7	24	1.7	0.4	1.205	0.1183	23.0	1.7	1.410	0.0344	0.134	0.2735
Cameron	2009	9	Border	2	24	0.0	0.0	--	--	16.7	2.0	0.863	0.7645	--	--
Cameron	2009	9	Whole-field	4	24	0.0	0.0	--	--	7.4	0.9	1.144	0.1874	--	--
Cameron	2009	10	Border	1	32	0.0	0.0	--	--	3.8	1.7	1.453 <sup>a</sup>	0.0194	--	--
Cameron	2009	10	Whole-field	5	32	0.0	0.0	--	--	9.6	0.7	1.240	0.0989	--	--
Tift	2010	11	Border	5	77	0.5	0.1	1.350	0.0647	10.5	0.7	1.580 <sup>a</sup>	0.0153	0.318 <sup>b</sup>	0.0047
Tift	2010	11	Whole-field	1	77	0.2	0.1	1.353	0.0695	19.1	2.2	1.271	0.0937	0.362 <sup>b</sup>	0.0027

Field location	Year	Field	Treatment	Total weeks sampled	Total sample points	Stink bug density				% Injury				Association	
						Mean	SE	$I_a$	$P_a$	Mean	SE	$I_a$	$P_a$	$X$	P( $X$ )
Pee Dee	2009	12	Border	7	17	2.3	1.1	1.700 <sup>a</sup>	0.0067	20.9	2.4	1.771 <sup>a</sup>	0.0196	0.604 <sup>b</sup>	0.0044
Tift	2009	13	Border	5	27	0.9	0.2	0.984	0.4677	11.9	1.3	1.004	0.4218	0.022	0.4559
Cameron	2010	14	Border	4	32	0.2	0.1	1.332	0.0484	12.1	1.3	1.444 <sup>a</sup>	0.0245	0.619 <sup>b</sup>	0.0002
Pee Dee	2010	15	Border	6	17	0.8	0.3	0.984	0.0412	23.1	2.6	0.898	0.5499	0.042	0.4434
Tift	2010	16	Border	7	64	0.4	0.1	1.695 <sup>a</sup>	0.0039	11.3	0.8	1.573 <sup>a</sup>	0.0126	0.222	0.0402
Pee Dee	2011	17	Border	6	17	0.7	0.4	0.884	0.5934	1.2	0.4	1.270	0.1319	0.043	0.4384
Tift	2011	18	Border	7	58	0.2	0.1	0.916	0.5515	3.4	0.4	1.037	0.3286	0.098	0.2520
Barnwell	2007	19	Whole-field	7	20	6.8	0.9	1.083	0.2864	24.0	1.5	1.244	0.1215	0.446	0.0313
Lee	2007	20	Whole-field	7	19	0.8	0.3	0.910	0.6427	19.3	1.3	1.031	0.3699	-0.276	0.8779
Barnwell	2008	21	Whole-field	6	27	10.8	1.5	0.935	0.5547	24.7	1.3	1.110	0.2298	0.215	0.1490
Lee	2008	22	Whole-field	7	22	4.0	0.8	1.806 <sup>a</sup>	0.0018	27.8	2.1	1.781 <sup>a</sup>	0.0022	0.486 <sup>b</sup>	0.0110
Tift	2009	23	Whole-field	6	47	0.5	0.1	1.257	0.0793	12.1	1.0	1.093	0.2648	0.032	0.4116
Tift	2009	24	Whole-field	6	28	0.4	0.2	0.821	0.8694	13.6	1.1	0.929	0.5943	0.058	0.3837
Blackville	2010	25	Whole-field	4	28	0.5	0.2	0.991	0.4049	19.0	1.9	1.515	0.0268	0.340	0.0376
Tift	2011	26	Whole-field	5	53	0.3	0.1	1.267	0.1108	7.0	1.0	1.431	0.0575	0.579 <sup>b</sup>	<0.0001

$I_a$  = Overall index of dispersion indicating aggregated (>1), random (1) or uniform (<1) pattern. <sup>a</sup>Significance in aggregation determined by  $\alpha = 0.05$  ( $P < 0.025$  or  $P > 0.975$ ).

$P_a$  = p-value for null hypothesis of spatial randomness.

$X$  = Overall index of aggregation between each paired dataset. <sup>b</sup>Significance in association is positive for  $X > 0$  ( $P < 0.025$ ) or negative for  $X < 0$  ( $P > 0.975$ ).

Missing data represented by ‘-’ indicate that insect counts were insufficient to generate aggregation indices.

Table 3.4. SADIE summary data analyses for selected weekly stink bug dispersion indices and associated boll injury (at least one significant  $I_a$  or  $X$ ) across all treatments.

Field location	Year	Field	Treatment	WOB	Total sample points	Stink bug density				% Injury				Association	
						Mean	SE	$I_a$	$P_a$	Mean	SE	$I_a$	$P_a$	$X$	P( $X$ )
Bamberg	2007	1	None	5	17	0.9	0.3	1.377	0.0788	52.9	6.9	0.972	0.4625	0.608 <sup>b</sup>	0.0173
Bamberg	2007	1	None	6	17	0.7	0.2	0.939	0.5128	23.5	3.9	0.968	0.4734	0.554 <sup>b</sup>	0.0168
Tifton	2007	3	None	1	21	0.0	0.0	--	--	4.8	1.6	1.727 <sup>a</sup>	0.0032	--	--
Bamberg	2008	4	None	6	15	0.2	0.1	0.746	0.9677	34.0	7.0	1.677 <sup>a</sup>	0.0144	-0.117	0.6495
Barnwell	2008	5	None	3	11	0.7	0.3	1.255	0.0781	10.9	1.6	1.298	0.1042	-0.705 <sup>b</sup>	0.9900
Tifton	2008	7	None	4	23	0.4	0.1	1.621 <sup>a</sup>	0.0171	43.9	5.4	0.809	0.8319	-0.072	0.6220
Tift	2010	8	None	4	24	0.2	0.1	1.211	0.1091	17.1	2.7	1.703 <sup>a</sup>	0.0027	0.188	0.1976
Cameron	2009	10	Border	3	32	0.0	0.0	--	--	3.8	1.7	1.453 <sup>a</sup>	0.0194	--	--
Cameron	2009	10	Whole-field	4	32	0.0	0.0	--	--	8.4	1.8	1.482 <sup>a</sup>	0.0189	--	--
Tift	2010	11	Border	2	77	0.0	0.0	0.850	0.7600	2.9	0.9	0.846	0.7716	-0.410 <sup>b</sup>	0.9988
Tift	2010	11	Border	3	77	0.0	0.0	--	--	12.2	1.2	2.269 <sup>a</sup>	0.0002	--	--
Tift	2010	11	Border	5	77	0.3	0.1	1.546 <sup>a</sup>	0.0183	22.7	2.8	1.359	0.0558	0.300 <sup>b</sup>	0.0104
Tift	2010	11	Whole-field	6	77	0.2	0.1	1.353	0.0695	19.1	2.2	1.271	0.0937	0.362 <sup>b</sup>	0.0027
Pee Dee	2009	12	Border	5	17	0.5	0.3	1.808 <sup>a</sup>	0.0047	22.9	4.3	1.294	0.1359	0.291	0.0941
Pee Dee	2009	12	Border	6	17	0.9	0.6	1.710 <sup>a</sup>	0.0002	17.1	4.8	1.871 <sup>a</sup>	0.0112	0.657	0.0356
Tift	2009	13	Border	4	27	0.2	0.1	0.813	0.9196	18.5	3.2	0.942	0.5624	0.395 <sup>b</sup>	0.0230
Cameron2	2010	14	Border	5	32	0.2	0.1	1.161	0.1636	22.2	4.5	1.459 <sup>a</sup>	0.0201	0.479 <sup>b</sup>	0.0042
Tift	2010	16	Border	2	64	0.0	0.0	0.851	0.7766	8.8	1.7	1.653 <sup>a</sup>	0.0057	0.100	0.2842
Tift	2010	16	Border	4	64	0.1	0.0	1.564 <sup>a</sup>	0.0126	14.1	1.6	1.523 <sup>a</sup>	0.0166	0.238	0.0410

Field location	Year	Field	Treatment	WOB	Total sample points	Stink bug density				% Injury				Association	
						Mean	SE	$I_a$	$P_a$	Mean	SE	$I_a$	$P_a$	$X$	P( $X$ )
Tift	2010	16	Border	5	64	0.0	0.0	0.941	0.5386	11.9	1.4	1.424	0.0325	0.277 <sup>b</sup>	0.0116
Pee Dee	2011	17	Border	3	17	0.4	0.2	0.934	0.5053	2.4	1.1	1.866 <sup>a</sup>	0.0092	0.084	0.3749
Barnwell	2007	19	Whole-field	3	20	0.8	0.2	0.979	0.4543	68.3	5.3	1.752 <sup>a</sup>	0.0040	0.116	0.3055
Barnwell	2008	21	Whole-field	3	27	1.1	0.3	0.740 <sup>a</sup>	0.9779	10.7	1.5	1.053	0.3060	-0.064	0.6211
Barnwell	2008	21	Whole-field	4	27	4.8	0.9	0.729 <sup>a</sup>	0.9849	39.3	3.5	1.469	0.0364	0.307	0.0731
Lee	2008	22	Whole-field	2	22	0.1	0.1	0.902	0.6395	26.0	2.9	0.974	0.4634	0.499 <sup>b</sup>	0.0106
Lee	2008	22	Whole-field	4	22	0.7	0.3	1.793 <sup>a</sup>	0.0003	23.0	2.4	0.979	0.4597	0.268	0.1147
Lee	2008	22	Whole-field	5	22	0.2	0.1	1.154	0.1753	28.4	3.7	1.221	0.1436	0.447 <sup>b</sup>	0.0179
Lee	2008	22	Whole-field	6	22	0.3	0.1	1.268	0.092	18.4	3.8	1.674 <sup>a</sup>	0.0050	0.440 <sup>b</sup>	0.0213
Lee	2008	22	Whole-field	7	22	0.8	0.3	1.633 <sup>a</sup>	0.0097	30.9	3.8	1.844 <sup>a</sup>	0.0017	0.288	0.0896
Tift	2009	24	Whole-field	7	28	0.0	0.0	--	--	4.3	1.6	1.566 <sup>a</sup>	0.0075	--	--
Tift	2011	26	Whole-field	2	53	0.1	0.0	0.675 <sup>a</sup>	0.9899	1.5	0.6	0.897	0.6073	-0.120	0.7505
Tift	2011	26	Whole-field	4	53	0.0	0.0	1.668 <sup>a</sup>	0.0154	5.5	1.3	1.108	0.2442	0.126	0.1928
Tift	2011	26	Whole-field	5	53	0.1	0.1	1.036	0.3461	12.1	2.0	1.208	0.1498	0.567 <sup>b</sup>	0.0001
Tift	2011	26	Whole-field	6	53	0.1	0.0	1.215	0.1497	11.0	1.9	1.158	0.1942	0.434 <sup>b</sup>	0.0015

$I_a$  = Overall index of dispersion indicating aggregated (>1), random (1) or uniform (<1) pattern. <sup>a</sup>Significance in aggregation determined by  $\alpha = 0.05$  ( $P < 0.025$  or  $P > 0.975$ ).

$P_a$  = p-value for null hypothesis of spatial randomness.

$X$  = Overall index of aggregation between each paired dataset. <sup>b</sup>Significance in association is positive for  $X > 0$  ( $P < 0.025$ ) or negative for  $X < 0$  ( $P > 0.975$ ).

Missing data represented by ‘-’ indicate that insect counts were insufficient to generate aggregation indices.

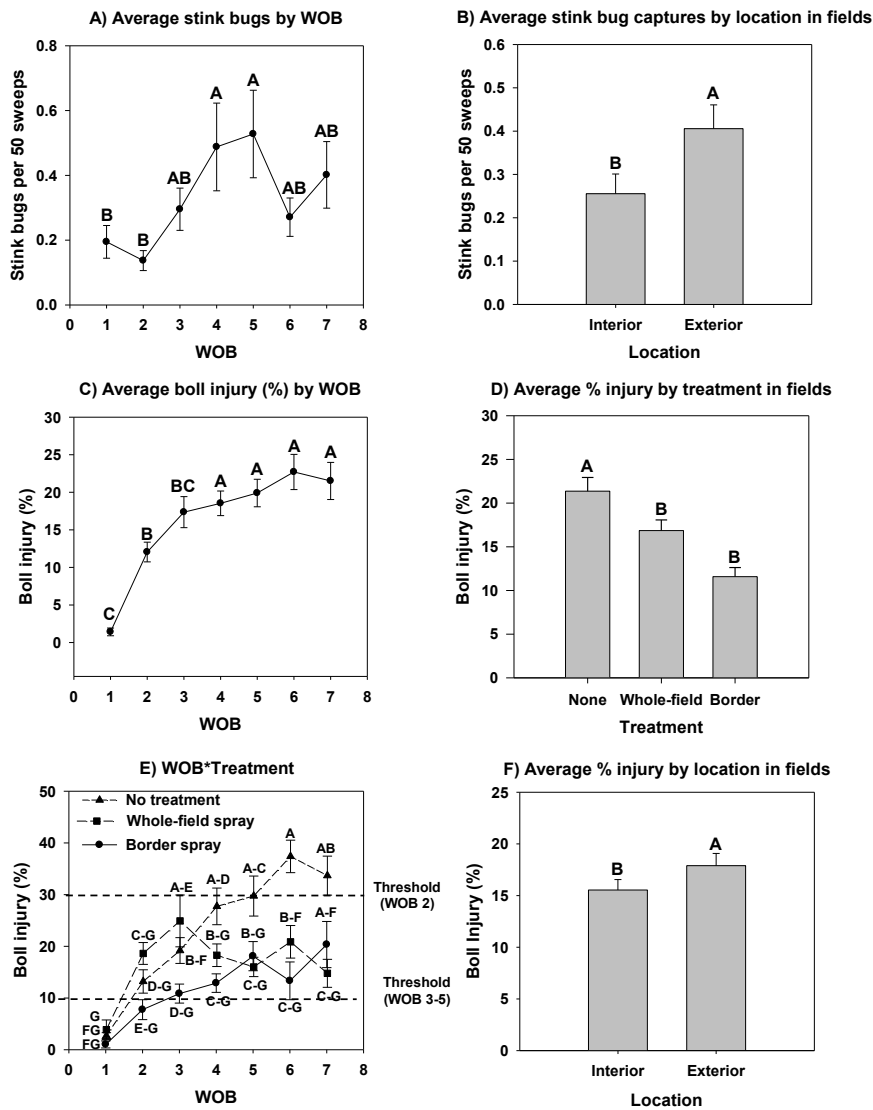


Fig. 3.1. Effects of A) week of bloom and B) location within fields on average numbers of stink bugs ( $\pm$  SEM) collected with sweep nets in cotton. Effects of C) week of bloom and D) insecticide treatment on average percentage of boll injury ( $\pm$  SEM). Effects of E) the interaction between week of bloom and insecticide treatment, and F) location within fields on average percentage of boll injury in cotton. Data from 26 fields in SC and GA, 2007-2011.

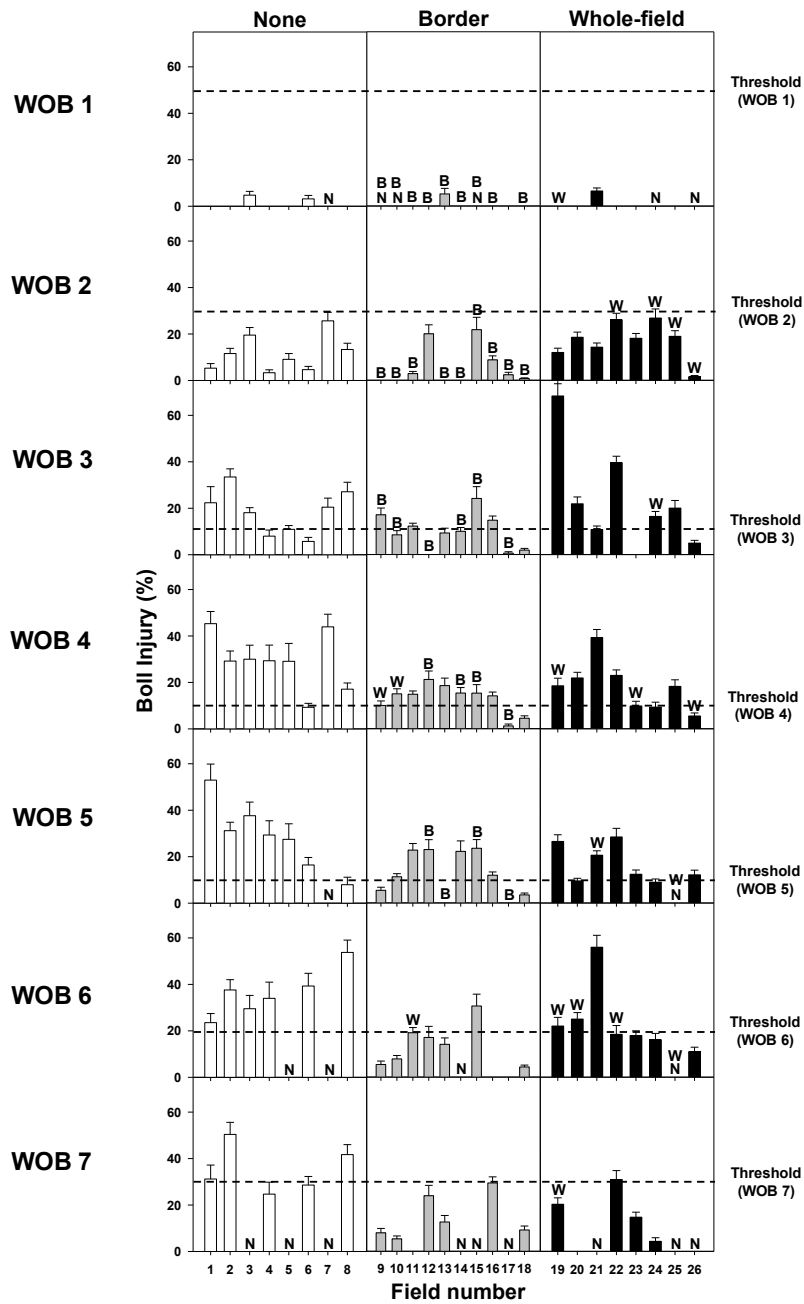


Fig. 3.2. Mean boll injury ( $\pm$  SEM) for each field per week of bloom (WOB) and treatment. Border-treatment applications are indicated by ‘B’. Whole-field treatment applications are indicated by ‘W’. Weeks where no sampling took place are indicated by ‘N’. Boll injury treatment thresholds are indicated by dotted lines per week of bloom. Data from 26 fields in SC and GA, 2007-2011.

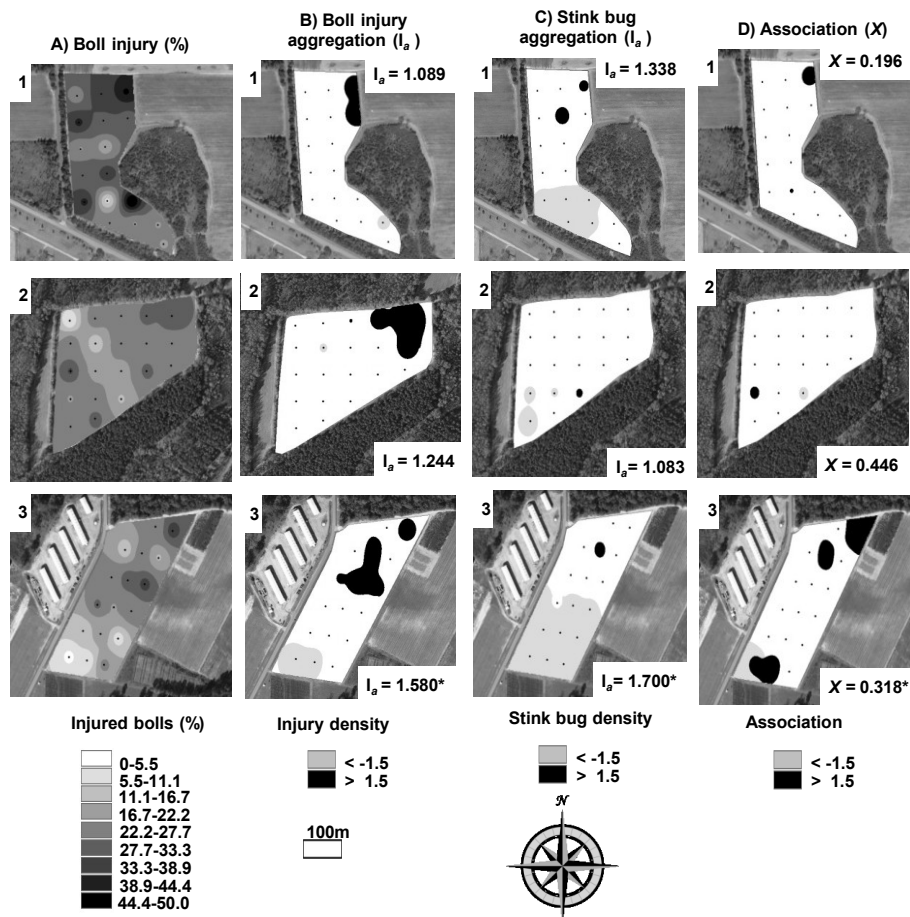


Fig. 3.3. Spatial interpolations of A) boll injury (averaged over sample dates), B) SADIE local aggregation indices for boll injury (averaged over sample dates), C) SADIE local aggregation indices for stink bugs (all species summed over sample dates) and D) SADIE indices of association between stink bugs and boll damage for 1) Untreated field #1 in Bamberg County, SC, 2007, 2) Field #19 receiving whole-field insecticide applications in Barnwell County, SC 2007, and 3) Field #12 receiving border-spray applications of insecticides in Florence County, SC, in 2009. Significant overall aggregation indices ( $P < 0.025$  or  $P > 0.975$ ) are indicated by ‘\*’. Black dots represent sample points in the grid.

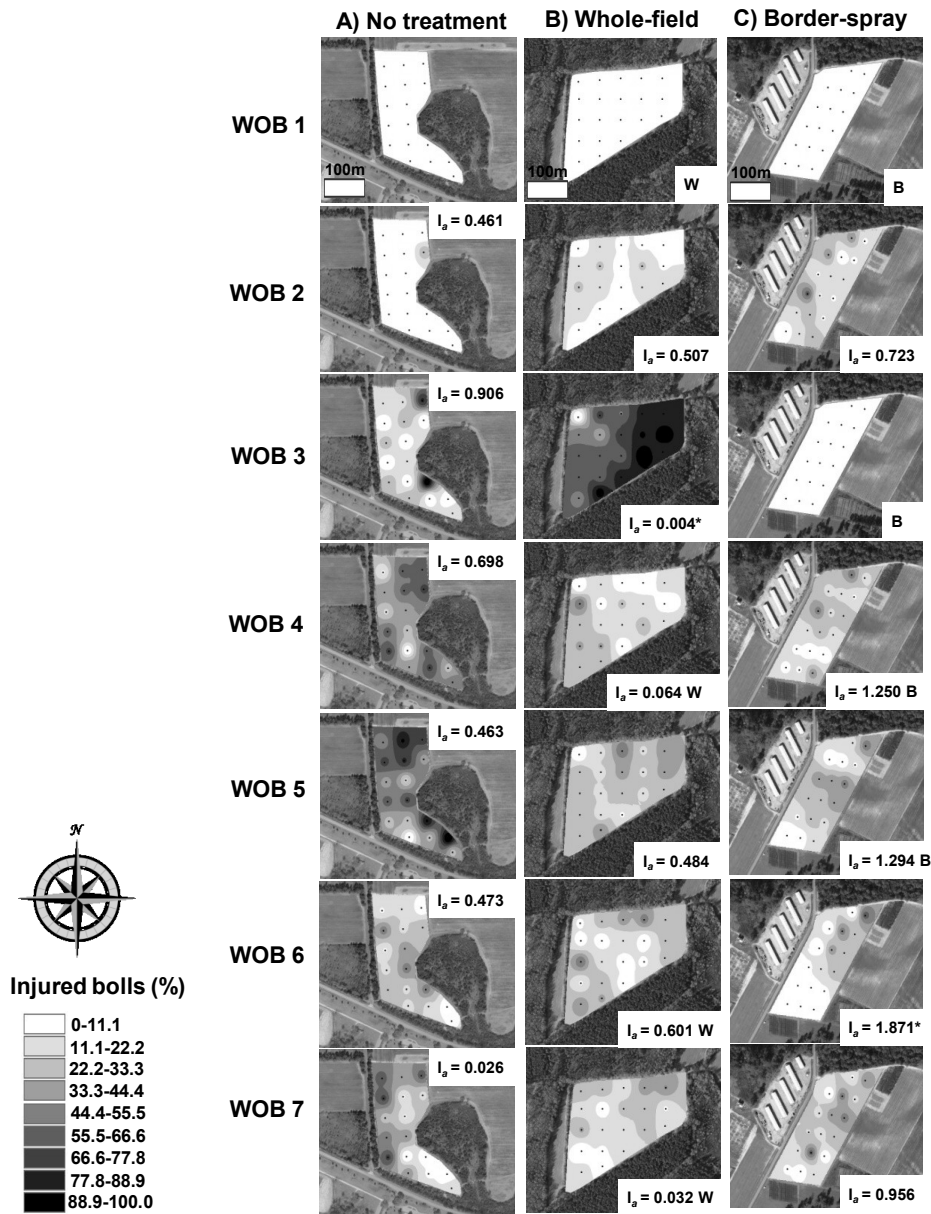


Fig. 3.4. Spatial interpolations of boll injury by week of bloom for A) Untreated field #1 in Bamberg County, SC 2007, B) Field #19 receiving whole-field insecticide applications in Barnwell County, SC 2007, and C) Field #12 receiving border-spray applications of insecticides in Florence County, SC, in 2009. Significant overall aggregation indices ( $P < 0.025$  or  $P > 0.975$ ) are indicated by ‘\*’. Black dots represent sample points in the grid. Border-treatment applications are indicated by ‘B’. Whole-field treatment applications are indicated by ‘W’.



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## CHAPTER IV

### HARMONIC RADAR TAGGING FOR TRACKING MOVEMENT OF *Nezara viridula*

#### (HEMIPTERA: PENTATOMIDAE)

##### Introduction

Studies addressing spatial distributions of insects in agricultural landscapes often use sampling methods such as sweep nets, drop cloths, and pheromone traps (Knight and Croft 1987, Fitt et al. 1989, Gage et al. 1990, Hoffman et al. 1992, Byers 1993, Reay-Jones et al. 2009, Reay-Jones 2012) or mark-recapture methods (Jolly 1965, Begon 1979, Nichols 1992) which cause disturbance that may influence normal behavior. Various tracking (Wallin and Ekblom 1988, Riecken and Raths 1996, Webb and Shine 1997) and remote sensing methods (Riley 1989) have been used to study movement, migration, and behavior of organisms. Among such methods, harmonic radar, a form of radio tracking, has allowed detailed study of the ecology of small vertebrates (Engelstoft et al. 1999) and insects (Mascanzoni and Wallin 1986, Carreck and Poppy 1996, Roland et al. 1996, Riley et al. 1998, Osborne et al. 1999).

Harmonic radar operates by transmitting a directional radar signal to the radar tag, consisting of an antenna (also called an aerial) and a Schottky barrier diode. The diode, powered by the radar signal, requires no power to generate a responding transmission (Colpitts and Boiteau 2004). The receiving unit can generate audible tones at different intensities when receiving this response to allow the searching individual to localize the tag. Commercially made hand-held harmonic radar units are now available. One unit, marketed by RECCO AB (Lidingo, Sweden), has the intended function of locating

avalanche victims using passive transponder tags built into their ski equipment. The RECCO unit, operating at about one watt, is not as powerful as custom-made units which can generate up to 4000 watts (Colpitts and Boiteau 2004, Boiteau et al. 2011). However, the RECCO unit is capable of being used in the field without additional equipment or external battery packs. While tags intended for mounting on ski gear are too large to be carried by insects, small custom made tags have been used with RECCO transceivers to successfully track snails (Lövei et al. 1997) and carabid beetles (O’Neal et al. 2004). These techniques were proposed to study the movement of stink bugs, an increasingly important pest group in cotton, *Gossypium hirsutum* (L.), production in the southeastern United States (Greene et al. 1999, Bundy and McPherson 2000). We assume that their sturdy physical structure and relatively large size would allow a radar tag of suitable size to be carried and their movement within a cotton field to be monitored. A better understanding of the movement of stink bugs among wild and cultivated host plants may help to develop more efficient scouting and treatment programs.

Attaching these tags requires an adhesive compatible with stink bug physiology. An ideal adhesive must be durable, non-toxic to the organism, easy to dispense, and not impact the behavior of the organism being studied (Hagler and Jackson 2001, Boiteau et al. 2009). No studies have been published to our knowledge on the impact of adhesives on stink bug mortality and behavior. The objective of this study was to determine the feasibility of harmonic radar tracking to monitor the movement of the southern green stink bug, *Nezara viridula* (L.).

## Materials and methods

Stink bugs. Adults of *N. viridula* (7-10 d after eclosion) were obtained from a colony reared in an environmental chamber (25°C, 14:10 L:D h) at the Clemson University Edisto Research and Education Center (REC) in Blackville, SC. Because size may impact the ability to remain mobile while carrying a radar tag, stink bugs were sorted by gender, and their length and weight recorded. A t-test was used to compare length and weight between males and females ( $n = 290$  per gender) (PROC TTEST, SAS Institute 2008).

Adhesive testing. Adhesives tested included Super-Glue (Elmer's products Inc., Westerville, OH, USA), Fix-All rubber adhesive (Pacer Technology LLC, Rancho Cucamonga, CA, USA), Weldwood contact cement (DAP Products, Inc., Baltimore, MD, USA), Liquid Skin first aid medical adhesive (Chemence Inc., Alpharetta, GA, USA), and Gorilla brand super glue (The Gorilla Glue Company, Cincinnati, OH, USA). Hot glue, used in a study of carabid beetles (O'Neal et al. 2004), was not evaluated as it was not possible to apply it in small enough quantities to prevent interference with the wings on the stink bugs. Careful hand application was used as pipette application was impossible with several of the adhesives due to thickness or instant bonding to the tip. Five drops for each adhesive were weighed on a scale to determine variability expected during application. To evaluate both mortality and adhesion, a single droplet of adhesive was applied to the pronotum of each stink bug. Five stink bugs of each gender in each of two cohorts were used for each type of adhesive. Trials were conducted with and without a Sanyo ISS350 (Sanyo Electric Co., Ltd., Moriguchi, Japan) diode (sans antenna) from



the radar reflecting tag. All stink bugs were kept in individual standard plastic medical specimen cups fitted with mesh lids and were supplied with a fresh green bean, *Phaseolus vulgaris* (L.), for diet. The cups were kept in plastic containers in the environmental chamber at the Edisto REC. Stink bugs were checked daily for mortality and the number of tags lost for 21 d. Stink bugs without glue were used as a control for mortality assessment.

Glue weights were analyzed using a one-way analysis of variance (ANOVA) with glue type as a fixed factor (PROC MIXED, SAS Institute 2008). Mortality, with and without a tag, and tag adhesion were expressed as a proportion of deaths or lost tags at 21 d and analyzed using a two-way generalized mixed linear model with a binomial distribution and a logit link, with gender of the insect and glue type as fixed effects and cohort as a random effect (PROC GLIMMIX, SAS Institute 2008). Satterthwaite's correction was used to calculate degrees of freedom. Means were separated using Tukey's honestly significant difference (HSD) test (Tukey 1953).

Harmonic radar tag design. As the RECCO unit operates at an output of 917 MHz, an ideal antenna for maximum receptive range would be 32 cm long (Pocock et al. 2011). For space savings, antennas are often made as half-wavelengths (ARRL 2011); however, an antenna of 16 cm would still be too long for a stink bug to carry. Because the RECCO unit cannot be tuned for higher frequencies to permit shorter antennas, the tag design had to be compromised for range and mobility. Assorted wire thicknesses (0.02 mm to 0.01 mm) and materials (steel, copper, and silver-plated copper) and two Schottky barrier diodes, a Toshiba DSF01S30SC (Toshiba Corporation, Tokyo, Japan) and a

Sanyo ISS350 (Sanyo Electric Co., Ltd., Moriguchi, Japan) compatible with the RECCO unit were tested. A pilot study (data not shown) to determine basic operational parameters for the tags revealed that a dipole design using two 2 cm long and 0.02 mm thick steel wire aerials encumbered movement and impacted behavior, preventing the insect from climbing or moving through vegetation, while a monopole design using the same thickness of wire 4 cm long was better, but still limited movement. Uncoated copper wire bent too easily to be substituted for steel. After much trial and error, improvements were obtained using the Sanyo diode and a lightweight 6 cm long, 0.01 mm thick silver-plated copper wire monopole attached at the anode terminal (used in all subsequent trials), which provided a range of  $6.4 \pm 0.9$  [SEM] m with a weight of  $0.017 \pm 0.001$  g ( $n = 5$ ). Silver-plated copper wire provided greater flexibility than steel wire, giving fewer encumbrances to movement despite an increase in antenna length. Wire glue (Indolon Technologies, Melrose, MA, USA), a graphite based epoxy, was used rather than soldering to attach the wires to the diode terminals to further reduce weight and prevent heat damage to the diode and the fine wire antenna from soldering.

To assemble the tags, a surface was prepared by taping a sticky note (3M Company, St. Paul, MN, USA) to a flat block of Styrofoam, with the adhesive side facing upwards (Fig. 2.5.1). The diode was placed contact-side down, held lightly in place by the sticky note adhesive. With the help of a magnifying glass and forceps, a wire was placed on the diode's attachment terminal. Wire-glue was applied sparingly to the junction and allowed to dry. The tag was carefully turned over, and more wire-glue was applied to the junction and allowed to dry fully to improve the electrical connection

before applying Gorilla super glue (chosen based on results of the Adhesive Testing section) for reinforcement. The tag was placed on a small piece of thin paper, and super glue was added as small droplets, covering the contact portion and a small amount of the wire. The paper was cut around the finished tag as tearing it off the preparation surface could damage the antenna connection. Before completed tags were attached to stink bugs, each tag was taken to and tested at a site at the Edisto REC previously scanned with the handheld radar unit to remove any source of false signals (cans, wire, or other reflective scrap). Tags were placed on a 10 cm high foam block and were scanned with the RECCO to verify proper tag function by listening for a return signal 5 m away.

Reflector tag attachment. To reduce handling trauma to the insects, a mounting clamp for holding the stink bugs during tag attachment was fashioned from a solid foam block and three strips of soft foam rubber (Fig. 2.5.2). Two of the foam strips pivoted on pins set at the ends. The stink bug was quickly positioned between the foam bars which were closed on the projecting points of the pronotum, away from the wing joints. The bars were locked with two additional pins. A third bar of foam was slid between the two clamping bars from behind the stink bug to support the antenna wire during attachment. A single droplet of adhesive was applied to the pronotum, and the diode was gently pressed into place. After 10 min, the rear block was slid back. If the tag showed any evidence of looseness, the block was replaced under the wire for an additional 10 min. If the connection remained solid, the two side pins were removed and the two foam bars were quickly moved apart to drop the stink bug without disturbing the wire. The stink bugs were then moved to an empty plastic container to allow for curing of the adhesive

and to check that all movements were unimpeded. The angle of wire was adjusted if necessary to allow for free movement.

Mobility testing. To evaluate the potential for impediments to movement, such as excessive tag weight limiting walking or the shape of the tag blocking wing motion (Boiteau et al. 2010), mobility of stink bugs was evaluated prior to field releases. Walking was tested using a 70×36×54 cm tracking field made from two large ‘Kritter Keeper’ plastic specimen containers (LLLReptile Company, Chelsea Vista CA) with their tops removed, placed one atop the other at the opening. A 1-cm grid covered the entire bottom, top, and three sides of the enclosed box. One side was left clear for observation. Three treatments were assessed on both genders of adult *N. viridula*: (1) glue and tags, (2) glue only, and (3) untreated controls. Stink bugs were placed in the arena one at a time and observed for 5 min, with the number of centimeters covered recorded. Ten replications of each treatment for both males and females were conducted. This test was conducted over two days on 28 and 29 June 2012, with one cohort containing five stink bugs of each gender in each of three treatments tested per day.

Flight ability was tested in an open hay field at the Edisto REC. This test was also conducted over two days on 11 and 16 July 2012 with one cohort per day, each containing a total of ten stink bugs of each gender in each of the same three treatments used for walking mobility tests. Stink bugs were released 2 m above a marked point on the ground. A spotter followed each stink bug as it flew to mark the location where the flight stopped. Flight distance was determined using a measuring wheel.

Walking and flying distances were analyzed using an analysis of variance, with gender and treatment as fixed effects and day tested as a random effect (PROC MIXED, SAS Institute 2008). Satterthwaite's correction was used to calculate degrees of freedom. Means were separated using Tukey's honestly significant difference (HSD) test (Tukey 1953).

Field release. Ten stink bugs of each gender were either marked with a unique identification number written on the pronotum in nontoxic waterproof archival ink (Sakura Pigma, Sakura Color Products Corp.) or marked and tagged with completed radar reflector tags. Releases were made at two locations in a fallow field 12 m apart, with vegetation approximately 40 cm in height that was not seeding, and at two equally spaced locations in cotton (AM 1511B2RF [*Americot, Inc. Lubbock, TX, USA*]) that had entered the second week of bloom. Within each habitat, tagged stink bugs were released at one location and marked stink bugs at the other location to ensure that initial sampling around the release location for marked stink bugs did not damage tagged stink bugs. Releases were made in the afternoons of 19 July 2012 and 26 July 2012, with the same locations used for each cotton or fallow field release. The area around the release points was scanned with the RECCO detector prior to release to identify and remove any source of false signals.

At intervals of 1 hr, and 1, 3, 7 and 14 d, the release areas were scanned with the RECCO unit starting at the release point. To ensure location of a stink bug, scans in cotton were made over two rows at a time starting 6 m east of the release point, each scan moving along 12-m of row before moving two rows over and repeating the scan in the

other direction. This scanning pattern was used on both sides of the release point making a search area of 12 m<sup>2</sup>. Fallow radar scans encompassed a circle 6 m in diameter.

Movement was determined by measuring straight line distance (meters) travelled from the release point to the locations where tagged stink bugs were found, with locations relative to the release point noted on a 1-m grid and the identification numbers of the insects retrieved recorded. After scanning for tagged stink bugs, sweep-net (38.1 cm diameter) sampling in the fallow area (three sweeps or 1.1 m at a time), or drop cloth sampling in cotton (0.91 m of row, two rows at a time), was conducted starting 6 m away from the marked insect release points as with the radar sweeps, with distance from the release point noted. If no marked or tagged insects were located, the search area was expanded a further 12 m around the release point using the same search techniques.

Distance covered by the stink bugs was analyzed using a three-way analysis of variance with gender, habitat (cotton or fallow) treatment (tagged or marked stink bugs) as fixed effects and day of release as a random effect (PROC MIXED, SAS Institute 2008). Satterthwaite's correction was used to calculate degrees of freedom. Means were separated using Tukey's honestly significant difference (HSD) test (Tukey 1953). Data from one of the releases in fallow were not included in the analysis as no stink bugs were recovered.

## Results

Stink bug size. Length of stink bugs did not vary between genders ( $14.5 \pm 1.15$  [SEM] mm;  $t = 1.3504$ ;  $df = 578$ ;  $P = 0.1774$ ). Weight did not also not vary between genders ( $0.16 \pm 0.03$  [SEM] g;  $t = 0.8944$ ;  $df = 578$ ;  $P = 0.3715$ ).

Adhesive testing. Weights of glue droplets were not significantly different among glue brands ( $0.002 \pm 0.001$  g;  $F = 2.70$ ;  $df = 4,20$ ;  $P = 0.06$ ). Stink bug mortality after 21 d was not significantly different among glue treatments including controls ( $F = 1.97$ ;  $df = 5,12$ ;  $P = 0.1564$ ) or between genders of stink bugs ( $F = 1.14$ ;  $df = 1,12$ ;  $P = 0.3071$ ) with a total of 8 deaths recorded for untreated controls, 17 for contact cement, 10 for Fix-All, 10 for Liquid Skin, 12 for super glue, and 8 for Gorilla super glue, with 20 replicates each. No interaction between glue and gender was recorded ( $F = 1.76$ ;  $df = 5,12$ ;  $P = 0.1956$ ). The number of dropped tags was not significantly different between glues ( $F = 1.41$ ;  $df = 4,10$ ;  $P = 0.2997$ ) or genders ( $F = 0.64$ ;  $df = 1,10$ ;  $P = 0.4439$ ) with no interaction ( $F = 0.14$ ;  $df = 4,10$ ;  $P = 0.9654$ ) with a total of 14 lost tags recorded for contact cement, 11 for Fix-All, 8 for Liquid Skin, 10 for super glue, and 6 for Gorilla super glue, with 20 replicates each. As such, the decision on the use of Gorilla super glue was based on handling characteristics.

Mobility testing. Walking distance in the laboratory and flying distance in the field varied significantly ( $P < 0.05$ ) between males and females, but not among treatments (Table 2.5.1). Females walked (1.15-fold) or flew (1.64-fold) further than males (Fig. 3). Interactions among gender and treatments were not significant.

Field release. For tagged insects recovery rate varied from 10% to 55% at one or 24 hours in fallow, with 0% to 25% of marked insects recovered in the same period. In cotton, recovery rates for tagged insects at one or 24 hours ranged from 25% to 75%, with marked insects recovered at rates from 25% to 55% (Table 2.5.2). Movement based on recovered stink bugs ranged from 0 to 233 m with an overall mean of  $2.8 \pm 2.2$  m. The distance dispersed was not significantly impacted by gender ( $F = 0.09$ ;  $df = 1, 95.2$ ;  $P = 0.7595$ ), habitat ( $F = 0.09$ ;  $df = 1, 95.8$ ;  $P = 0.7634$ ) treatment ( $F = 0.09$ ;  $df = 1, 95.3$ ;  $P = 0.7673$ ), nor any of the interactions ( $P > 0.05$ ). No released insects were found after 24 hours, despite expanding the radar sweeping and beat cloth samples into neighboring fields.

### Discussion

Our results indicated that *N. viridula* was tolerant to a wide range of adhesives. Prior studies of glues for radar tagging noted that the toxicity and durability of any compound varied considerably with insect species (Boiteau et al. 2009). Any adhesive used in tag attachment must offer the least possible impact to the organism. Viscosity, drying time, and application of the adhesive made a difference in the ease of dispensing and repeatability of application. Rubber cement and contact cement remained tacky for an unpredictable interval after application. Stink bugs treated with cement and contact cement became stuck to the walls of the cages in the environmental chamber, and ‘strings’ of adhesive would often adhere to the wings, impairing the ability to fly. Overflow from the runny nature of super glue and surgical glue caused similar problems



if the adhesive flowed into the wing joints. Gorilla brand super glue offered the most consistent application, as it dried quickly and was viscous enough to remain in place without running during applications. The adhesive is sold in a thick bottle with a clog-resistant nozzle design, further improving application. Gorilla super glue was selected for use in all mobility tests. This adhesive has been used to affix radio frequency tags in other studies, attaching external devices to the hunting billbug, *Sphenophorus venatus vestitus* Chittenden, and to seal wounds made surgically in the cuticle of tawny mole crickets, *Scapteriscus vicinus* Scudder (Silcox et al. 2011). Stink bugs successfully fed after glue application, but no observations were taken to determine if any differences in feeding behavior resulted, nor if mating was impacted. More research is needed to determine potential impacts other than mobility that adhesive applications may have on *N. viridula*.

Boiteau et al. (2010) observed that radar tag designs must accommodate physical constraints posed by the model insect. The 6 cm x 0.01 mm silver plated copper monopole described here worked well for movement, but the design can be refined. At least two insects died during field release testing, with two tags found inside fire ant, *Solenopsis invicta* Buren mounds, suggesting the insects had either succumbed to predation, or had been scavenged. Lost tags were also found in cotton (3) and fallow (5) after 24 hours, with the antenna wire tangled around plant stems. The entanglement suggests that while the tags accomplished the goal of being lightweight and flexible enough to not impact dispersal, further refinements are still possible. Several other designs were investigated, but none were adequate in maximizing mobility. A monopole

antenna of a design similar to O'Neal et al. (2004) using the Sanyo diode with a 0.02 mm thick steel wire of 8 cm in length provided 10 m of range. This design allowed for climbing in the laboratory but remained too encumbering to allow flight. An inductive loop was noted to increase range at very high frequencies (Colpitts and Boiteau 2004), but at the frequencies the RECCO transceiver operated, any such addition decreased range and increased the chance of the antenna entangling the moving insect in plants. A monopole antenna design of 4 cm in length using the same diode with 0.02 mm thick steel wire increased the body weight by 10% with a range of 6 m. Of 15 individuals released with steel-wire tags into soybeans (*Glycine max* [L.] Merrill) in a small pilot study (data not shown), none moved more than 1.5 m. Tags likely interfered with the behavior of the stink bugs, as untagged individuals were never found after release using drop-cloth sampling. Detection range increased and the load decreased to 8% of body mass by using the smaller Toshiba diode with a 2-cm 0.02 mm steel wire dipole, with one pole coiled to reduce length. Despite being less cumbersome, the coil was too delicate for field deployment as it was easily bent, reducing effective range to only 1 m if disturbed.

Although no statistics were reported comparing size among genders, DeWitt and Armbrust (1973) indicate that male *N. viridula* can be smaller than females, suggesting that females may be better suited to carry the tag without impediment to flight. Differences in body length and mass were, however, statistically insignificant in our trial. Our stink bugs and those of DeWitt and Armbrust (1973) were obtained from laboratory colonies, and it is unknown if discrepancies exist in the relative size of *N. viridula* of each gender between laboratory reared and wild-caught insects. Despite this, pre-release

mobility testing demonstrated female stink bugs travelling longer distances by walking and by flying than males. No difference in dispersion between males and females was recorded in field releases. This discrepancy might stem from the multiple insects we were unable to find (Table 2.5.2), due either to dispersal beyond the sampling range or our inability to detect them.

Harmonic radar has the potential to be a viable technology for tracking pentatomids in agricultural environments. Using easily available materials, it was possible to create a tag that allowed for unencumbered dispersal in farmscapes. Future work using this technology may determine real-time movement across farmscapes, providing valuable ecological data on stink bug behavior. At present, no low-cost harmonic radar transceiver is capable of handling both short and long range tracking that field studies would require. Improvements to the radar transceiver should be investigated in conjunction with further refinements of harmonic radar tag designs. For example, it may be possible to produce a tag on an adhesive label, using thin foil as an antenna. Such a tag would be readily applied to insects in the field without the need to return to the laboratory, and would not suffer from the entanglement problems of long-wire antennas. It could also be possible to incorporate RFID (radio frequency identification) technology into the tags to allow a researcher to identify the insect without disturbing it after locating the tag remotely with a transceiver. Such technology has successfully been used to monitor movement of other insects (e.g. Vinatier et al. 2010). Building on the successful tag design will allow for better investigations on the movement of stink bugs in farmscapes.

Table 4.1: Statistical comparisons of *N. viridula* gender and harmonic radar tag attachment on distance travelled in mobility trials.

Movement	Effect	DF	F-value	<i>P</i> -value
Flying	Gender	1, 114	7.10	0.0088
	Treatment	2, 114	0.34	0.7137
	Gender × Treatment	2, 114	0.44	0.6447
	Gender	1, 54	12.93	0.0007
Walking	Treatment	2, 54	1.73	0.1866
	Gender × Treatment	2, 54	0.53	0.5909
	Treatment			

Table 4.2: Number of stink bugs tagged and recovered per habitat and release date, with minimum, maximum, and average distances (males and females combined) moved (m) in 1 and 24 hours. No tagged or marked stink bugs were recovered in any situation after 24 hours.

Habitat and release date	Treatment	Recovered stink bugs		Distance (m)					
		1 hour	24 hour	1 hr			24 hr		
				Average (±SE)	Min	Max	Average (±SE)	Min	Max
Fallow (1)	Tag	11 (55%)	5 (25%)	3.7 ± 2.5	0.3	28.9	0.6 ± 0.1	0.3	0.9
Fallow (1)	Mark	0 (0%)	0 (0%)	-	-	-	-	-	-
Cotton (1)	Tag	11 (55%)	7 (35%)	21.4 ± 21.1	0.0	232.8	2.3 ± 1.5	0.3	11.5
Cotton (1)	Mark	9 (45%)	6 (30%)	0.2 ± 0.1	0.0	0.6	0.4 ± 0.1	0.0	0.9
Fallow (2)	Tag	5 (25%)	2 (10%)	0.6 ± 0.1	0.0	1.2	0.9 ± 0.2	0.3	1.8
Fallow (2)	Mark	2 (10%)	0 (0%)	0.3 ± 0.0	0.3	0.3	-	-	-
Cotton (2)	Tag	15 (75%)	5 (25%)	0.4 ± 0.1	0.0	1.8	0.3 ± 0.1	0.0	1.5
Cotton (2)	Mark	11 (55%)	5 (25%)	0.7 ± 0.5	0.0	6.1	0.6 ± 0.1	0.3	1.2

Distance travelled not significantly different between genders, crops, treatments and interactions ( $P > 0.05$ ).

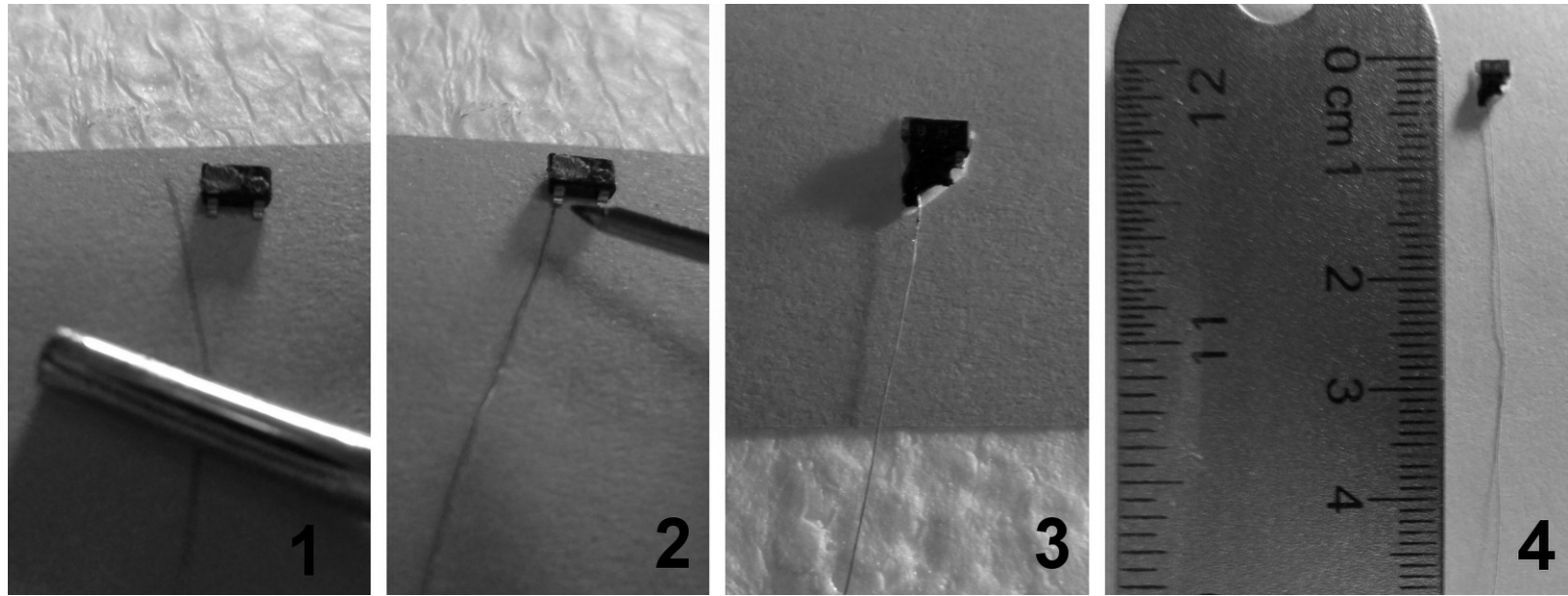


Fig. 4.1: Construction of harmonic radar tag for stink bug tracking. 1) A sticky note is placed adhesive-side up on a foam block. Diode and wire are arranged using the sticky note to hold the small components in place. 2) Wire glue is applied to the juncture between the wire and diode and allowed to dry. 3) The tag is carefully transferred to thin paper, and additional wire glue and cyanoacrylate is applied to strengthen the juncture. The paper is cut from around the diode. 4) The antenna can now be trimmed to length and tested.

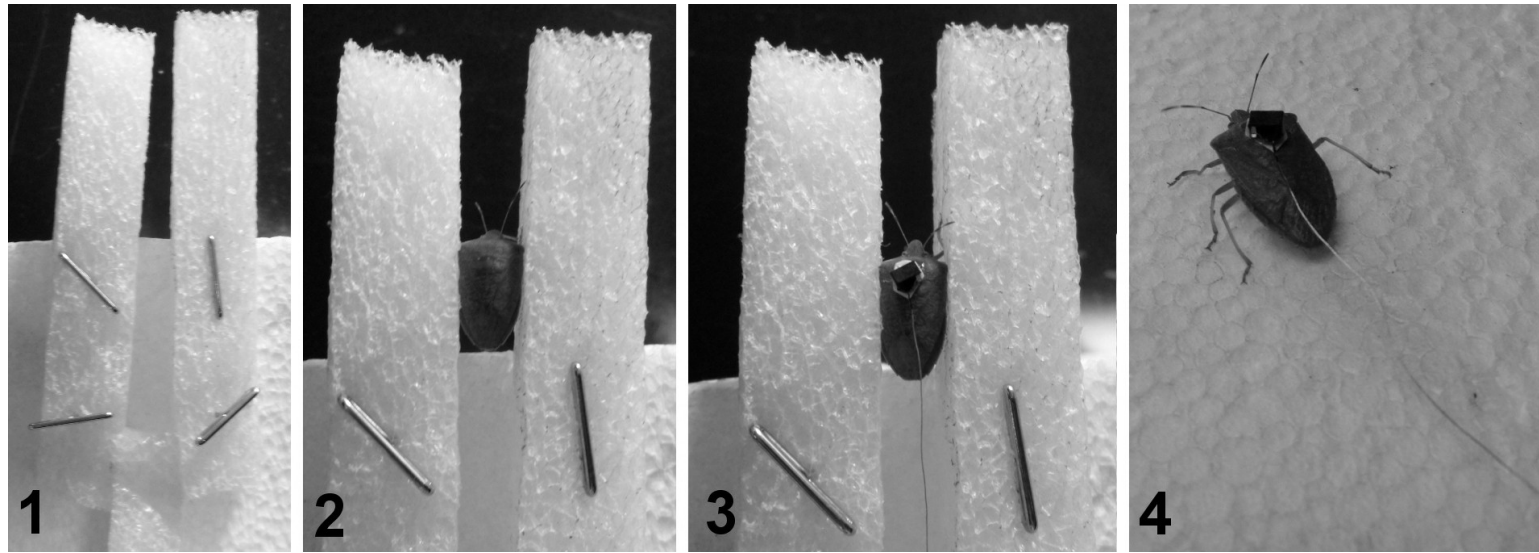
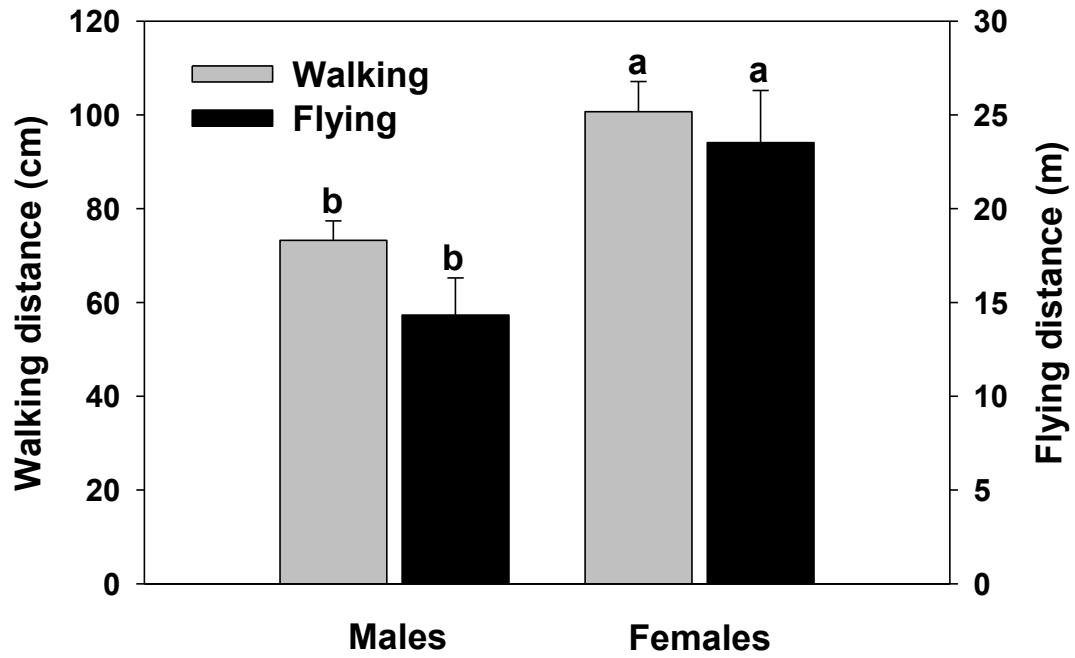


Fig. 4.2: Use of foam clamp to aid attachment of harmonic radar tags on stink bugs. 1) Two foam bars are positioned and held in place on a foam block by pivot pins. 2) Stink bug positioned between the bars. 3) A single droplet of glue is applied to the pronotum, with the tag positioned and pressed into place. 4) Upon release, the stink bug is checked for mobility and tag adhesion, the position of the antenna is adjusted if required.

Fig. 4.3: Average distance ( $\pm$  SEM) covered by male and female *N. viridula* flying (m) and walking (cm) in laboratory mobility tests. Mean bars with different letters were significantly different ( $P < 0.05$ ; Tukey's [1953] HSD).





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## CHAPTER V

### HOST PREFERENCE OF THE PARASITOID *Trichopoda pennipes* (DIPTERA: TACHINIDAE) WITH *Euschistus servus* AND *Nezara viridula* (HEMIPTERA PENTATOMIDAE)<sup>1</sup>

#### Introduction

*Trichopoda pennipes* (F.) (Diptera: Tachinidae), an endoparasitoid of stink bug nymphs and adults, is one of the most-studied natural enemies of the southern green stink bug, *Nezara viridula* (L.), in the United States (Worthley 1924, Todd and Lewis 1976, Buschman and Witcomb 1980, Panizzi and Slansky 1985, Jones 1988). *Trichopoda pennipes* is native to North and South America, and has been introduced to Hawaii, Australia, several Pacific islands, South Africa, Europe, and Israel for the control of *N. viridula* (Jones 1988, Colazza et al. 1996, Freidberg et al. 2011). *Trichopoda pennipes* produces up to three generations per year depending on location, with the last generation of the season remaining as second instars, overwintering inside the body of the host stink bug until late spring or early summer shortly after the stink bug breaks diapause (Dietrick and van den Bosch 1957). Each female fly lays 100 eggs on average, which are typically placed singly on late instars or adults (Pickett et al. 1996). The larva bores from the egg through the cuticle and into the stink bug, feeding on the host for about two weeks. After reaching the third instar, the larva exits the host between the posterior abdominal segments or the genitalia, killing the stink bug (Todd and Lewis 1976). Pupation takes place in the top three cm of soil. Upon emergence, adults feed on nectar (Tillman 2011).<sup>1</sup>

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Several mechanisms of host selection exist in insect parasitoids, with documented chemical and physical cues (Vinson 1976). Males of *N. viridula* produce an aggregation pheromone that attracts conspecific adults (both sexes) and late instars (Mitchell and Mau 1971, Harris and Todd 1980). This pheromone is also cross-attractive to the green stink bug, *Chinavia hilaris* (Say), and several egg and adult parasitoids including *T. pennipes* (Buschman and Whitcomb 1980, Colazza et al. 1999, Ehler 2000). The parasitoid uses the stink bug aggregation pheromone primarily to find its host, and secondarily as a mating kairomone, with both sexes of the fly being attracted to the chemical (Harris and Todd 1980, Aldrich et al. 1987, Tillman et al. 2010). Harris and Todd (1980) noted that, although the attraction of *T. pennipes* to *N. viridula* was continuous throughout the day, the attraction was strongest shortly before dark.

In addition to *N. viridula*, *C. hilaris*, and the brown stink bug *Euschistus servus* (Say), the parasitoid has been documented using the squash bug *Anasa tristis* (DeGeer) (Worthley 1924), and the red-banded stink bug, *Piezodorus guildinii* (Westwood) (Panizzi and Slansky 1985) as hosts. Parasitism rates, determined by identifying *T. pennipes* eggs on the cuticle of the stink bug, were documented for *N. viridula* as high as 44% by Todd and Lewis (1976), while *E. servus* collected simultaneously had parasitization rates of only 0.29%. Limited data have been collected as to the rates of successful emergence of *T. pennipes* in *E. servus*. Buschman and Whitcomb (1980) did not collect any *T. pennipes* emerging from 130 *E. servus* in Florida. McPherson et al. (1982) documented five tachinid species (including *T. pennipes*) emerging from *E. servus* collected in Louisiana, though rates were not reported. While published survey data show

that *N. viridula* is more commonly parasitized by *T. pennipes* than *E. servus*, no study has yet attempted to identify *T. pennipes* preference behavior among host species. This study aims to clarify the host preference and parasitoid emergence rates of *T. pennipes* with *N. viridula* and *E. servus* in the laboratory.

### Material and methods

Stink bugs used in the preference trials originated from laboratory colonies at the Clemson University Edisto Research and Education Center (REC) in Blackville, SC. Tachinids were reared from field-collected *N. viridula* and *E. servus*, known hosts of several tachinid species (Eger and Ables 1981, Jones et al. 1996). Collections of stink bugs occurred in fallow areas, wheat, *Triticum aestivum* (L.), and corn, *Zea mays* (L.), from mid-May to mid-July 2012 at the Clemson University Edisto REC. All pupae were reared to adulthood in a plastic rearing cage (30 x 30 x 30 cm) lined with paper towels in an environmental chamber (25°C, 14:10 L:D h). A dish of cotton balls saturated in a 30% sugar water solution was provided to feed any adults that emerged (Modi and Tesh 1983). Upon emergence, *T. pennipes* flies were isolated in a separate cage to observe mating activity, and were provided with sugar water and droplets of honey.

Laboratory-reared stink bugs used in trials were held for approximately one week after the end of nymphal development for cuticular sclerotization before being offered to *T. pennipes*. Two cohorts of mated (based on observations) females of *T. pennipes* were used in choice and no-choice trials in five arenas formed from five plastic cups (9 cm in diameter x 7 cm high) with screen mesh lids. A small dish with cotton soaked in sugar

water was provided as a source of moisture and carbohydrates for *T. pennipes* and a single green bean, *Phaseolus vulgaris* (L.), was placed in the arena as food for the stink bugs. For no-choice trials, *E. servus* and *N. viridula* were offered separately in male-only and female-only pairs ( $n = 20$  pairs per cohort). Choice trials consisted of *N. viridula* and *E. servus* being offered in pairs of different species or a male and a female of the same species ( $n = 30$  pairs per cohort). A single mated female of *T. pennipes* was placed in each arena. Trials were repeated five times per cohort for a total of 10 replications. Arenas were washed with soap and water and dried between trials. Female flies of each cohort (11 and 9 flies per cohort) were returned to the rearing cage between trials and randomly selected for each arena and trial to randomize exposure to arenas and stink bugs.

After 24 hours, stink bugs were removed from an arena and the number of *T. pennipes* eggs deposited per stink bug was counted. Stink bugs were isolated in standard plastic specimen cups fitted with a mesh lid and provided green beans for diet until the emergence of a parasitoid larva or until death. In cases where the stink bug died but a larva did not emerge, the stink bug was held for an additional 24 hours for any larval parasitoids to complete their development. If none were observed, the stink bug was dissected to determine if a larva was present inside. Dates of egg deposition were recorded, as were the dates of stink bug death or parasitoid emergence. Additionally, external signs indicative of parasitism were noted. Voucher specimens were deposited with the arthropod collection at Clemson University.



Data analyses. Numbers of *T. pennipes* eggs per stink bug were analyzed for significant departure from random by performing chi-square tests (Zar 1999). The expected frequency of eggs per stink bug was one-half for choice trials (df = 1) and one-fourth for no-choice trials (df = 3).

## Results

Field-collected *T. pennipes* emerged from *N. viridula* exclusively. All pupae obtained from field-collected *E. servus* were identified upon adult emergence as *Cylindromyia euchenor* (Walker) (Diptera: Tachinidae). One specimen of an unknown species of *Trichopoda* was also obtained from *N. viridula*, but was not included in laboratory trials.

The chi-square test indicated a departure from random in no-choice tests (Fig. 2.3.1), with 8.3-fold more eggs deposited on *N. viridula* than on *E. servus*. Significant differences between the two species also held for choice trials, but differences were not significant between genders of the same species (Fig. 2.3.2). A total of 779 eggs were laid on *N. viridula* across choice and no-choice trials, compared with 94 on *E. servus*.

Out of 200 (100 *N. viridula*, 100 *E. servus*) stink bugs tested, one or more tachinid eggs were deposited on 92 *N. viridula* and 26 *E. servus*. Twenty-six larvae emerged from egg-bearing stink bugs (24 from *N. viridula* and two from *E. servus*). Of the 24 larvae from *N. viridula*, 20 of the egg-bearing stink bugs had seven eggs or fewer, while four larvae emerged from stink bugs with more than seven eggs. Larvae emerged from *E. servus* with one egg each. The other 92 egg-bearing stink bugs (68 *N. viridula*, 24 *E.*

*servus*), dissected after death, yielded 21 living larvae (one from *E. servus* in a no-choice trial, and 20 from *N. viridula*, including one with two larvae). Of the 82 stink bugs with no visible eggs after the 24 hour trial, none had evidence of parasitism after death, based on dissections.

Numbers of tachinid eggs deposited on stink bugs varied considerably among individual stink bugs, ranging from 0 to 43 per host. Flies laid eggs on non-host surfaces as well as on hosts, with eggs found on rearing cage walls and on seven *T. pennipes* pupae that had not yet emerged as adults. One emerging pupa had 24 eggs on it as the fly attempted to leave the pupal case.

Physical evidence for emergence of the larvae from the egg was noticed in the dissections. Eggs laid on *N. viridula* often showed a dark spot where the parasitoid larvae had burrowed through the cuticle of the host after hatching. As this was noticed late in the experiment, no data were kept of the numbers of stink bugs demonstrating these spots, or how many occurred per number of eggs on the stink bug, but it was generally noted that no signs of cuticular penetration were observed on *E. servus*, even after several days of carrying eggs.

### Discussion

*Trichopoda pennipes* exhibited a clear preference for *N. viridula* over *E. servus*, with 8.3-fold more eggs laid on *N. viridula*. The presence of *T. pennipes* eggs on the cuticle has been used as a reliable method of determining parasitized status in past studies with a high level of accuracy in *N. viridula* (Harris and Todd 1981). All stink bugs

without eggs had no external or internal evidence of parasitism, indicating that the eggs were likely not present at any point, as opposed to being dislodged in handling or movement. The low number of tachinid larvae in or emerging from *E. servus* provided further evidence of the unsuitability of *E. servus* as a frequent host, as did the lack of entry wounds on the cuticle underneath the eggs. In our study, the presence of *T. pennipes* eggs on *E. servus* was not a reliable indicator of host suitability.

Prior studies have noted *T. pennipes* as using the male *N. viridula* aggregation pheromone to find its host (Mitchell and Mau 1971). Therefore, we expected oviposition to be higher around male *N. viridula*, as documented by Todd and Lewis (1976), which did not occur. The limited selection of hosts in the arena, as well as the fact that *T. pennipes* mating had already occurred away from *N. viridula*, might have influenced these results.

Superparasitism has been documented in *T. pennipes* when high populations of parasitoids occur relative to host bugs (Shahjahtan 1968). Superparasitism occurs when multiple larvae enter and attempt to develop in the same host. Only one larva typically can survive in a single bug, and superparasitization decreases parasitoid survival rate (Shahjahtan 1968). In that study, the percentage of emerged larvae dropped from 64-78% at two to five larvae per bug to 38% when seven larvae attempted to develop in a host. The host's fecundity was about 59% that of unparasitized hosts, but egg fertility remained unaffected (Shahjahan 1968). Adult parasitism rates of *N. viridula* by *T. pennipes* have been as high as 69% in South Carolina (Shahjahan 1968). Of 112 overwintering adults of *N. viridula* in the same study, 22% had at least one *T. pennipes* egg attached to the cuticle

(Jones et al. 1996). In Blackville, SC, where our experiments took place, parasitism rates of *N. viridula* had previously been documented at 37-65% (Jones et al. 1996). In our experiments, multiple *T. pennipes* eggs laid on a single stink bug occurred in 106 out of 200 stink bugs tested (17 for *E. servus* and 88 for *N. viridula*). This level of superparasitism could have been attributed to the limitations imposed by the arena. In the wild, the parasitoid would have had the freedom to leave after depositing an egg on a host. Of 36 *N. viridula* hosting more than seven eggs, only four larvae (11%) emerged from the host, suggesting that superparasitism might have been responsible for the low emergence rate. Larval emergence from 56 stink bugs hosting one to seven eggs increased to 36%. The two *T. pennipes* larvae that emerged from *E. servus* were from stink bugs hosting one egg each.

The deposition of eggs on non-host surfaces has been documented previously in tachinids (Stireman et al. 2006). The eggs are placed on surfaces that the host will frequent, and parasitism takes place when the host crawls over the egg-bearing surface, triggering the egg to hatch. *Trichopoda pennipes* is not one of the species for which this behavior had been observed. While it is unknown what prompted this ovipositional behavior, there is no evidence that this behavior was anything other than an artifact of confinement and it is likely incidental.

Many tachinid flies have localized variations in host preference, with some populations using different hosts in different areas of the parasitoid range (Jones 1988, Ehler 2000). *Trichopoda pennipes* is likely a complex of cryptic species, as different hosts have been documented in different regions (Jones 1988, Panizzi and Slansky 1995,

Pickett et al. 1996). Our results with parasitoids in South Carolina might not be valid elsewhere. More research is required to determine if our observed host preferences are consistent over a wide geographic area. If the species complex theory is valid, and *E. servus* is found to be a desirable host for some regional variations of *T. pennipes*, procedures to differentiate these cryptic species and their host preferences could be a fruitful avenue of investigation.

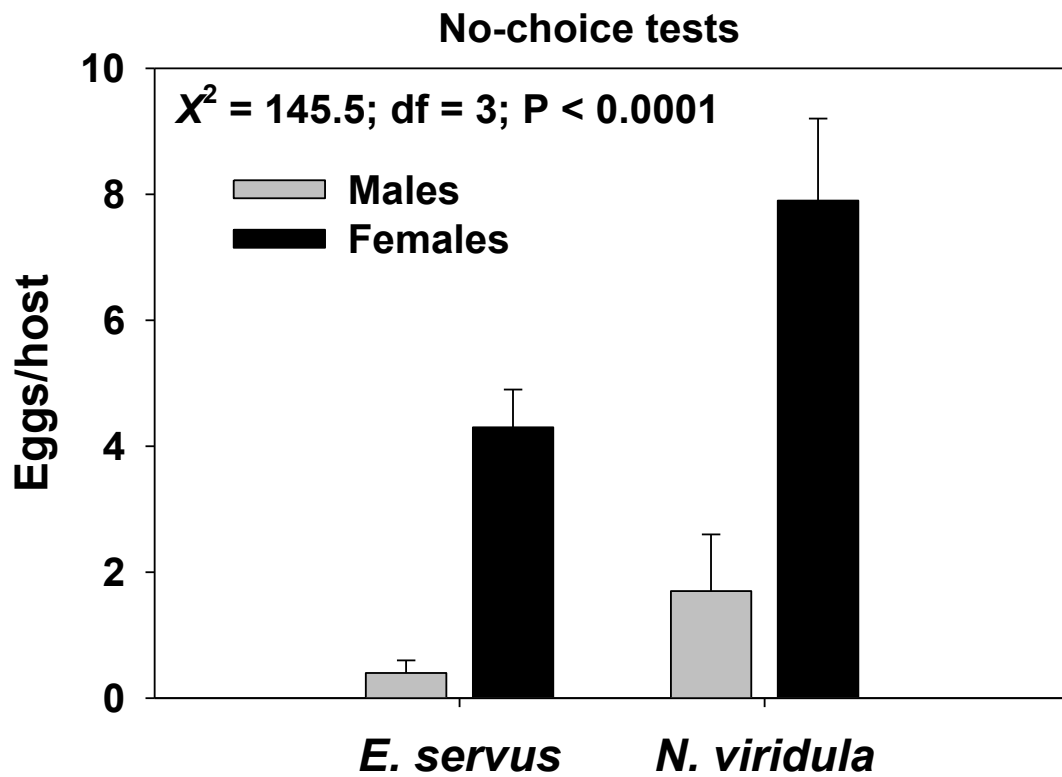


Fig. 5.1: Average numbers of eggs ( $\pm$  SEM) laid per host by *Trichopoda pennipes* in no-choice (same species, same gender) trials.

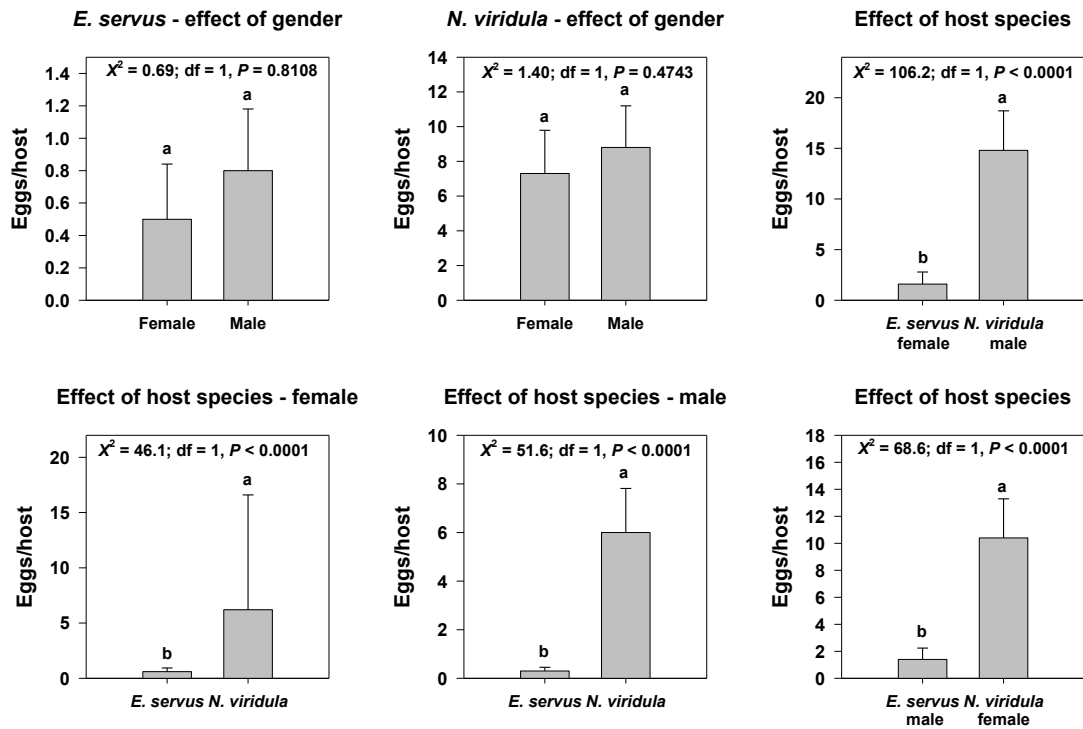


Fig. 5.2: Effect of gender and species (*Nezara viridula* and *Euschistus servus*) on average numbers of eggs ( $\pm$  SEM) laid per host by *Trichopoda pennipes* in choice trials. Means followed by the same letter are not significantly ( $P > 0.05$ ) different based on chi-square tests.

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## CHAPTER VI

OVIPOSITION BY *Cylindromyia euchenor* (WALKER) (DIPTERA: TACHINIDAE)

IN THE BROWN STINK BUG, *Euschistus servus* (SAY) (HEMIPTERA:  
PENTATOMIDAE)<sup>2</sup>

Several stink bug species (Hemiptera: Pentatomidae) are key pests of crops in the United States (McPherson and McPherson 2000). Parasitoids of stink bugs can play a major role in reducing pest populations (Tillman 2008). *Trichopoda pennipes* (F.) (Diptera: Tachinidae), an endoparasitoid of stink bug nymphs and adults, is one of the most common natural enemies of *Nezara viridula* (L.) in the United States and also can parasitize *Chinavia hilaris* (Say) (McPherson 1982, Jones et al. 1996). *Trichopoda pennipes* is native to North and South America and has been introduced to Hawaii, Australia, several Pacific islands, South Africa, and Europe (Jones et al. 1996, Tschorsnig et al. 2012). Several additional species of tachinid flies that parasitize stink bugs also exist in the United States (Eger and Ables 1981), but research has generally been limited compared with *T. pennipes*.

*Cylindromyia euchenor* (Walker) had previously been found in *Euschistus servus* (Say) (Eger and Ables 1981). Unlike most tachinids that deposit eggs on or near their hosts, members of the genus *Cylindromyia* have an ovipositor formed from an abdominal sternite, which, assisted by serrated curved claspers, implants eggs directly into hosts (Aldrich 1926, Herting 1957, Herting 1983). *Trichopoda pennipes* rarely parasitizes *E. servus* (Jones et al. 1996, Tillman 2008). Research on other parasitoids targeting this

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pyrethroid-tolerant stink bug (Willrich et al. 2003) is needed. Only limited observations have been published on the oviposition behavior of *C. euchenor* (Dupuis 1963). Our work studied ovipositional behavior and host preferences in laboratory-reared females of *C. euchenor*. Our observations were limited to three females over approximately two weeks.

Pupae of various parasitoids were obtained from *N. viridula* and *E. servus* collected from uncultivated grasses and weeds in fallow fields, wheat, *Triticum aestivum* (L.), and corn, *Zea mays* (L.), from mid-May to late June 2012. All obtained parasitoids were reared to adulthood. Trials were conducted in an environmental chamber at the Clemson University Edisto Research and Education Center (25°C, 14:10 L:D h) in a plastic rearing cage (30 x 30 x 30 cm Bug dorm, BioQuip) lined with paper towels. Tachinid pupae were provided with a dish of cotton balls saturated in sugar water to feed adults that emerged. Upon emergence, adults of *C. euchenor* were provided with sugar water and droplets of honey and isolated in a separate cage to observe mating activity. Three female and four male *C. euchenor* were obtained from *E. servus*, with *T. pennipes* emerging from *N. viridula* exclusively.

*Cylindromyia euchenor* mated on the second day after emergence, and females were subjected to choice and no-choice trials to determine host preference. Laboratory-reared stink bugs (one week after end of nymphal development) were introduced into cages with *C. euchenor*. Pheromonal contamination between different stink bug species may have occurred despite changing the paper towels lining the cage with each trial. Because viable tachinid pupae were obtained only from *E. servus* and *N. viridula*, these

two species were offered to a female *C. euchenor* in differing combinations of gender and species. For no-choice trials, *E. servus* and *N. viridula* were offered separately in male-only and female-only pairs (n = 22 with two female *C. euchenor*, n = 10 with one female). Choice trials consisted of *N. viridula* and *E. servus* being offered in pairs of different species or a male and a female of the same species (n = 22 with two female *C. euchenor*, n = 10 with one female). Pairs were left in the cage for 30 minutes or until an attack was witnessed.

After an attack, the stink bug was removed from the cage and isolated in a plastic cup fitted with a mesh lid and provided green beans, *Phaseolus vulgaris* (L.), until the emergence of a parasitoid pupa or until death. If the stink bug died but pupae did not emerge, the stink bug was dissected to determine if a parasitoid larva was inside the stink bug. Five selected stink bugs were dissected immediately after an observed attack in an attempt to find any injuries that might indicate the oviposition site.

Video recordings of the parasitizing behavior of the female fly were made with two different digital cameras (nine recordings at a 1280x720 resolution [Samsung, Samsung Town, Seoul] and five recordings at 432x192 resolution at 300 frames per second [Exilim, Casio, Tokyo]). Only no-choice tests with *E. servus* of the same gender and choice tests between male and female *E. servus* yielded footage of attack or rejection behavior. Three attacks, four rejections, and five examples of pre-attack cleaning behavior were documented across the 14 recordings.

Of the three *C. euchenor* females successfully reared from pupae, adult longevity varied, with the three females living 6, 7, and 10 days. Across all trials, only *E. servus*

was attacked. A total of 67 attacks were completed on *E. servus* out of 128 stink bugs presented in 64 paired choice and no choice trials for an average of 22.3 ( $\pm$  7.5 SE) attacks for each of the three females. One *C. euchenor* female emerged from its pupal case, mated the next day, and attacked 22 *E. servus* in two days in choice and no-choice trials, rejecting five females and one male on the fourth day. On the fifth day, it continued to examine *E. servus* of both sexes but made no attacks, dying the sixth day. The longest-lived female, at 10 days, also began to attack *E. servus* after mating on day two, after which attacks occurred every day except day six, when all stink bugs were rejected in both choice and no-choice trials. This female attacked 30 *E. servus* total. In all three females, examination of *E. servus* continued even after oviposition had ceased.

The sequence of parasitism, out of 67 attacks across all trials, started with cleaning of the wings and hind legs, followed by the ovipositor, with the front legs being cleaned last. This behavior restarted when interrupted. One female performed this sequence 11 times in 45 minutes when disrupted. Approach toward *E. servus* occurred from any direction by walking rather than flying, with the front legs extended toward the stink bug as the female *C. euchenor* advanced. The fly would touch *E. servus* with its forelegs, or crawl over it several times, flying away each time. When this behavior was observed, the fly cleaned its forelegs again before reexamining the stink bug. In contrast, the oviposition of *T. pennipes* occurs by alighting on a stink bug, laying an egg, and leaving quickly (Pickett et al. 1996, Stireman et al. 2006), with the entire sequence from initial contact to egg laying taking less than a second (G. Pilkay et al. unpubl.). *Cylindromyia euchenor* grasped *E. servus*, often clasping it even as it flipped over (Fig.

1). The female of *C. euchenor* oriented so that it was on the dorsal side of the abdomen of *E. servus*, with the parasitoid's abdominal tip towards the head of the host, and placed the ovipositor in the vicinity of the junction between the head and thorax. Contact duration was 2-5 sec and the position was maintained even if the stink bug landed on top of the fly when struggling. The influence of the presence of plants on ovipositional behavior, as would occur in the wild, is unknown. However, *E. servus* resting on green beans was attacked in the manner previously described. Male and female *E. servus* were attacked equally, though the inability to move each trial to a separate cage may have biased or influenced the behavior. One attack during a choice trial between male and female *E. servus* involved the fly examining and rejecting a female *E. servus* twice before attacking a male a short distance away with no prior examination.

Immediately after an attack, no eggs were visible on the surface of the host or internally via dissection. The entry wounds presumably formed during internal oviposition were not apparent. Eight days after the initial attack, dissections of four *E. servus* immediately after death showed no *C. euchenor* larvae. Three dead *E. servus* had *C. euchenor* larvae inside 14 days after the initial attack. The larvae were still alive in two of those cases. Two *C. euchenor* larvae emerged from *E. servus* out of the 67 attacks. Dissection of these *E. servus* after pupal emergence showed that most of the fat body of the host had been consumed, with the larvae having cleared ~1/3 of the abdominal volume.

*Cylindromyia* has been noted as a genus of 'nonlethal' parasitoids that often leave the host alive, with *Cylindromyia brassicaria* (Fabricius) as an example in *Dolycoris*

*baccarum* (L.) (Aldrich 1926). Both *E. servus* in our study, from which larvae emerged, died within 24 hours. However, this may have been due to a combination of stress from both parasitism and laboratory conditions. Such stress could also potentially explain the low numbers of emerged parasitoids (Eger and Ables 1981). Stireman et al. (2006) noted that hosts of tachinids could remain reproductive for some time after parasitization, but the females we dissected often showed damage to the ovaries from larval feeding.

In no-choice trials, *N. viridula* was ignored by all *C. euchenor* females. In choice trials where *E. servus* and *N. viridula* were offered together, *C. euchenor* examined and attacked only *E. servus*. Cross attraction among many tachinid parasitoid species has been documented in response to aggregation pheromones of *N. viridula* (Harris and Todd 1980, Tillman et al. 2010). Many tachinid flies have localized variations in host preference, with some populations using different hosts in different areas of the range (Jones et al. 1996). Such a situation also may apply to *C. euchenor*. It is unknown as to what host cues lead to *C. euchenor* oviposition.



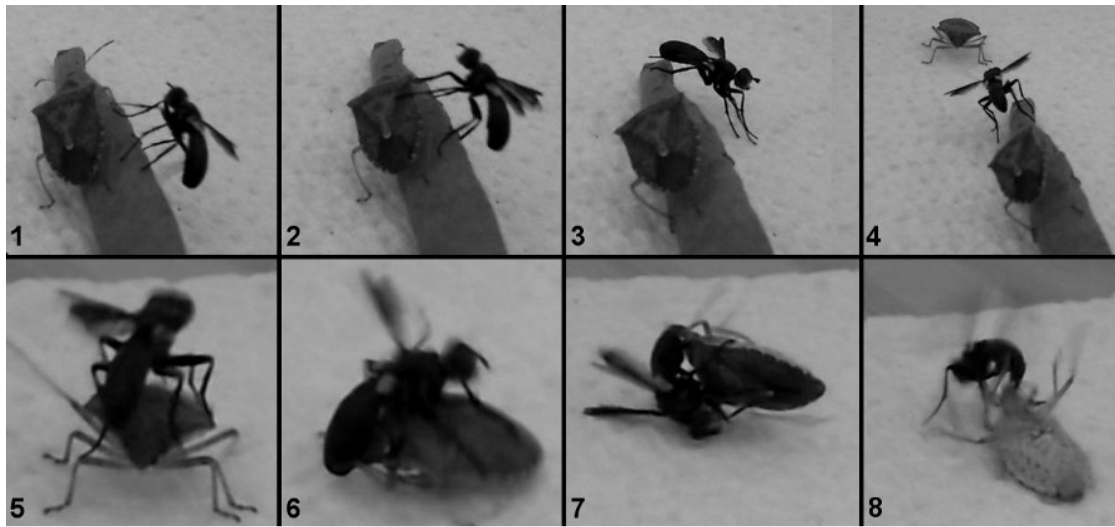


Fig. 1

Fig. 6.1: Sequence of oviposition by *Cylindromyia euchenor* in *Euschistus servus* in a male/female choice test. 1) The parasitoid approaches the female stink bug with front legs outstretched. 2) Contact is made by the front legs. 3) Rejecting the female host, the parasitoid cleans its front legs. 4) The parasitoid approaches the male stink bug. 5) Accepting the male host, the parasitoid mounts the stink bug. 6) Once on the stink bug's dorsum, the parasitoid adopts a characteristic posture with the fly's head facing the posterior of the stink bug. The parasitoid's abdomen curls under the stink bug, placing the ovipositor in the vicinity of the junction between the head and thorax. 7) This posture is maintained as the stink bug struggles, even if the fly is caught under the stink bug as it turns over. 8) The fly releases the stink bug, withdrawing the ovipositor, and escapes.

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## CHAPTER VII

### CONCLUSIONS

Stink bug management represents the complexity inherent in the control of any polyphagous pest. The movements of pest species within crops, the control methods to be applied, the natural enemies that may supplement chemical control, and the techniques used to determine the interactions of all these factors are still being investigated, with much more yet to be described or discovered. My study of the spatial and temporal dynamics of stink bugs demonstrated how the sequence of plant hosts, documented at smaller scales in prior studies, applied to much larger areas. My sampling grids captured the distributions of stink bugs across farmscapes over time, but the transects used to determine distance and crop/adjacent effects at a smaller scale failed to detect any significant distance effects from any farmscape, possibly due to low densities across the study. Despite the limitations of my data, biases of stink bugs towards soybean interfaces were clearly detected in Lee and Barnwell Counties, SC. In Tift County, GA, no crop/adjacent effects were detected. Tift County was the only farmscape where grass borders were maintained with insecticide treatments and regularly mown. These grass borders, separating each of the transects in Tift County, might have disrupted stink bug dispersion between crops. As the farmscape structure of the southeastern United States does not permit placement of crops beyond the flight range of stink bugs, the potential use of disruptive borders to mitigate stink bug dispersal might be a fruitful avenue of future investigation.

My study supports previous findings of bivoltinism in *E. servus*, with two distinct peak populations of nymphs recorded, with the first generation of nymphs forming in wheat, and the last pre-diapause generation forming in soybeans. I was not able to confirm bivoltinism in *C. hilaris*. *Chinavia hilaris* is likely bivoltine in South Carolina and Georgia, but early-season populations developed away from my sampling points and were undetected until second-generation adults moved into the farmscape. *Nezara viridula*, like *C. hilaris*, often did not demonstrate an initial peak of adults before the first peak of nymphs was recorded. The post-diapause generation of adults also likely fed in non-agricultural hosts before oviposition in crops. Multiple nymphal peaks suggest *N. viridula* is multivoltine in South Carolina and Georgia. Future studies of the life cycles of *N. viridula* and *C. hilaris* should include sampling of additional hosts, so that any emerging adult populations early in the season may be detected.

Despite the lack of significant difference among distances from field edge in densities in the farmscape sampling study, sampling in the border application study using data taken from a wider range of years and locations showed densities in cotton fields and their associated boll injury greater in the exterior portions compared with the interior portions of fields. This aggregated pattern of dispersal on the field border suggests that border applications of insecticides might be sufficient to control stink bugs in cotton prior to invasion of the entire field. Despite variability in insecticide applications in commercial fields, reduction of boll injury achieved with border treatments was identical to that afforded by applications to the entire field, while using an average of 77% less active ingredient. The percentage of area treated varied proportionally with the size of a

field. Larger fields had comparatively less area treated with the same sprayer than smaller ones, but the pattern of reduced damage held true across multiple field sizes in multiple states and counties. Neither border nor whole-field treatments suppressed boll damage below the economic threshold in all cases, suggesting that improvements to the application times or insecticide rates can still be made. Two fields received border treatments early in the season, followed by a whole-field treatment in the fourth week of bloom (WOB) when a large percentage of bolls were vulnerable to injury by stink bugs. Injury in one field dropped below threshold after the whole-field treatment, while the other remained slightly above threshold (+2%). While two example fields provided limited data, results suggested that border applications of insecticide provided control during the early part of the season before whole-field application was needed during the more damage-sensitive periods of crop growth. The possibility of using border treatments early in the season, to be supplemented with more expensive whole-field treatments applied only when needed, should be investigated further.

The use of boll injury in cotton has been favored by crop scouts, as it is semi-permanent in nature, while stink bugs are highly mobile and difficult to sample. In the farmscape sampling study, the movement of stink bugs over time was inferred from sampling, but could not be directly observed. Improvements in harmonic radar tracking could allow researchers in the future to monitor the movements of tagged stink bugs in real time, facilitating observations of true dispersal patterns. My results from investigating and improving this technology indicated that *N. viridula* tolerated a wide range of adhesives to attach tags. The technique I developed of clamping the insect to be

tagged with a foam fixture reduced the need for handling, reduced mortality and simplified the application of tags. Although *N. viridula* was observed walking, flying, and feeding with tags, no observations were taken to determine if any differences in feeding behavior resulted, nor if mating was influenced. More research is needed to determine the potential behavioral impacts of adhesives and tags on *N. viridula*, and to determine if other stink bug species are as tolerant to adhesives. The tag design I developed was the best that I could make by hand with existing technology. Improvements in tag construction through the use of adhesive surfaces for component arrangement and the elimination of heavy lead solder through the use of graphite adhesives allowed me to create a tag that did not impair walking or flying, but the design can still be refined. At present, the tag uses a fine wire as an antenna, which could be tangled in vegetation. Mass production could allow for the creation of radar tracking tags built into self-adhesive labels, using thin foil as an antenna. Such a tag could be readily applied to insects in the field without the need to return to the laboratory. This tag would not suffer from the entanglement problems of long-wire antennas. Radio frequency identification technology (RFID) could also be directly incorporated into the tags to allow a researcher to identify a tracked insect after locating the tag remotely, minimizing disturbance through recapture.

The primary remaining limitation to the application of harmonic radar tracking technology is the transceiver. Ideally, tracking a stink bug in a multi-crop farmscape would require a harmonic radar unit with the capacity for high power output over a wide area to first locate the insects from a distance. The unit would then have to switch to a

much lower output and narrower scanning area so that the insect could be accurately located at close range. True real-time monitoring of movement also requires a means to identify the insect without disturbing normal behavior through recapture, similar to what can be accomplished through RFID. At present, no low-cost harmonic radar transceiver is capable of handling all these requirements. During testing, we attempted to increase the power of the RECCO unit. A repurposed 33-cm band linear amplifier was connected to the RECCO's output. The output signal, measuring approximately 100 watts, was directed using a 900 MHz yagi antenna (Terrawave solutions, San Antonio, Texas). The original RECCO receiver was used to detect the return signal. Aluminum foil coverings were added to the input and output wires of the amplifier to shield them from radio frequencies, with ferrite beads added to the power cables, as the extra wire lengths were resonant on the same frequencies on which the tags operated. Despite the addition of shielding, the amplified signal was too powerful for the receiver, producing feedback and unpredictable false signals. The RECCO unit has no attenuator function to narrow reception and filter strong signals. This is not a design flaw, as it was constructed as avalanche rescue equipment. Investigating 'false' signals, possibly generated by metal on skiers, buried by avalanches, who may not have the correct tags attached to their clothing, may offer another means of finding victims, while restricting reception decreases the chance of possible rescue. The inability to consistently identify false signals prevented the use of the RECCO with an amplifier to enhance tag detection. Improvements to the radar transceiver should be investigated in conjunction with further refinements of harmonic radar tag designs. The transceiver would require an adjustable power range



with an attenuator to allow for selective signal filtration, combined with a close-range RFID reader that would not be impaired by the radar signal. A set of yagi antennas selected by a switch for both transmission and reception could also be incorporated to change the scanning area. Such capability would allow a researcher to follow multiple stink bugs from a distance while still allowing localization and identification of individual insects when required. All this is possible with existing technology, but no manufacturer which has adopted it in this integrated manner. At present, I have reached the technological and physical limits of stink bug tracking with commercially available harmonic radar tracking equipment. Further improvements to our tools must be made for harmonic radar to be a viable system for further research.

*Trichopoda pennipes*, one of the best-studied of adult stink bug parasitoids, exhibited a clear preference for *N. viridula* over *E. servus* in my study. *Cylindromyia euchenor*, which has never been studied for host preference or ovipositional behavior, favored *E. servus* exclusively over *N. viridula*. These results indicate that we cannot look to a single species of parasitoid to control our stink bug complex. The presence of *T. pennipes* eggs on the cuticle has been used as a reliable method of determining parasitized status in past studies with a high level of accuracy in *N. viridula*, but *C. euchenor* injects an egg using a sternotheca, leaving no external evidence of parasitism in *E. servus*. The presence of eggs, the primary method of determining the percentage of parasitism in field studies, is not a viable method when dealing with a multi-species parasitoid complex, and no external evidence of *C. euchenor* parasitism was observed in my study. Additionally, *T. pennipes* has been documented as having localized variations

in host preference, but no information exists if *C. euchenor* demonstrates geographically distinct host preferences. A great deal remains unknown about both species, and many more tachinid stink bug parasitoids exist in the southeastern United States, which have had no studies conducted on their behavior or preferences. Border applications of insecticides might be less detrimental to parasitoids, which are more sensitive to insecticide applications than their stink bug hosts, than whole-field applications by preserving the untreated interior of the field as a refuge. If these natural enemies could be preserved in the farmscape, they may offer a supplement to insecticide applications, further reducing the amount of material required to protect crops from stink bug injury. Understanding the biology and ecology of these natural enemies is critical to understanding their role in our agroecosystems.

My research in the course of this program has raised many additional questions and answered some, but I do not consider this to be a flaw. Rather, it provides me with many possible avenues of future investigation as I advance my career. I have successfully expanded the information in spatial and temporal dynamics, and evaluated a method of insecticide application that may enable the elusive goal of providing economic control while reducing the amount of active ingredient applied. I have advanced the design of radar tags to the point where they may no longer impair the movements of stink bugs, and I have brought new information to light about our local parasitoid complexes, including the first published description of the behavior of *C. euchenor* and one of the few descriptions of the behavior of any species of the genus *Cylindromyia* in English. In all

these projects, I have seen where more research is required, and I hope to have the opportunity in the future to continue to investigate these issues in the future.

## APPENDICES

## Appendix A

### SAS Code for Chapter II

#### Program 1: Edge Effect Test for Barnwell County, SC.

```
dm'log;clear;output;clear';
Title'Edge Test';
options nodate nonumber ps=55 ls=78;
data EdgeBarnwell;
input Location$ Year FieldID$ Transect$ Distance Crop$ Adjacent$ NumTimes BSB
SGSB GSB ALL BSNB SGSBN GSNB ALLN AllBugs;
AvgBAd= BSB/NumTimes;
AvgBNy= BSNB/NumTimes;
AvgSGAd= SGSB/NumTimes;
AvgSGN= SGSBN/NumTimes;
AvgGAd= GSB/NumTimes;
AvgGN= GSNB/NumTimes;
AvgBug= AllN/NumTimes;
AvgNym= AllN/NumTimes;
cropadj=crop||adjacent;
cards;
Barnwell 2009 10B2009 28 0 SB Cotton 6 2 1 0 3 0 0 0 0 3
Barnwell 2009 10B2009 28 5 SB Cotton 6 3 0 2 5 0 0 0 0 5
Barnwell 2009 10B2009 28 10 SB Cotton 6 1 0 1 2 0 0 0 0 2
Barnwell 2009 10B2009 28 25 SB Cotton 6 1 2 0 3 0 0 0 0 3
Barnwell 2009 10B2009 29 0 SB Cotton 6 9 0 5 14 0 0 0 0 14
Barnwell 2009 10B2009 29 5 SB Cotton 6 1 0 1 2 0 0 0 0 2
Barnwell 2009 10B2009 29 10 SB Cotton 6 0 0 0 0 0 0 0 0 0
Barnwell 2009 10B2009 29 25 SB Cotton 6 0 1 0 1 0 0 0 0 1
Barnwell 2009 10B2009 30 0 SB Cotton 6 2 0 0 2 0 0 0 0 2
Barnwell 2009 10B2009 30 5 SB Cotton 6 2 0 2 4 0 0 0 0 4
Barnwell 2009 10B2009 30 10 SB Cotton 6 0 0 1 1 0 0 0 0 1
Barnwell 2009 10B2009 30 25 SB Cotton 6 0 0 1 1 0 0 0 0 1
Barnwell 2009 1B2009 1 0 Cotton Corn 7 0 0 0 0 0 0 0 0 0
Barnwell 2009 1B2009 1 5 Cotton Corn 7 0 0 0 0 0 0 0 0 0
Barnwell 2009 1B2009 1 10 Cotton Corn 7 0 0 0 0 0 0 0 0 0
Barnwell 2009 1B2009 1 25 Cotton Corn 7 1 0 0 1 0 0 0 0 1
Barnwell 2009 1B2009 2 0 Cotton Corn 7 0 0 0 0 0 0 0 0 0
Barnwell 2009 1B2009 2 5 Cotton Corn 7 1 0 0 1 0 0 0 0 1
Barnwell 2009 1B2009 2 10 Cotton Corn 7 0 0 0 0 0 0 0 0 0
Barnwell 2009 1B2009 2 25 Cotton Corn 7 1 0 0 1 0 0 0 0 1
Barnwell 2009 1B2009 3 0 Cotton Corn 7 1 0 0 1 0 0 0 0 1
Barnwell 2009 1B2009 3 5 Cotton Corn 7 1 0 1 2 0 0 0 0 2
Barnwell 2009 1B2009 3 10 Cotton Corn 7 1 0 0 1 0 0 0 0 1
```

Barnwell	2009	1B2009	3	25	Cotton	Corn	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	2B2009	4	0	Cotton	SB	7	6	0	0	6	0	0	0	0	6
Barnwell	2009	2B2009	4	5	Cotton	SB	7	5	0	3	8	1	0	0	1	9
Barnwell	2009	2B2009	4	10	Cotton	SB	7	1	0	1	2	0	0	1	1	3
Barnwell	2009	2B2009	4	25	Cotton	SB	7	2	0	1	3	0	0	0	0	3
Barnwell	2009	2B2009	5	0	Cotton	SB	7	1	0	1	2	0	0	0	0	2
Barnwell	2009	2B2009	5	5	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	2B2009	5	10	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	2B2009	5	25	Cotton	SB	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	2B2009	6	0	Cotton	SB	7	2	0	0	2	0	0	0	0	2
Barnwell	2009	2B2009	6	5	Cotton	SB	7	0	0	1	1	0	0	0	0	1
Barnwell	2009	2B2009	6	10	Cotton	SB	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	2B2009	6	25	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	3B2009	7	0	Cotton	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	3B2009	7	5	Cotton	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	3B2009	7	10	Cotton	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	3B2009	7	25	Cotton	Fallow	7	0	0	1	1	0	0	0	0	1
Barnwell	2009	3B2009	8	0	Cotton	Fallow	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	3B2009	8	5	Cotton	Fallow	7	2	0	0	2	0	0	0	0	2
Barnwell	2009	3B2009	8	10	Cotton	Fallow	7	1	0	1	2	0	0	0	0	2
Barnwell	2009	3B2009	8	25	Cotton	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	3B2009	9	0	Cotton	Fallow	7	2	1	0	3	0	0	0	0	3
Barnwell	2009	3B2009	9	5	Cotton	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	3B2009	9	10	Cotton	Fallow	7	0	0	1	1	0	0	0	0	1
Barnwell	2009	3B2009	9	25	Cotton	Fallow	7	5	0	0	5	0	0	0	0	5
Barnwell	2009	4B2009	10	0	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	4B2009	10	5	Cotton	SB	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	4B2009	10	10	Cotton	SB	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	4B2009	10	25	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	4B2009	11	0	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	4B2009	11	5	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	4B2009	11	10	Cotton	SB	7	0	0	1	1	0	0	0	0	1
Barnwell	2009	4B2009	11	25	Cotton	SB	7	2	0	0	2	0	0	0	0	2
Barnwell	2009	4B2009	12	0	Cotton	SB	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	4B2009	12	5	Cotton	SB	7	0	1	1	2	0	0	0	0	2
Barnwell	2009	4B2009	12	10	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	4B2009	12	25	Cotton	SB	7	2	0	0	2	0	0	0	0	2
Barnwell	2009	5B2009	13	0	Cotton	Corn	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	5B2009	13	5	Cotton	Corn	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	5B2009	13	10	Cotton	Corn	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	5B2009	13	25	Cotton	Corn	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	5B2009	14	0	Cotton	Corn	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	5B2009	14	5	Cotton	Corn	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	5B2009	14	10	Cotton	Corn	7	0	0	0	0	0	0	0	0	0

Barnwell	2009	5B2009	14	25	Cotton	Corn	7	2	0	0	2	0	0	0	0	2
Barnwell	2009	5B2009	15	0	Cotton	Corn	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	5B2009	15	5	Cotton	Corn	7	3	0	0	3	0	0	0	0	3
Barnwell	2009	5B2009	15	10	Cotton	Corn	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	5B2009	15	25	Cotton	Corn	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	16	0	Cotton	SB	7	2	0	2	4	0	0	0	0	4
Barnwell	2009	6B2009	16	5	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	16	10	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	16	25	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	17	0	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	17	5	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	17	10	Cotton	SB	7	1	0	0	1	0	0	0	0	1
Barnwell	2009	6B2009	17	25	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	18	0	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	18	5	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	6B2009	18	10	Cotton	SB	7	0	0	1	1	0	0	0	0	1
Barnwell	2009	6B2009	18	25	Cotton	SB	7	0	0	0	0	0	0	0	0	0
Barnwell	2009	7B2009	19	0	Fallow	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	7B2009	19	5	Fallow	Cotton	6	1	0	0	1	0	0	0	0	1
Barnwell	2009	7B2009	19	10	Fallow	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	7B2009	19	25	Fallow	Cotton	6	1	0	0	1	0	3	0	3	4
Barnwell	2009	7B2009	20	0	Fallow	Cotton	6	4	0	0	4	1	0	0	1	5
Barnwell	2009	7B2009	20	5	Fallow	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	7B2009	20	10	Fallow	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	7B2009	20	25	Fallow	Cotton	6	0	0	0	0	0	0	0	0	0
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Barnwell	2009	8B2009	22	0	SB	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	8B2009	22	5	SB	Cotton	6	0	0	1	1	0	0	0	0	1
Barnwell	2009	8B2009	22	10	SB	Cotton	6	0	0	1	1	0	0	0	0	1
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Barnwell	2009	8B2009	23	0	SB	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	8B2009	23	5	SB	Cotton	6	2	0	0	2	0	0	0	0	2
Barnwell	2009	8B2009	23	10	SB	Cotton	6	2	0	0	2	1	0	0	1	3
Barnwell	2009	8B2009	23	25	SB	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	8B2009	24	0	SB	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	8B2009	24	5	SB	Cotton	6	1	0	0	1	0	0	0	0	1
Barnwell	2009	8B2009	24	10	SB	Cotton	6	2	0	0	2	0	0	0	0	2
Barnwell	2009	8B2009	24	25	SB	Cotton	6	0	0	1	1	0	1	0	1	2
Barnwell	2009	9B2009	25	0	SB	Cotton	6	0	0	1	1	0	0	0	0	1
Barnwell	2009	9B2009	25	5	SB	Cotton	6	0	0	1	1	0	0	0	0	1
Barnwell	2009	9B2009	25	10	SB	Cotton	6	0	0	0	0	0	0	0	0	0

Barnwell	2009	9B2009	25	25	SB	Cotton	6	2	0	1	3	0	0	0	0	3
Barnwell	2009	9B2009	26	0	SB	Cotton	6	0	0	2	2	0	0	0	0	2
Barnwell	2009	9B2009	26	5	SB	Cotton	6	0	1	0	1	0	0	0	0	1
Barnwell	2009	9B2009	26	10	SB	Cotton	6	3	0	0	3	0	0	0	0	3
Barnwell	2009	9B2009	26	25	SB	Cotton	6	0	0	1	1	0	0	1	1	2
Barnwell	2009	9B2009	27	0	SB	Cotton	6	0	0	0	0	0	0	1	1	1
Barnwell	2009	9B2009	27	5	SB	Cotton	6	0	0	0	0	0	0	0	0	0
Barnwell	2009	9B2009	27	10	SB	Cotton	6	1	0	1	2	0	0	1	1	3
Barnwell	2009	9B2009	27	25	SB	Cotton	6	1	0	0	1	0	0	0	0	1
Barnwell	2010	1B2010	1	0	Cotton	Fallow	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	1B2010	1	5	Cotton	Fallow	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	1B2010	1	10	Cotton	Fallow	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	1B2010	1	25	Cotton	Fallow	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	1B2010	2	0	Cotton	Fallow	5	1	0	0	1	0	0	0	0	1
Barnwell	2010	1B2010	2	5	Cotton	Fallow	5	3	0	0	3	0	0	0	0	3
Barnwell	2010	1B2010	2	10	Cotton	Fallow	5	1	0	2	3	0	0	0	0	3
Barnwell	2010	1B2010	2	25	Cotton	Fallow	5	1	0	1	2	0	0	0	0	2
Barnwell	2010	2B2010	3	0	Cotton	Corn	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	2B2010	3	5	Cotton	Corn	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	2B2010	3	10	Cotton	Corn	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	2B2010	3	25	Cotton	Corn	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	2B2010	4	0	Cotton	Corn	5	0	0	1	1	0	0	0	0	1
Barnwell	2010	2B2010	4	5	Cotton	Corn	5	1	0	0	1	0	0	0	0	1
Barnwell	2010	2B2010	4	10	Cotton	Corn	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	2B2010	4	25	Cotton	Corn	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	3B2010	5	0	Cotton	Peanut	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	3B2010	5	5	Cotton	Peanut	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	3B2010	5	10	Cotton	Peanut	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	3B2010	5	25	Cotton	Peanut	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	3B2010	10	0	Cotton	Peanut	5	1	0	0	1	0	0	0	0	1
Barnwell	2010	3B2010	10	5	Cotton	Peanut	5	1	0	0	1	0	0	0	0	1
Barnwell	2010	3B2010	10	10	Cotton	Peanut	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	3B2010	10	25	Cotton	Peanut	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	4B2010	11	0	Cotton	Peanut	5	1	0	0	1	0	0	0	0	1
Barnwell	2010	4B2010	11	5	Cotton	Peanut	5	1	0	0	1	0	0	0	0	1
Barnwell	2010	4B2010	11	10	Cotton	Peanut	5	2	0	0	2	0	0	0	0	2
Barnwell	2010	4B2010	11	25	Cotton	Peanut	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	4B2010	12	0	Cotton	Peanut	5	0	0	0	0	0	0	0	0	0
Barnwell	2010	4B2010	12	5	Cotton	Peanut	5	1	0	0	1	0	0	0	0	1
Barnwell	2010	4B2010	12	10	Cotton	Peanut	5	2	0	0	2	0	0	0	0	2
Barnwell	2010	4B2010	12	25	Cotton	Peanut	5	0	1	0	1	0	0	0	0	1
Barnwell	2010	5B2010	6	0	SB	Peanut	10	2	0	6	8	0	0	0	0	8
Barnwell	2010	5B2010	6	5	SB	Peanut	10	2	0	8	10	0	0	0	0	10
Barnwell	2010	5B2010	6	10	SB	Peanut	10	0	0	1	1	0	0	0	0	1



Barnwell	2010	5B2010	6	25	SB	Peanut	10	0	0	5	5	0	1	0	1	6
Barnwell	2010	5B2010	7	0	SB	Fallow	10	1	0	0	1	0	0	0	0	1
Barnwell	2010	5B2010	7	5	SB	Fallow	10	0	0	0	0	0	0	0	0	0
Barnwell	2010	5B2010	7	10	SB	Fallow	10	0	0	1	1	0	0	0	0	1
Barnwell	2010	5B2010	7	25	SB	Fallow	10	0	0	2	2	0	0	0	0	2
Barnwell	2010	6B2010	8	0	SB	Peanut	10	1	0	15	16	0	0	0	0	16
Barnwell	2010	6B2010	8	5	SB	Peanut	10	5	0	7	12	0	0	0	0	12
Barnwell	2010	6B2010	8	10	SB	Peanut	10	9	1	11	21	2	0	1	3	24
Barnwell	2010	6B2010	8	25	SB	Peanut	10	1	1	0	2	0	0	0	0	2
Barnwell	2010	6B2010	9	0	SB	Peanut	10	1	0	2	3	0	0	0	0	3
Barnwell	2010	6B2010	9	5	SB	Peanut	10	1	0	5	6	0	0	0	0	6
Barnwell	2010	6B2010	9	10	SB	Peanut	10	3	1	4	8	0	0	0	0	8
Barnwell	2010	6B2010	9	25	SB	Peanut	10	2	0	3	5	0	0	2	2	7
Barnwell	2011	1B2011	7	0	Cotton	Fallow	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	1B2011	7	5	Cotton	Fallow	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	1B2011	7	10	Cotton	Fallow	4	1	0	0	1	0	0	0	0	1
Barnwell	2011	1B2011	7	25	Cotton	Fallow	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	1B2011	8	0	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	1B2011	8	5	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	1B2011	8	10	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	1B2011	8	25	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	2B2011	9	0	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	2B2011	9	5	Cotton	Peanut	4	0	0	2	2	0	0	0	0	2
Barnwell	2011	2B2011	9	10	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	2B2011	9	25	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	2B2011	10	0	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	2B2011	10	5	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	2B2011	10	10	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	2B2011	10	25	Cotton	Peanut	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	3B2011	4	0	Cotton	Corn	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	3B2011	4	5	Cotton	Corn	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	3B2011	4	10	Cotton	Corn	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	3B2011	4	25	Cotton	Corn	4	0	0	0	0	0	0	0	0	0
Barnwell	2011	4B2011	1	0	Peanut	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	4B2011	1	5	Peanut	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	4B2011	1	10	Peanut	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	4B2011	1	25	Peanut	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	4B2011	2	0	Peanut	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	4B2011	2	5	Peanut	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	4B2011	2	10	Peanut	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	4B2011	2	25	Peanut	Fallow	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	5B2011	5	0	Peanut	Cotton	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	5B2011	5	5	Peanut	Cotton	7	0	0	0	0	0	0	0	0	0
Barnwell	2011	5B2011	5	10	Peanut	Cotton	7	0	0	0	0	0	0	0	0	0

```

Barnwell 2011 5B2011 5 25 Peanut Cotton 7 0 0 0 0 0 0 0 0 0
Barnwell 2011 6B2011 3 0 Fallow Corn 7 0 0 0 0 0 0 0 0 0
Barnwell 2011 6B2011 3 5 Fallow Corn 7 0 0 0 0 0 0 0 0 0
Barnwell 2011 6B2011 3 10 Fallow Corn 7 1 0 0 1 0 0 0 0 1
Barnwell 2011 6B2011 3 25 Fallow Corn 7 1 0 0 1 0 0 0 0 1
Barnwell 2011 7B2011 6 0 Fallow Cotton 7 0 0 0 0 0 0 0 0 0
Barnwell 2011 7B2011 6 5 Fallow Cotton 7 0 0 0 0 1 0 0 1 1
Barnwell 2011 7B2011 6 10 Fallow Cotton 7 2 0 0 2 0 0 0 0 2
Barnwell 2011 7B2011 6 25 Fallow Cotton 7 0 0 0 0 1 0 0 1 1
;
PROC GLIMMIX;
CLASS Year FieldID Transect Distance CropAdj;
MODEL *Variable* = Cropadj Distance Distance*Cropadj/ DFM=KENWARDROGER;
RANDOM year Distance*Year FieldID(Year) Cropadj*FieldID(Year)
Transect(Cropadj*FieldID*Year);
LSMEANS Distance CropAdj distance*cropadj;
ESTIMATE 'sig vs others' cropadj *coefficients assigned as needed* /divisor=*assigned
as needed*;
RUN;QUIT;

```

Program 2: Edge Effect Test for Lee County, SC.

```
dm'log;clear;output;clear';
Title'Edge Test';
options nodate nonumber ps=55 ls=78;
data EdgeLee;
input Location$ Year FieldID$ Transect$ Distance Crop$ Adjacent$ NumTimes BSB
SGSB GSB ALL BSBN SGSBN GSBN ALLN AllBugs;
AvgBAd= BSB/NumTimes;
AvgBNy= BSBN/NumTimes;
AvgSGAd= SGSB/NumTimes;
AvgSGN= SGSBN/NumTimes;
AvgGAd= GSB/NumTimes;
AvgGN= GSBN/NumTimes;
AvgBug= All/NumTimes;
AvgNym= AllN/NumTimes;
cropadj=crop||adjacent;
cards;
Lynchburg 2009 1L2009 1 0 Cotton SB 13 10 0 0 10 1 0 0 1 11
Lynchburg 2009 1L2009 1 5 Cotton SB 13 2 2 12 16 0 0 0 0 16
Lynchburg 2009 1L2009 1 10 Cotton SB 13 2 0 0 2 1 0 0 1 3
Lynchburg 2009 1L2009 1 25 Cotton SB 13 0 0 2 2 0 0 0 0 2
Lynchburg 2009 1L2009 2 0 Cotton SB 13 6 2 20 28 0 1 0 1 29
Lynchburg 2009 1L2009 2 5 Cotton SB 13 0 0 0 0 0 0 0 0 0
Lynchburg 2009 1L2009 2 10 Cotton SB 13 2 0 4 6 0 0 0 0 6
Lynchburg 2009 1L2009 2 25 Cotton SB 13 0 0 0 0 0 0 0 0 0
Lynchburg 2009 1L2009 3 0 Cotton SB 13 2 2 2 6 0 0 0 0 6
Lynchburg 2009 1L2009 3 5 Cotton SB 13 2 2 0 4 0 0 0 0 4
Lynchburg 2009 1L2009 3 10 Cotton SB 13 0 0 2 2 0 0 0 0 2
Lynchburg 2009 1L2009 3 25 Cotton SB 13 0 0 2 2 0 0 1 1 3
Lynchburg 2009 2L2009 4 0 SB Cotton 13 4 4 24 32 15 22 19 56 88
Lynchburg 2009 2L2009 4 5 SB Cotton 13 10 4 4 18 17 21 4 42 60
Lynchburg 2009 2L2009 4 10 SB Cotton 13 12 6 4 22 5 12 10 27 49
Lynchburg 2009 2L2009 4 25 SB Cotton 13 6 2 2 10 1 12 11 24 34
Lynchburg 2009 2L2009 5 0 SB Cotton 13 2 0 4 6 14 8 15 37 43
Lynchburg 2009 2L2009 5 5 SB Cotton 13 4 0 0 4 1 8 1 10 14
Lynchburg 2009 2L2009 5 10 SB Cotton 13 2 2 2 6 2 1 2 5 11
Lynchburg 2009 2L2009 5 25 SB Cotton 13 2 0 0 2 1 12 4 17 19
Lynchburg 2009 2L2009 6 0 SB Cotton 13 2 8 4 14 18 25 8 51 65
Lynchburg 2009 2L2009 6 5 SB Cotton 13 10 2 0 12 8 11 0 19 31
Lynchburg 2009 2L2009 6 10 SB Cotton 13 6 2 2 10 3 10 4 17 27
Lynchburg 2009 2L2009 6 25 SB Cotton 13 2 0 0 2 1 3 3 7 9
Lynchburg 2009 3L2009 7 0 Corn WSB 12 0 0 0 0 0 0 0 0 0
Lynchburg 2009 3L2009 7 5 Corn WSB 12 0 0 0 0 1 0 0 1 1
Lynchburg 2009 3L2009 7 10 Corn WSB 12 0 0 0 0 0 1 0 1 1
```

Lynchburg	2009	3L2009	7	25	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	3L2009	8	0	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	3L2009	8	5	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	3L2009	8	10	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	3L2009	8	25	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	3L2009	9	0	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	3L2009	9	5	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	3L2009	9	10	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	3L2009	9	25	Corn	WSB	12	0	0	0	0	0	0	0	0	0	0	0
Lynchburg	2009	4L2009	10	0	WSB	Corn	12	0	2	0	2	1	1	0	2	4		
Lynchburg	2009	4L2009	10	5	WSB	Corn	12	4	0	0	4	5	2	1	8	12		
Lynchburg	2009	4L2009	10	10	WSB	Corn	12	6	2	0	8	7	3	1	11	19		
Lynchburg	2009	4L2009	10	25	WSB	Corn	12	2	0	0	2	1	6	1	8	10		
Lynchburg	2009	4L2009	11	0	WSB	Corn	12	4	0	0	4	1	0	0	1	5		
Lynchburg	2009	4L2009	11	5	WSB	Corn	12	4	0	0	4	1	2	0	3	7		
Lynchburg	2009	4L2009	11	10	WSB	Corn	12	4	0	0	4	5	1	1	7	11		
Lynchburg	2009	4L2009	11	25	WSB	Corn	12	4	0	0	4	5	3	1	9	13		
Lynchburg	2009	4L2009	12	0	WSB	Corn	12	0	0	0	0	7	3	0	10	10		
Lynchburg	2009	4L2009	12	5	WSB	Corn	12	6	0	0	6	3	2	0	5	11		
Lynchburg	2009	4L2009	12	10	WSB	Corn	12	8	0	0	8	4	0	0	4	12		
Lynchburg	2009	4L2009	12	25	WSB	Corn	12	4	0	0	4	5	3	0	8	12		
Lynchburg	2009	5L2009	13	0	SB	Woods	12	6	0	2	8	12	0	9	21	29		
Lynchburg	2009	5L2009	13	5	SB	Woods	12	2	0	0	2	7	0	2	9	11		
Lynchburg	2009	5L2009	13	10	SB	Woods	12	4	0	0	4	10	1	0	11	15		
Lynchburg	2009	5L2009	13	25	SB	Woods	12	4	0	0	4	6	1	0	7	11		
Lynchburg	2009	5L2009	14	0	SB	Woods	12	8	0	4	12	10	0	6	16	28		
Lynchburg	2009	5L2009	14	5	SB	Woods	12	10	0	4	14	15	5	2	22	36		
Lynchburg	2009	5L2009	14	10	SB	Woods	12	8	0	4	12	11	2	0	13	25		
Lynchburg	2009	5L2009	14	25	SB	Woods	12	0	0	0	0	15	0	0	15	15		
Lynchburg	2009	5L2009	15	0	SB	Woods	12	8	2	18	28	13	0	3	16	44		
Lynchburg	2009	5L2009	15	5	SB	Woods	12	8	0	8	16	6	3	1	10	26		
Lynchburg	2009	5L2009	15	10	SB	Woods	12	6	0	4	10	5	8	3	16	26		
Lynchburg	2009	5L2009	15	25	SB	Woods	12	4	0	4	8	15	3	1	19	27		
Lynchburg	2009	6L2009	16	0	SB	Woods	11	0	2	0	2	0	0	0	0	2		
Lynchburg	2009	6L2009	16	5	SB	Woods	11	4	0	0	4	0	0	0	0	4		
Lynchburg	2009	6L2009	16	10	SB	Woods	11	4	2	0	6	4	2	3	9	15		
Lynchburg	2009	6L2009	16	25	SB	Woods	11	0	4	2	6	4	3	1	8	14		
Lynchburg	2009	6L2009	17	0	SB	Woods	11	0	0	0	0	0	0	1	1	1		
Lynchburg	2009	6L2009	17	5	SB	Woods	11	0	0	0	0	3	0	1	4	4		
Lynchburg	2009	6L2009	17	10	SB	Woods	11	2	0	0	2	6	3	5	14	16		
Lynchburg	2009	6L2009	17	25	SB	Woods	11	2	4	0	6	2	2	2	6	12		
Lynchburg	2009	6L2009	18	0	SB	Woods	11	0	4	0	4	3	0	0	3	7		
Lynchburg	2009	6L2009	18	5	SB	Woods	11	0	2	0	2	0	1	0	1	3		
Lynchburg	2009	6L2009	18	10	SB	Woods	11	0	0	2	2	6	7	1	14	16		

Lynchburg	2009	6L2009	18	25	SB	Woods	11	0	2	0	2	3	4	1	8	10
Lynchburg	2010	1L2010	1	0	Corn	Cotton	9	0	0	0	0	0	0	0	0	0
Lynchburg	2010	1L2010	1	5	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	1L2010	1	10	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	1L2010	1	25	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	1L2010	2	0	Corn	Woods	9	2	0	0	2	0	0	0	0	2
Lynchburg	2010	1L2010	2	5	Corn	Cotton	9	0	0	0	0	0	0	0	0	0
Lynchburg	2010	1L2010	2	10	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	1L2010	2	25	Corn	Cotton	9	0	0	0	0	0	0	0	0	0
Lynchburg	2010	1L2010	3	0	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	1L2010	3	5	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	1L2010	3	10	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	1L2010	3	25	Corn	Cotton	9	0	0	0	0	0	0	0	0	0
Lynchburg	2010	2L2010	4	0	Cotton	Corn	10	3	0	0	3	1	0	0	1	4
Lynchburg	2010	2L2010	4	5	Cotton	Corn	10	0	1	0	1	0	0	0	0	1
Lynchburg	2010	2L2010	4	10	Cotton	Corn	10	3	2	0	5	0	0	0	0	5
Lynchburg	2010	2L2010	4	25	Cotton	Corn	10	3	1	0	4	0	0	0	0	4
Lynchburg	2010	2L2010	5	0	Cotton	Corn	10	4	0	0	4	0	0	0	0	4
Lynchburg	2010	2L2010	5	5	Cotton	Corn	10	3	0	0	3	0	0	0	0	3
Lynchburg	2010	2L2010	5	10	Cotton	Corn	10	2	0	1	3	0	0	0	0	3
Lynchburg	2010	2L2010	5	25	Cotton	Corn	10	2	0	0	2	0	0	0	0	2
Lynchburg	2010	2L2010	6	0	Cotton	Corn	10	6	0	0	6	0	0	0	0	6
Lynchburg	2010	2L2010	6	5	Cotton	Corn	10	3	0	2	5	0	0	0	0	5
Lynchburg	2010	2L2010	6	10	Cotton	Corn	10	2	5	0	7	0	0	0	0	7
Lynchburg	2010	2L2010	6	25	Cotton	Corn	10	1	0	0	1	0	0	0	0	1
Lynchburg	2010	3L2010	7	0	SB	Cotton	11	9	2	0	11	6	1	4	11	22
Lynchburg	2010	3L2010	7	5	SB	Cotton	11	2	0	0	2	6	1	2	9	11
Lynchburg	2010	3L2010	7	10	SB	Cotton	11	1	2	0	3	4	2	6	12	15
Lynchburg	2010	3L2010	7	25	SB	Cotton	11	1	1	0	2	5	1	4	10	12
Lynchburg	2010	3L2010	8	0	SB	Cotton	11	6	1	0	7	5	0	4	9	16
Lynchburg	2010	3L2010	8	5	SB	Cotton	11	2	2	0	4	7	1	5	13	17
Lynchburg	2010	3L2010	8	10	SB	Cotton	11	4	1	0	5	5	0	0	5	10
Lynchburg	2010	3L2010	8	25	SB	Cotton	11	3	3	0	6	1	1	5	7	13
Lynchburg	2010	3L2010	9	0	SB	Cotton	11	4	1	0	5	6	1	4	11	16
Lynchburg	2010	3L2010	9	5	SB	Cotton	11	2	1	1	4	2	1	4	7	11
Lynchburg	2010	3L2010	9	10	SB	Cotton	11	7	1	3	11	3	0	6	9	20
Lynchburg	2010	3L2010	9	25	SB	Cotton	11	1	3	1	5	5	0	2	7	12
Lynchburg	2010	4L2010	10	0	Cotton	SB	11	3	3	0	6	0	0	0	0	6
Lynchburg	2010	4L2010	10	5	Cotton	SB	11	4	3	0	7	0	0	0	0	7
Lynchburg	2010	4L2010	10	10	Cotton	SB	11	2	0	0	2	0	0	0	0	2
Lynchburg	2010	4L2010	10	25	Cotton	SB	11	2	0	0	2	0	0	0	0	2
Lynchburg	2010	4L2010	11	0	Cotton	SB	11	7	0	0	7	1	0	0	1	8
Lynchburg	2010	4L2010	11	5	Cotton	SB	11	1	1	0	2	0	0	0	0	2
Lynchburg	2010	4L2010	11	10	Cotton	SB	11	2	0	0	2	0	0	0	0	2

Lynchburg	2010	4L2010	11	25	Cotton	SB	11	3	1	2	6	0	0	0	0	6
Lynchburg	2010	4L2010	12	0	Cotton	SB	11	6	1	0	7	0	0	0	0	7
Lynchburg	2010	4L2010	12	5	Cotton	SB	11	3	1	0	4	0	0	0	0	4
Lynchburg	2010	4L2010	12	10	Cotton	SB	11	2	0	0	2	0	0	0	0	2
Lynchburg	2010	4L2010	12	25	Cotton	SB	11	1	1	0	2	0	2	0	2	4
Lynchburg	2010	5L2010	13	0	Cotton	Corn	11	2	0	0	2	0	0	0	0	2
Lynchburg	2010	5L2010	13	5	Cotton	Corn	11	2	0	0	2	0	0	0	0	2
Lynchburg	2010	5L2010	13	10	Cotton	Corn	11	1	0	0	1	0	0	0	0	1
Lynchburg	2010	5L2010	13	25	Cotton	Corn	11	3	0	0	3	0	0	0	0	3
Lynchburg	2010	5L2010	14	0	Cotton	Corn	11	3	0	0	3	0	1	0	1	4
Lynchburg	2010	5L2010	14	5	Cotton	Corn	11	1	0	0	1	0	0	0	0	1
Lynchburg	2010	5L2010	14	10	Cotton	Corn	11	0	0	0	0	0	0	0	0	0
Lynchburg	2010	5L2010	14	25	Cotton	Corn	11	0	0	0	0	0	0	0	0	0
Lynchburg	2010	5L2010	15	0	Cotton	Corn	11	3	0	0	3	0	0	0	0	3
Lynchburg	2010	5L2010	15	5	Cotton	Corn	11	2	0	0	2	0	0	0	0	2
Lynchburg	2010	5L2010	15	10	Cotton	Corn	11	3	0	0	3	0	0	0	0	3
Lynchburg	2010	5L2010	15	25	Cotton	Corn	11	2	1	0	3	0	0	0	0	3
Lynchburg	2010	6L2010	16	0	Corn	Cotton	9	0	0	0	0	0	0	0	0	0
Lynchburg	2010	6L2010	16	5	Corn	Cotton	9	2	0	0	2	0	0	0	0	2
Lynchburg	2010	6L2010	16	10	Corn	Cotton	9	4	0	0	4	0	0	0	0	4
Lynchburg	2010	6L2010	16	25	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	6L2010	17	0	Corn	Cotton	9	3	0	0	3	0	0	0	0	3
Lynchburg	2010	6L2010	17	5	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	6L2010	17	10	Corn	Cotton	9	2	0	0	2	0	0	0	0	2
Lynchburg	2010	6L2010	17	25	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	6L2010	18	0	Corn	Cotton	9	0	0	0	0	0	0	0	0	0
Lynchburg	2010	6L2010	18	5	Corn	Cotton	9	2	0	0	2	0	0	0	0	2
Lynchburg	2010	6L2010	18	10	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	6L2010	18	25	Corn	Cotton	9	1	0	0	1	0	0	0	0	1
Lynchburg	2010	7L2010	19	0	SB	Woods	11	0	0	0	0	1	0	0	1	1
Lynchburg	2010	7L2010	19	5	SB	Woods	11	8	0	0	8	5	1	6	12	20
Lynchburg	2010	7L2010	19	10	SB	Woods	11	4	0	0	4	13	2	1	16	20
Lynchburg	2010	7L2010	19	25	SB	Woods	11	0	1	0	1	8	1	0	9	10
Lynchburg	2010	7L2010	20	0	SB	Woods	11	0	0	0	0	0	0	0	0	0
Lynchburg	2010	7L2010	20	5	SB	Woods	11	0	0	0	0	5	1	4	10	10
Lynchburg	2010	7L2010	20	10	SB	Woods	11	2	1	0	3	13	2	0	15	18
Lynchburg	2010	7L2010	20	25	SB	Woods	11	1	0	0	1	12	0	0	12	13
Lynchburg	2010	7L2010	21	0	SB	Woods	11	0	1	0	1	1	0	1	2	3
Lynchburg	2010	7L2010	21	5	SB	Woods	11	2	2	0	4	10	6	0	16	20
Lynchburg	2010	7L2010	21	10	SB	Woods	11	7	1	1	9	6	3	8	17	26
Lynchburg	2010	7L2010	21	25	SB	Woods	11	0	1	0	1	5	1	0	6	7
Lynchburg	2010	8L2010	22	0	SB	Woods	12	3	3	0	6	4	0	3	7	13
Lynchburg	2010	8L2010	22	5	SB	Woods	12	6	2	2	10	9	1	4	14	24
Lynchburg	2010	8L2010	22	10	SB	Woods	12	10	2	1	13	2	0	4	6	19

Lynchburg	2010	8L2010	22	25	SB Woods	12	6	0	0	6	6	0	2	8	14
Lynchburg	2010	8L2010	23	0	SB Woods	12	2	0	0	2	3	0	0	3	5
Lynchburg	2010	8L2010	23	5	SB Woods	12	2	0	0	2	5	1	3	9	11
Lynchburg	2010	8L2010	23	10	SB Woods	12	11	4	0	15	5	0	8	13	28
Lynchburg	2010	8L2010	23	25	SB Woods	12	8	0	1	9	2	1	3	6	15
Lynchburg	2010	8L2010	24	0	SB Woods	12	2	1	0	3	0	0	0	0	3
Lynchburg	2010	8L2010	24	5	SB Woods	12	4	1	0	5	1	0	0	1	6
Lynchburg	2010	8L2010	24	10	SB Woods	12	10	7	1	18	3	4	1	8	26
Lynchburg	2010	8L2010	24	25	SB Woods	12	12	1	0	13	6	0	1	7	20
Lynchburg	2010	9L2010	1a	0	Wheat Woods	7	1	1	0	2	2	1	0	3	5
Lynchburg	2010	9L2010	1a	5	Wheat Woods	7	3	3	0	6	9	4	0	13	19
Lynchburg	2010	9L2010	1a	10	Wheat Woods	7	2	0	1	3	2	1	0	3	6
Lynchburg	2010	9L2010	1a	25	Wheat Woods	7	0	0	0	0	2	0	0	2	2
Lynchburg	2010	9L2010	2a	0	Wheat Cotton	7	4	2	0	6	1	0	0	1	7
Lynchburg	2010	9L2010	2a	5	Wheat Woods	7	1	4	0	5	2	0	0	2	7
Lynchburg	2010	9L2010	2a	10	Wheat Woods	7	3	5	0	8	2	0	0	2	10
Lynchburg	2010	9L2010	2a	25	Wheat Woods	7	2	0	0	2	4	0	0	4	6
Lynchburg	2010	9L2010	3a	0	Wheat Woods	7	6	0	0	6	1	0	0	1	7
Lynchburg	2010	9L2010	3a	5	Wheat Woods	7	3	3	0	6	0	1	0	1	7
Lynchburg	2010	9L2010	3a	10	Wheat Woods	7	0	0	0	0	1	0	0	1	1
Lynchburg	2010	9L2010	3a	25	Wheat Woods	7	5	2	0	7	10	0	0	10	17
Lynchburg	2011	1L2011	1	0	Cotton Woods	7	7	0	2	9	1	0	1	2	11
Lynchburg	2011	1L2011	1	5	Cotton Woods	7	4	0	4	8	0	0	0	0	8
Lynchburg	2011	1L2011	1	10	Cotton Woods	7	2	0	3	5	0	0	0	0	5
Lynchburg	2011	1L2011	1	25	Cotton Woods	7	0	0	1	1	0	0	0	0	1
Lynchburg	2011	1L2011	2	0	Cotton Woods	7	11	0	4	15	0	0	0	0	15
Lynchburg	2011	1L2011	2	5	Cotton Woods	7	2	0	5	7	0	0	0	0	7
Lynchburg	2011	1L2011	2	10	Cotton Woods	7	3	0	4	7	0	0	0	0	7
Lynchburg	2011	1L2011	2	25	Cotton Woods	7	6	0	3	9	0	0	0	0	9
Lynchburg	2011	1L2011	3	0	Cotton Woods	7	3	0	5	8	0	0	0	0	8
Lynchburg	2011	1L2011	3	5	Cotton Woods	7	0	0	0	0	0	0	0	0	0
Lynchburg	2011	1L2011	3	10	Cotton Woods	7	3	0	3	6	0	0	0	0	6
Lynchburg	2011	1L2011	3	25	Cotton Woods	7	1	0	2	3	0	0	0	0	3
Lynchburg	2011	2L2011	4	0	Cotton SB	7	3	0	2	5	0	0	0	0	5
Lynchburg	2011	2L2011	4	5	Cotton SB	7	0	0	0	0	0	0	0	0	0
Lynchburg	2011	2L2011	4	10	Cotton SB	7	0	0	0	0	0	0	0	0	0
Lynchburg	2011	2L2011	4	25	Cotton SB	7	1	0	0	1	0	0	0	0	1
Lynchburg	2011	2L2011	5	0	Cotton SB	7	3	0	0	3	0	0	0	0	3
Lynchburg	2011	2L2011	5	5	Cotton SB	7	0	0	2	2	0	0	0	0	2
Lynchburg	2011	2L2011	5	10	Cotton SB	7	0	0	0	0	0	0	0	0	0
Lynchburg	2011	2L2011	5	25	Cotton SB	7	1	0	0	1	0	0	0	0	1
Lynchburg	2011	2L2011	6	0	Cotton SB	7	5	0	9	14	0	0	0	0	14
Lynchburg	2011	2L2011	6	5	Cotton SB	7	0	0	1	1	0	0	0	0	1
Lynchburg	2011	2L2011	6	10	Cotton SB	7	1	0	1	2	0	0	0	0	2

Lynchburg 2011 2L2011 6 25 Cotton SB 7 1 0 1 2 0 0 0 0 2  
 Lynchburg 2011 3L2011 7 0 SB Cotton 9 6 0 6 12 27 0 7 34 46  
 Lynchburg 2011 3L2011 7 5 SB Cotton 9 2 0 5 7 14 0 9 23 30  
 Lynchburg 2011 3L2011 7 10 SB Cotton 9 5 0 1 6 36 0 5 41 47  
 Lynchburg 2011 3L2011 7 25 SB Cotton 9 11 0 2 13 36 0 5 41 54  
 Lynchburg 2011 3L2011 8 0 SB Cotton 9 6 0 6 12 20 0 9 29 41  
 Lynchburg 2011 3L2011 8 5 SB Cotton 9 8 0 1 9 22 0 12 34 43  
 Lynchburg 2011 3L2011 8 10 SB Cotton 9 3 0 3 6 14 0 1 15 21  
 Lynchburg 2011 3L2011 8 25 SB Cotton 9 1 0 1 2 14 0 6 20 22  
 Lynchburg 2011 3L2011 9 0 SB Cotton 9 5 0 8 13 20 1 12 33 46  
 Lynchburg 2011 3L2011 9 5 SB Cotton 9 7 0 3 10 23 0 6 29 39  
 Lynchburg 2011 3L2011 9 10 SB Cotton 9 6 0 3 9 39 0 3 42 51  
 Lynchburg 2011 3L2011 9 25 SB Cotton 9 8 0 1 9 10 0 3 13 22  
 Lynchburg 2011 4L2011 10 0 Cotton SB 8 2 0 4 6 0 0 0 0 6  
 Lynchburg 2011 4L2011 10 5 Cotton SB 8 2 0 3 5 0 0 0 0 5  
 Lynchburg 2011 4L2011 10 10 Cotton SB 8 1 0 0 1 0 0 0 0 1  
 Lynchburg 2011 4L2011 10 25 Cotton SB 8 1 0 0 1 0 0 0 0 1  
 Lynchburg 2011 4L2011 11 0 Cotton SB 8 5 0 5 10 0 0 0 0 10  
 Lynchburg 2011 4L2011 11 5 Cotton SB 8 0 0 0 0 0 0 0 0 0  
 Lynchburg 2011 4L2011 11 10 Cotton SB 8 0 0 0 0 0 0 0 0 0  
 Lynchburg 2011 4L2011 11 25 Cotton SB 8 0 0 3 3 0 0 0 0 3  
 Lynchburg 2011 4L2011 12 0 Cotton SB 8 2 0 1 3 0 0 0 0 3  
 Lynchburg 2011 4L2011 12 5 Cotton SB 8 0 0 1 1 0 0 0 0 1  
 Lynchburg 2011 4L2011 12 10 Cotton SB 8 0 0 0 0 0 0 0 0 0  
 Lynchburg 2011 4L2011 12 25 Cotton SB 8 0 0 0 0 0 0 0 0 0  
 Lynchburg 2011 5L2011 13 0 SB Cotton 9 12 0 8 20 31 0 11 42 62  
 Lynchburg 2011 5L2011 13 5 SB Cotton 9 1 0 3 4 19 0 13 32 36  
 Lynchburg 2011 5L2011 13 10 SB Cotton 9 4 0 1 5 28 0 2 30 35  
 Lynchburg 2011 5L2011 13 25 SB Cotton 9 5 0 0 5 17 0 1 18 23  
 Lynchburg 2011 5L2011 14 0 SB Cotton 9 4 0 6 10 10 0 27 37 47  
 Lynchburg 2011 5L2011 14 5 SB Cotton 9 5 0 6 11 4 0 10 14 25  
 Lynchburg 2011 5L2011 14 10 SB Cotton 9 3 0 0 3 19 0 6 25 28  
 Lynchburg 2011 5L2011 14 25 SB Cotton 9 5 0 3 8 24 0 6 30 38  
 Lynchburg 2011 5L2011 15 0 SB Cotton 9 7 0 3 10 18 0 14 32 42  
 Lynchburg 2011 5L2011 15 5 SB Cotton 9 2 0 1 3 15 0 11 26 29  
 Lynchburg 2011 5L2011 15 10 SB Cotton 9 0 0 0 0 12 0 5 17 17  
 Lynchburg 2011 5L2011 15 25 SB Cotton 9 3 0 2 5 31 0 2 33 38  
 Lynchburg 2011 6L2011 16 0 Cotton SB 8 3 0 1 4 1 0 0 1 5  
 Lynchburg 2011 6L2011 16 5 Cotton SB 8 1 0 1 2 0 0 0 0 2  
 Lynchburg 2011 6L2011 16 10 Cotton SB 8 0 0 0 0 0 0 0 0 0  
 Lynchburg 2011 6L2011 16 25 Cotton SB 8 0 0 1 1 0 0 0 0 1  
 Lynchburg 2011 6L2011 17 0 Cotton SB 8 1 0 2 3 0 0 0 0 3  
 Lynchburg 2011 6L2011 17 5 Cotton SB 8 2 0 0 2 0 0 0 0 2  
 Lynchburg 2011 6L2011 17 10 Cotton SB 8 1 0 0 1 0 0 0 0 1



```

Lynchburg 2011 6L2011 17 25 Cotton SB 8 0 0 0 0 0 0 0 0 0
Lynchburg 2011 6L2011 18 0 Cotton SB 8 1 0 0 1 0 0 0 0 1
Lynchburg 2011 6L2011 18 5 Cotton SB 8 1 0 0 1 0 0 0 0 1
Lynchburg 2011 6L2011 18 10 Cotton SB 8 0 0 1 1 0 0 0 0 1
Lynchburg 2011 6L2011 18 25 Cotton SB 8 0 0 0 0 0 0 0 0 0
Lynchburg 2011 7L2011 19 0 SB Cotton 9 6 0 8 14 20 0 10 30 44
Lynchburg 2011 7L2011 19 5 SB Cotton 9 7 0 2 9 11 0 9 20 29
Lynchburg 2011 7L2011 19 10 SB Cotton 9 10 0 12 22 21 0 9 30 52
Lynchburg 2011 7L2011 19 25 SB Cotton 9 5 0 0 5 20 0 1 21 26
Lynchburg 2011 7L2011 20 0 SB Cotton 9 3 0 5 8 4 0 14 18 26
Lynchburg 2011 7L2011 20 5 SB Cotton 9 5 0 1 6 10 0 1 11 17
Lynchburg 2011 7L2011 20 10 SB Cotton 9 8 0 0 8 13 0 5 18 26
Lynchburg 2011 7L2011 20 25 SB Cotton 9 5 0 0 5 14 0 2 16 21
Lynchburg 2011 7L2011 21 0 SB Cotton 9 1 0 5 6 11 0 2 13 19
Lynchburg 2011 7L2011 21 5 SB Cotton 9 2 0 2 4 9 0 2 11 15
Lynchburg 2011 7L2011 21 10 SB Cotton 9 5 0 5 10 18 1 2 21 31
Lynchburg 2011 7L2011 21 25 SB Cotton 9 4 0 4 8 13 0 6 19 27
;
PROC GLIMMIX;
CLASS Year FieldID Transect Distance CropAdj;
MODEL *Variable* = Cropadj Distance Distance*Cropadj/ DFM=KENWARDROGER;
RANDOM year Distance*Year FieldID(Year) Cropadj*FieldID(Year)
Transect(Cropadj*FieldID*Year);
LSMEANS Distance CropAdj distance*cropadj;
ESTIMATE 'sig vs others' cropadj *coefficients assigned as needed* /divisor=*assigned
as needed*;
RUN;QUIT;

```

Program 3: Edge Effect Test for Tift County, GA.

```
dm'log;clear;output;clear';
Title'EdgeTift;
options nodate nonumber ps=55 ls=78;
data border;
input Location$ Year FieldID$ Transect$ Distance Crop$ Adjacent$ NumTimes BSB
SGSB GSB ALL BSBN SGSBN GSN ALLN AllBugs;
AvgBAd= BSB/NumTimes;
AvgBNy= BSBN/NumTimes;
AvgSGAd= SGSB/NumTimes;
AvgSGN= SGSBN/NumTimes;
AvgGAd= GSB/NumTimes;
AvgGN= GSN/NumTimes;
AvgBug= All/NumTimes;
AvgNym= AllN/NumTimes;
cropadj=crop||adjacent;
cards;
GA 2009 12009 1 0 Border ab 16 1 1 0 2 0 0 0 0 2
GA 2009 12009 2 0 Border ab 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 3 0 Border ab 16 1 1 0 2 1 0 0 1 3
GA 2009 12009 1a 5 Cotton Sorghum 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 1a 10 Cotton Sorghum 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 1a 25 Cotton Sorghum 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 1b 5 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 1b 10 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 1b 25 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 2a 5 Cotton Sorghum 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 2a 10 Cotton Sorghum 16 0 1 0 1 0 0 0 0 1
GA 2009 12009 2a 25 Cotton Sorghum 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 2b 5 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 2b 10 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 2b 25 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 3a 5 Cotton Sorghum 16 1 0 0 1 0 0 0 0 1
GA 2009 12009 3a 10 Cotton Sorghum 16 1 0 0 1 0 0 0 0 1
GA 2009 12009 3a 25 Cotton Sorghum 16 1 1 0 2 0 0 0 0 2
GA 2009 12009 3b 5 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 3b 10 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 12009 3b 25 Sorghum Cotton 16 0 0 0 0 0 0 0 0 0
GA 2009 22009 4 0 Border ab 16 4 0 0 4 0 0 0 0 4
GA 2009 22009 5 0 Border ab 16 0 0 0 0 0 0 0 0 0
GA 2009 22009 6 0 Border ab 16 1 0 0 1 0 0 0 0 1
GA 2009 22009 4a 5 Cotton Peanut 16 0 0 0 0 0 0 0 0 0
GA 2009 22009 4a 10 Cotton Peanut 16 1 0 0 1 0 0 0 0 1
GA 2009 22009 4a 25 Cotton Peanut 16 0 0 0 0 0 0 0 0 0
```

GA	2009	22009	4b	5	Peanut Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	4b	10	Peanut Cotton	16	1	0	0	1	0	0	0	0	1
GA	2009	22009	4b	25	Peanut Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	5a	5	Cotton Peanut	16	1	1	0	2	0	0	0	0	2
GA	2009	22009	5a	10	Cotton Peanut	16	1	1	0	2	0	0	0	0	2
GA	2009	22009	5a	25	Cotton Peanut	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	5b	5	Peanut Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	5b	10	Peanut Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	5b	25	Peanut Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	6a	5	Cotton Peanut	16	2	0	0	2	0	0	0	0	2
GA	2009	22009	6a	10	Cotton Peanut	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	6a	25	Cotton Peanut	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	6b	5	Peanut Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	6b	10	Peanut Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	22009	6b	25	Peanut Cotton	16	1	0	0	1	0	0	0	0	1
GA	2009	32009	7	0	Border ab	16	2	2	0	4	0	0	0	0	4
GA	2009	32009	8	0	Border ab	16	1	0	0	1	0	0	0	0	1
GA	2009	32009	9	0	Border ab	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	7a	5	Cotton WM	16	2	1	0	3	0	1	0	1	4
GA	2009	32009	7a	10	Cotton WM	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	7a	25	Cotton WM	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	7b	5	WM Cotton	16	0	1	0	1	0	0	0	0	1
GA	2009	32009	7b	10	WM Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	7b	25	WM Cotton	16	1	0	0	1	0	0	0	0	1
GA	2009	32009	8a	5	Cotton WM	16	1	0	0	1	0	0	0	0	1
GA	2009	32009	8a	10	Cotton WM	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	8a	25	Cotton WM	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	8b	5	WM Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	8b	10	WM Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	8b	25	WM Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	9a	5	Cotton WM	16	1	1	0	2	0	0	0	0	2
GA	2009	32009	9a	10	Cotton WM	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	9a	25	Cotton WM	16	0	0	0	0	0	0	0	0	0
GA	2009	32009	9b	5	WM Cotton	16	1	0	0	1	0	0	0	0	1
GA	2009	32009	9b	10	WM Cotton	16	0	0	1	1	0	0	0	0	1
GA	2009	32009	9b	25	WM Cotton	16	0	0	0	0	0	0	0	0	0
GA	2009	42009	10	0	Border ab	16	1	0	0	1	0	1	1	2	3
GA	2009	42009	11	0	Border ab	16	1	0	0	1	0	0	0	0	1
GA	2009	42009	12	0	Border ab	16	2	2	0	4	1	0	0	1	5
GA	2009	42009	10a	5	Cotton SB	16	0	0	0	0	0	0	0	0	0
GA	2009	42009	10a	10	Cotton SB	16	1	0	0	1	0	0	0	0	1
GA	2009	42009	10a	25	Cotton SB	16	1	0	0	1	0	0	0	0	1
GA	2009	42009	10b	5	SB Cotton	16	1	0	0	1	1	0	0	1	2
GA	2009	42009	10b	10	SB Cotton	16	0	1	0	1	0	0	0	0	1

GA 2009 42009 10b 25 SB Cotton 16 0 0 0 0 0 0 0 0 0  
GA 2009 42009 11a 5 Cotton SB 16 3 0 1 4 0 0 0 0 4  
GA 2009 42009 11a 10 Cotton SB 16 0 0 0 0 0 0 0 0 0  
GA 2009 42009 11a 25 Cotton SB 16 2 0 0 2 0 0 0 0 2  
GA 2009 42009 11b 5 SB Cotton 16 1 0 0 1 1 0 0 1 2  
GA 2009 42009 11b 10 SB Cotton 16 1 0 0 1 0 0 0 0 1  
GA 2009 42009 11b 25 SB Cotton 16 0 0 0 0 0 0 0 0 0  
GA 2009 42009 12a 5 Cotton SB 16 0 0 0 0 0 0 0 0 0  
GA 2009 42009 12a 10 Cotton SB 16 1 0 0 1 0 0 0 0 1  
GA 2009 42009 12a 25 Cotton SB 16 0 0 0 0 0 0 0 0 0  
GA 2009 42009 12b 5 SB Cotton 16 0 0 0 0 2 0 0 2 2  
GA 2009 42009 12b 10 SB Cotton 16 1 0 0 1 0 0 0 0 1  
GA 2009 42009 12b 25 SB Cotton 16 0 0 0 0 0 0 0 0 0  
GA 2009 52009 13 0 Border ab 16 0 0 0 0 2 0 0 2 2  
GA 2009 52009 14 0 Border ab 16 0 1 0 1 0 0 0 0 1  
GA 2009 52009 15 0 Border ab 16 0 1 0 1 0 0 0 0 1  
GA 2009 52009 13a 5 Cotton Pecan 16 1 0 0 1 2 0 0 2 3  
GA 2009 52009 13a 10 Cotton Pecan 16 0 0 0 0 3 0 0 3 3  
GA 2009 52009 13a 25 Cotton Pecan 16 0 0 0 0 2 0 0 2 2  
GA 2009 52009 13b 5 Pecan Cotton 16 2 0 0 2 4 0 0 4 6  
GA 2009 52009 13b 10 Pecan Cotton 16 0 0 0 0 5 0 0 5 5  
GA 2009 52009 13b 25 Pecan Cotton 16 8 1 0 9 3 0 0 3 12  
GA 2009 52009 14a 5 Cotton Pecan 16 0 0 0 0 0 0 0 0 0  
GA 2009 52009 14a 10 Cotton Pecan 16 1 0 0 1 0 0 0 0 1  
GA 2009 52009 14a 25 Cotton Pecan 16 0 0 0 0 0 0 0 0 0  
GA 2009 52009 14b 5 Pecan Cotton 16 1 0 0 1 0 3 0 3 4  
GA 2009 52009 14b 10 Pecan Cotton 16 0 1 0 1 2 6 0 8 9  
GA 2009 52009 14b 25 Pecan Cotton 16 4 1 0 5 0 0 0 0 5  
GA 2009 52009 15a 5 Cotton Pecan 16 1 1 0 2 0 0 0 0 2  
GA 2009 52009 15a 10 Cotton Pecan 16 1 0 0 1 0 0 0 0 1  
GA 2009 52009 15a 25 Cotton Pecan 16 0 0 0 0 0 0 0 0 0  
GA 2009 52009 15b 5 Pecan Cotton 16 0 0 0 0 2 0 0 2 2  
GA 2009 52009 15b 10 Pecan Cotton 16 3 0 0 3 0 0 0 0 3  
GA 2009 52009 15b 25 Pecan Cotton 16 1 0 0 1 1 0 0 1 2  
GA 2010 12010 1 0 Border ab 18 3 0 0 3 3 0 0 3 6  
GA 2010 12010 2 0 Border ab 18 0 0 0 0 0 0 0 0 0  
GA 2010 12010 3 0 Border ab 18 1 0 0 1 2 0 0 2 3  
GA 2010 12010 1a 5 Cotton Pecan 18 0 0 0 0 0 0 0 0 0  
GA 2010 12010 1a 10 Cotton Pecan 18 1 0 2 3 4 0 0 4 7  
GA 2010 12010 1a 25 Cotton Pecan 18 0 0 0 0 0 0 0 0 0  
GA 2010 12010 1b 5 Pecan Cotton 18 5 0 0 5 1 0 0 1 6  
GA 2010 12010 1b 10 Pecan Cotton 18 2 0 0 2 5 0 0 5 7  
GA 2010 12010 1b 25 Pecan Cotton 18 0 0 0 0 0 0 0 0 0  
GA 2010 12010 2a 5 Cotton Pecan 18 0 1 0 1 0 0 0 0 1

GA 2010 12010 2a 10 Cotton Pecan	18 0 0 0 0 0 0 0 0 0
GA 2010 12010 2a 25 Cotton Pecan	18 2 0 0 2 0 0 0 0 2
GA 2010 12010 2b 5 Pecan Cotton	18 5 0 0 5 5 1 0 6 11
GA 2010 12010 2b 10 Pecan Cotton	18 2 0 0 2 1 0 0 1 3
GA 2010 12010 2b 25 Pecan Cotton	18 1 0 0 1 5 0 0 5 6
GA 2010 12010 3a 5 Cotton Pecan	18 3 0 0 3 2 0 0 2 5
GA 2010 12010 3a 10 Cotton Pecan	18 2 0 2 4 1 0 0 1 5
GA 2010 12010 3a 25 Cotton Pecan	18 0 0 0 0 2 0 0 2 2
GA 2010 12010 3b 5 Pecan Cotton	18 3 0 0 3 2 0 0 2 5
GA 2010 12010 3b 10 Pecan Cotton	18 1 0 0 1 3 0 0 3 4
GA 2010 12010 3b 25 Pecan Cotton	18 0 0 0 0 3 1 0 4 4
GA 2010 22010 4 0 Border ab	18 2 0 0 2 13 0 1 14 16
GA 2010 22010 5 0 Border ab	18 13 0 0 13 14 1 0 15 28
GA 2010 22010 6 0 Border ab	18 6 1 0 7 9 2 1 12 19
GA 2010 22010 4a 5 Cotton Peanut	18 0 0 0 0 0 0 0 0 0
GA 2010 22010 4a 10 Cotton Peanut	18 0 0 1 1 0 0 0 0 1
GA 2010 22010 4a 25 Cotton Peanut	18 0 0 0 0 0 0 0 0 0
GA 2010 22010 4b 5 Peanut Cotton	18 0 0 0 0 0 0 0 0 0
GA 2010 22010 4b 10 Peanut Cotton	18 0 0 1 1 0 0 0 0 1
GA 2010 22010 4b 25 Peanut Cotton	18 0 0 0 0 1 0 0 1 1
GA 2010 22010 5a 5 Cotton Peanut	18 0 0 0 0 0 0 0 0 0
GA 2010 22010 5a 10 Cotton Peanut	18 2 0 0 2 0 0 0 0 2
GA 2010 22010 5a 25 Cotton Peanut	18 0 0 0 0 0 0 0 0 0
GA 2010 22010 5b 5 Peanut Cotton	18 1 0 0 1 0 0 0 0 1
GA 2010 22010 5b 10 Peanut Cotton	18 1 0 0 1 3 0 0 3 4
GA 2010 22010 5b 25 Peanut Cotton	18 1 0 0 1 0 0 0 0 1
GA 2010 22010 6a 5 Cotton Peanut	18 0 3 0 3 0 0 0 0 3
GA 2010 22010 6a 10 Cotton Peanut	18 0 0 0 0 0 0 0 0 0
GA 2010 22010 6a 25 Cotton Peanut	18 0 1 0 1 0 0 0 0 1
GA 2010 22010 6b 5 Peanut Cotton	18 0 0 0 0 1 0 0 1 1
GA 2010 22010 6b 10 Peanut Cotton	18 1 0 0 1 0 0 0 0 1
GA 2010 22010 6b 25 Peanut Cotton	18 0 0 0 0 0 0 0 0 0
GA 2010 32010 7 0 Border ab	18 1 0 0 1 0 0 0 0 1
GA 2010 32010 8 0 Border ab	18 0 0 0 0 7 0 0 7 7
GA 2010 32010 9 0 Border ab	18 1 0 0 1 10 1 0 11 12
GA 2010 32010 7a 5 Cotton Peanut	18 2 1 0 3 0 0 0 0 3
GA 2010 32010 7a 10 Cotton Peanut	18 0 0 0 0 0 0 0 0 0
GA 2010 32010 7a 25 Cotton Peanut	18 0 0 0 0 0 0 0 0 0
GA 2010 32010 7b 5 Peanut Cotton	18 1 0 0 1 0 0 0 0 1
GA 2010 32010 7b 10 Peanut Cotton	18 1 0 0 1 0 0 0 0 1
GA 2010 32010 7b 25 Peanut Cotton	18 2 0 0 2 0 0 0 0 2
GA 2010 32010 8a 5 Cotton Peanut	18 2 0 0 2 0 0 0 0 2
GA 2010 32010 8a 10 Cotton Peanut	18 1 0 0 1 0 0 0 0 1
GA 2010 32010 8a 25 Cotton Peanut	18 0 0 0 0 0 0 0 0 0

GA 2010 32010 8b 5 Peanut Cotton 18 3 0 0 3 0 0 0 0 3  
GA 2010 32010 8b 10 Peanut Cotton 18 1 0 0 1 0 0 0 0 1  
GA 2010 32010 8b 25 Peanut Cotton 18 1 0 0 1 0 0 0 0 1  
GA 2010 32010 9a 5 Cotton Peanut 18 4 0 0 4 1 0 0 1 5  
GA 2010 32010 9a 10 Cotton Peanut 18 2 0 0 2 0 0 0 0 2  
GA 2010 32010 9a 25 Cotton Peanut 18 1 0 0 1 0 0 0 0 1  
GA 2010 32010 9b 5 Peanut Cotton 18 0 0 0 0 0 0 0 0 0  
GA 2010 32010 9b 10 Peanut Cotton 18 0 0 0 0 0 1 0 1 1  
GA 2010 32010 9b 25 Peanut Cotton 18 1 0 0 1 0 0 0 0 1  
GA 2011 12011 1 0 Border ab 7 0 0 0 0 4 0 0 4 4  
GA 2011 12011 1 5 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 1 5 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 1 10 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 1 10 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 1 25 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 1 25 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 2 0 Border ab 7 1 0 0 1 4 0 0 4 5  
GA 2011 12011 2 5 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 2 5 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 2 10 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 2 10 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 2 25 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 2 25 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 3 0 Border ab 7 1 0 0 1 1 0 0 1 2  
GA 2011 12011 3 5 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 3 5 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 3 10 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 3 10 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 3 25 Cotton Pecan 7 0 0 0 0 0 0 0 0 0  
GA 2011 12011 3 25 Pecan Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 4 0 Border ab 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 4 5 Cotton PI 7 1 0 0 1 0 0 0 0 1  
GA 2011 22011 4 5 PI Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 4 10 Cotton PI 7 1 0 0 1 0 0 0 0 1  
GA 2011 22011 4 10 PI Cotton 7 0 0 0 0 1 0 0 1 1  
GA 2011 22011 4 25 Cotton PI 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 4 25 PI Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 5 0 Border ab 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 5 5 Cotton PI 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 5 5 PI Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 5 10 Cotton PI 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 5 10 PI Cotton 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 5 25 Cotton PI 7 0 0 0 0 0 0 0 0 0  
GA 2011 22011 5 25 PI Cotton 7 0 0 0 0 0 0 0 0 0

```

GA 2011 22011 6 0 Border ab 7 2 0 0 2 2 0 0 2 4
GA 2011 22011 6 5 Cotton PI 7 0 0 0 0 0 0 0 0 0
GA 2011 22011 6 5 PI Cotton 7 0 0 0 0 3 0 0 3 3
GA 2011 22011 6 10 Cotton PI 7 1 0 0 1 0 0 0 0 1
GA 2011 22011 6 10 PI Cotton 7 0 0 0 0 0 0 0 0 0
GA 2011 22011 6 25 Cotton PI 7 0 0 0 0 0 0 0 0 0
GA 2011 22011 6 25 PI Cotton 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 7 0 Border ab 7 1 0 0 1 0 0 0 0 1
GA 2011 32011 7 5 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 7 5 Peanut Cotton 7 0 0 0 0 1 0 0 1 1
GA 2011 32011 7 10 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 7 10 Peanut Cotton 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 7 25 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 7 25 Peanut Cotton 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 8 0 Border ab 7 0 0 0 0 1 0 0 1 1
GA 2011 32011 8 5 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 8 5 Peanut Cotton 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 8 10 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 8 10 Peanut Cotton 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 8 25 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 8 25 Peanut Cotton 7 1 0 0 1 0 0 0 0 1
GA 2011 32011 9 0 Border ab 7 6 0 0 6 1 0 0 1 7
GA 2011 32011 9 5 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 9 5 Peanut Cotton 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 9 10 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 9 10 Peanut Cotton 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 9 25 Cotton Peanut 7 0 0 0 0 0 0 0 0 0
GA 2011 32011 9 25 Peanut Cotton 7 1 0 0 1 1 0 0 1 2

```

```

;
PROC GLIMMIX;
CLASS Year FieldID Transect Distance CropAdj;
MODEL *Variable* = Cropadj Distance Distance*Cropadj/ DFM=KENWARDROGER;
RANDOM year Distance*Year FieldID(Year) Cropadj*FieldID(Year)
Transect(Cropadj*FieldID*Year);
LSMEANS Distance CropAdj distance*cropadj;
ESTIMATE 'sig vs others' cropadj *coefficients assigned as needed* /divisor=*assigned
as needed*;
RUN;QUIT;

```

Appendix B

SAS Code for Chapter III

Program 4: Border, whole-field, and untreated effects for stink bugs and boll injury.

```
dm'log;clear;output;clear';
Title'Grant Border Test';
options nodate nonumber ps=55 ls=78;
Data InOut;
input Location$ Year Field$ ID$ Trt$ WOB Loc$ Flags TotalBugs TotalInjury
TotalBollsPerFlag AvgBugs;
AvgBug= TotalBugs/Flags;
PropInj= TotalInjury/TotalBollsPerFlag;
Cards;
Bamberg 2007 A J None 1 In 4 0 0 0 0.000
Bamberg 2007 A J None 1 Out 13 2 0 0 0.154
Bamberg 2007 A J None 2 In 4 0 10 200 0.000
Bamberg 2007 A J None 2 Out 13 12 35 650 0.923
Bamberg 2007 A J None 3 In 4 1 25 200 0.250
Bamberg 2007 A J None 3 Out 13 8 165 650 0.615
Bamberg 2007 A J None 4 In 4 1 65 200 0.250
Bamberg 2007 A J None 4 Out 13 10 320 650 0.769
Bamberg 2007 A J None 5 In 4 2 100 200 0.500
Bamberg 2007 A J None 5 Out 13 13 350 650 1.000
Bamberg 2007 A J None 6 In 4 5 65 200 1.250
Bamberg 2007 A J None 6 Out 13 7 135 650 0.538
Bamberg 2007 A J None 7 In 4 10 30 200 2.500
Bamberg 2007 A J None 7 Out 13 4 235 650 0.308
Barnwell 2007 D K None 1 In 9 0 0 450 0.000
Barnwell 2007 D K None 1 Out 21 1 0 1050 0.048
Barnwell 2007 E R Spray 1 In 6 2 0 600 0.333
Barnwell 2007 E R Spray 1 Out 14 11 0 1400 0.786
Barnwell 2007 D K None 2 In 9 3 40 450 0.333
Barnwell 2007 D K None 2 Out 21 5 110 1050 0.238
Barnwell 2007 E R Spray 2 In 6 3 85 600 0.500
Barnwell 2007 E R Spray 2 Out 14 4 155 1400 0.286
Barnwell 2007 D K None 3 In 9 7 134 450 0.778
Barnwell 2007 D K None 3 Out 21 12 373 1050 0.571
Barnwell 2007 E R Spray 3 In 6 2 380 600 0.333
Barnwell 2007 E R Spray 3 Out 14 13 985 1400 0.929
Barnwell 2007 D K None 4 In 9 1 120 450 0.111
Barnwell 2007 D K None 4 Out 21 12 330 1050 0.571
Barnwell 2007 E R Spray 4 In 6 11 115 600 1.833
Barnwell 2007 E R Spray 4 Out 14 13 255 1400 0.929
```



Barnwell 2007 D K None 5 In 9 2 160 450 0.222  
 Barnwell 2007 D K None 5 Out 21 27 325 1050 1.286  
 Barnwell 2007 E R Spray 5 In 6 3 145 600 0.500  
 Barnwell 2007 E R Spray 5 Out 14 30 385 1400 2.143  
 Barnwell 2007 D K None 6 In 9 9 155 450 1.000  
 Barnwell 2007 D K None 6 Out 21 30 410 1050 1.429  
 Barnwell 2007 E R Spray 6 In 6 3 70 600 0.500  
 Barnwell 2007 E R Spray 6 Out 14 18 370 1400 1.286  
 Barnwell 2007 D K None 7 In 9 9 190 450 1.000  
 Barnwell 2007 D K None 7 Out 21 29 520 1050 1.381  
 Barnwell 2007 E R Spray 7 In 6 2 100 600 0.333  
 Barnwell 2007 E R Spray 7 Out 14 21 305 1400 1.500  
 Lee 2007 C T Spray 1 In 5 0 0 0 0.000  
 Lee 2007 C T Spray 1 Out 14 1 0 0 0.071  
 Lee 2007 C T Spray 2 In 5 0 21 95 0.000  
 Lee 2007 C T Spray 2 Out 14 1 46 270 0.071  
 Lee 2007 C T Spray 3 In 5 3 19 100 0.600  
 Lee 2007 C T Spray 3 Out 14 5 64 280 0.357  
 Lee 2007 C T Spray 4 In 5 0 18 100 0.000  
 Lee 2007 C T Spray 4 Out 14 3 65 280 0.214  
 Lee 2007 C T Spray 5 In 5 0 8 100 0.000  
 Lee 2007 C T Spray 5 Out 14 1 28 280 0.071  
 Lee 2007 C T Spray 6 In 5 0 26 100 0.000  
 Lee 2007 C T Spray 6 Out 14 1 69 280 0.071  
 Lee 2007 C T Spray 7 In 5 0 0 0 0.000  
 Lee 2007 C T Spray 7 Out 14 0 0 0 0.000  
 Tifton 2007 F L None 1 In 4 0 5 200 0.000  
 Tifton 2007 F L None 1 Out 17 0 45 850 0.000  
 Tifton 2007 F L None 2 In 4 0 65 200 0.000  
 Tifton 2007 F L None 2 Out 17 1 140 850 0.059  
 Tifton 2007 F L None 3 In 4 0 45 200 0.000  
 Tifton 2007 F L None 3 Out 17 2 145 850 0.118  
 Tifton 2007 F L None 4 In 4 0 80 200 0.000  
 Tifton 2007 F L None 4 Out 17 33 235 850 1.941  
 Tifton 2007 F L None 5 In 4 1 65 200 0.250  
 Tifton 2007 F L None 5 Out 17 15 330 850 0.882  
 Tifton 2007 F L None 6 In 4 0 70 200 0.000  
 Tifton 2007 F L None 6 Out 17 9 240 850 0.529  
 Bamberg 2008 G M None 1 In 3 0 0 150 0.000  
 Bamberg 2008 G M None 1 Out 12 1 0 600 0.083  
 Bamberg 2008 G M None 2 In 3 0 5 150 0.000  
 Bamberg 2008 G M None 2 Out 12 0 20 600 0.000  
 Bamberg 2008 G M None 3 In 3 7 10 150 2.333  
 Bamberg 2008 G M None 3 Out 12 9 50 600 0.750

Bamberg 2008 G M None 4 In 3 5 5 150 1.667  
 Bamberg 2008 G M None 4 Out 12 6 215 600 0.500  
 Bamberg 2008 G M None 5 In 3 2 30 150 0.667  
 Bamberg 2008 G M None 5 Out 12 7 190 600 0.583  
 Bamberg 2008 G M None 6 In 3 1 50 150 0.333  
 Bamberg 2008 G M None 6 Out 12 2 205 600 0.167  
 Bamberg 2008 G M None 7 In 3 2 35 150 0.667  
 Bamberg 2008 G M None 7 Out 12 1 150 600 0.083  
 Barnwell 2008 H N None 1 In 2 0 0 100 0.000  
 Barnwell 2008 H N None 1 Out 9 5 0 450 0.556  
 Barnwell 2008 J P None 1 In 11 1 25 550 0.091  
 Barnwell 2008 J P None 1 Out 17 5 19 850 0.294  
 Barnwell 2008 K S Spray 1 In 10 7 115 1000 0.700  
 Barnwell 2008 K S Spray 1 Out 17 6 60 1700 0.353  
 Barnwell 2008 H N None 2 In 2 0 5 100 0.000  
 Barnwell 2008 H N None 2 Out 9 6 45 450 0.667  
 Barnwell 2008 J P None 2 In 11 2 20 550 0.182  
 Barnwell 2008 J P None 2 Out 17 4 45 850 0.235  
 Barnwell 2008 K S Spray 2 In 10 3 160 1000 0.300  
 Barnwell 2008 K S Spray 2 Out 17 10 225 1700 0.588  
 Barnwell 2008 H N None 3 In 2 1 10 100 0.500  
 Barnwell 2008 H N None 3 Out 9 7 50 450 0.778  
 Barnwell 2008 J P None 3 In 11 2 0 550 0.182  
 Barnwell 2008 J P None 3 Out 17 9 80 850 0.529  
 Barnwell 2008 K S Spray 3 In 10 18 110 1000 1.800  
 Barnwell 2008 K S Spray 3 Out 17 12 180 1700 0.706  
 Barnwell 2008 H N None 4 In 2 0 15 100 0.000  
 Barnwell 2008 H N None 4 Out 9 15 145 450 1.667  
 Barnwell 2008 J P None 4 In 11 1 55 550 0.091  
 Barnwell 2008 J P None 4 Out 17 12 75 850 0.706  
 Barnwell 2008 K S Spray 4 In 10 30 405 1000 3.000  
 Barnwell 2008 K S Spray 4 Out 17 100 655 1700 5.882  
 Barnwell 2008 H N None 5 In 2 0 30 100 0.000  
 Barnwell 2008 H N None 5 Out 9 19 121 450 2.111  
 Barnwell 2008 J P None 5 In 11 3 80 550 0.273  
 Barnwell 2008 J P None 5 Out 17 28 150 850 1.647  
 Barnwell 2008 K S Spray 5 In 10 43 215 1000 4.300  
 Barnwell 2008 K S Spray 5 Out 17 63 340 1700 3.706  
 Barnwell 2008 J P None 6 In 11 0 130 550 0.000  
 Barnwell 2008 J P None 6 Out 17 11 420 850 0.647  
 Barnwell 2008 K S Spray 6 In 10 0 510 1000 0.000  
 Barnwell 2008 K S Spray 6 Out 17 0 1000 1700 0.000  
 Barnwell 2008 J P None 7 In 11 2 120 550 0.182  
 Barnwell 2008 J P None 7 Out 17 19 280 850 1.118

Lee 2008 L U Spray 1 In 6 6 0 0 1.000  
 Lee 2008 L U Spray 1 Out 16 23 0 0 1.438  
 Lee 2008 L U Spray 2 In 6 0 23 120 0.000  
 Lee 2008 L U Spray 2 Out 16 2 91 318 0.125  
 Lee 2008 L U Spray 3 In 6 4 43 120 0.667  
 Lee 2008 L U Spray 3 Out 16 6 131 320 0.375  
 Lee 2008 L U Spray 4 In 6 3 19 120 0.500  
 Lee 2008 L U Spray 4 Out 16 13 82 320 0.813  
 Lee 2008 L U Spray 5 In 6 1 27 120 0.167  
 Lee 2008 L U Spray 5 Out 16 4 98 320 0.250  
 Lee 2008 L U Spray 6 In 6 0 17 120 0.000  
 Lee 2008 L U Spray 6 Out 16 7 64 320 0.438  
 Lee 2008 L U Spray 7 In 6 1 29 120 0.167  
 Lee 2008 L U Spray 7 Out 16 17 107 320 1.063  
 Tifton 2008 I O None 2 In 6 0 75 300 0.000  
 Tifton 2008 I O None 2 Out 17 0 220 850 0.000  
 Tifton 2008 I O None 3 In 6 0 75 300 0.000  
 Tifton 2008 I O None 3 Out 17 1 160 850 0.059  
 Tifton 2008 I O None 4 In 6 2 60 300 0.333  
 Tifton 2008 I O None 4 Out 17 8 445 850 0.471  
 Cameron1 2009 Cam1 A Border 2 In 11 0 13 110 0.000  
 Cameron1 2009 Cam1 A Border 2 Out 13 0 26 130 0.000  
 Cameron1 2009 Cam1 A Border 3 In 11 0 19 110 0.000  
 Cameron1 2009 Cam1 A Border 3 Out 13 0 22 130 0.000  
 Cameron1 2009 Cam1 AA Spray 4 In 11 0 15 110 0.000  
 Cameron1 2009 Cam1 AA Spray 4 Out 13 0 9 130 0.000  
 Cameron1 2009 Cam1 AA Spray 5 In 11 0 3 110 0.000  
 Cameron1 2009 Cam1 AA Spray 5 Out 13 0 10 130 0.000  
 Cameron1 2009 Cam1 AA Spray 6 In 11 0 6 110 0.000  
 Cameron1 2009 Cam1 AA Spray 6 Out 13 0 7 130 0.000  
 Cameron1 2009 Cam1 AA Spray 7 In 11 0 9 110 0.000  
 Cameron1 2009 Cam1 AA Spray 7 Out 13 0 10 130 0.000  
 Cameron2 2009 Cam2 B Border 2 In 13 0 1 130 0.000  
 Cameron2 2009 Cam2 B Border 2 Out 19 0 11 190 0.000  
 Cameron2 2009 Cam2 BB Spray 3 In 13 0 8 130 0.000  
 Cameron2 2009 Cam2 BB Spray 3 Out 19 0 19 190 0.000  
 Cameron2 2009 Cam2 BB Spray 4 In 13 0 23 130 0.000  
 Cameron2 2009 Cam2 BB Spray 4 Out 19 0 25 190 0.000  
 Cameron2 2009 Cam2 BB Spray 5 In 13 0 15 130 0.000  
 Cameron2 2009 Cam2 BB Spray 5 Out 19 0 21 190 0.000  
 Cameron2 2009 Cam2 BB Spray 6 In 13 0 11 130 0.000  
 Cameron2 2009 Cam2 BB Spray 6 Out 19 0 14 190 0.000  
 Cameron2 2009 Cam2 BB Spray 7 In 13 0 10 130 0.000  
 Cameron2 2009 Cam2 BB Spray 7 Out 19 0 7 190 0.000

PeeDee 2009 Cot C Border 1 In 4 0 0 0 0.000  
 PeeDee 2009 Cot C Border 1 Out 13 3 0 0 0.231  
 PeeDee 2009 Cot C Border 2 In 4 0 6 39 0.000  
 PeeDee 2009 Cot C Border 2 Out 13 2 28 130 0.154  
 PeeDee 2009 Cot C Border 3 In 4 0 0 0 0.000  
 PeeDee 2009 Cot C Border 3 Out 13 0 0 0 0.000  
 PeeDee 2009 Cot C Border 4 In 4 2 7 40 0.500  
 PeeDee 2009 Cot C Border 4 Out 13 4 29 130 0.308  
 PeeDee 2009 Cot C Border 5 In 4 1 10 40 0.250  
 PeeDee 2009 Cot C Border 5 Out 13 8 29 130 0.615  
 PeeDee 2009 Cot C Border 6 In 4 1 11 40 0.250  
 PeeDee 2009 Cot C Border 6 Out 13 14 18 130 1.077  
 PeeDee 2009 Cot C Border 7 In 4 3 13 31 0.750  
 PeeDee 2009 Cot C Border 7 Out 13 1 23 125 0.077  
 Tift 2009 forks E Spray 1 In 24 0 0 240 0.000  
 Tift 2009 forks E Spray 1 Out 23 2 0 230 0.087  
 Tift 2009 SR G Border 1 In 11 0 5 110 0.000  
 Tift 2009 SR G Border 1 Out 16 5 9 160 0.313  
 Tift 2009 forks E Spray 2 In 24 1 44 240 0.042  
 Tift 2009 forks E Spray 2 Out 23 2 41 230 0.087  
 Tift 2009 mm F Spray 2 In 12 2 41 120 0.167  
 Tift 2009 mm F Spray 2 Out 16 5 34 160 0.313  
 Tift 2009 mm F Spray 3 In 12 0 17 120 0.000  
 Tift 2009 mm F Spray 3 Out 16 0 29 160 0.000  
 Tift 2009 SR G Border 3 In 11 0 13 110 0.000  
 Tift 2009 SR G Border 3 Out 16 1 12 160 0.063  
 Tift 2009 forks E Spray 4 In 24 0 18 240 0.000  
 Tift 2009 forks E Spray 4 Out 23 0 28 230 0.000  
 Tift 2009 mm F Spray 4 In 12 0 15 120 0.000  
 Tift 2009 mm F Spray 4 Out 16 1 11 160 0.063  
 Tift 2009 SR G Border 4 In 11 1 29 110 0.091  
 Tift 2009 SR G Border 4 Out 16 3 21 160 0.188  
 Tift 2009 forks E Spray 5 In 24 0 26 240 0.000  
 Tift 2009 forks E Spray 5 Out 23 2 32 230 0.087  
 Tift 2009 mm F Spray 5 In 12 0 13 120 0.000  
 Tift 2009 mm F Spray 5 Out 16 3 12 160 0.188  
 Tift 2009 forks E Spray 6 In 24 2 39 240 0.083  
 Tift 2009 forks E Spray 6 Out 23 9 45 230 0.391  
 Tift 2009 mm F Spray 6 In 12 0 25 120 0.000  
 Tift 2009 mm F Spray 6 Out 16 1 20 160 0.063  
 Tift 2009 SR G Border 6 In 11 1 14 110 0.091  
 Tift 2009 SR G Border 6 Out 16 4 24 160 0.250  
 Tift 2009 forks E Spray 7 In 24 3 39 240 0.125  
 Tift 2009 forks E Spray 7 Out 23 3 30 230 0.130

Tift 2009 mm F Spray 7 In 12 0 4 120 0.000  
Tift 2009 mm F Spray 7 Out 16 0 8 160 0.000  
Tift 2009 SR G Border 7 In 11 3 10 110 0.273  
Tift 2009 SR G Border 7 Out 16 6 24 160 0.375  
Blackville 2010 1 YY Spray 1 In 10 4 0 0 0.400  
Blackville 2010 1 YY Spray 1 Out 18 10 0 0 0.556  
Blackville 2010 1 YY Spray 2 In 10 0 24 100 0.000  
Blackville 2010 1 YY Spray 2 Out 18 0 29 180 0.000  
Blackville 2010 1 YY Spray 3 In 10 0 31 100 0.000  
Blackville 2010 1 YY Spray 3 Out 18 1 25 180 0.056  
Blackville 2010 1 YY Spray 4 In 10 0 22 100 0.000  
Blackville 2010 1 YY Spray 4 Out 18 0 29 180 0.000  
Cameron2 2010 1 W Border 2 In 11 0 0 110 0.000  
Cameron2 2010 1 W Border 2 Out 21 0 0 210 0.000  
Cameron2 2010 1 W Border 3 In 11 0 15 110 0.000  
Cameron2 2010 1 W Border 3 Out 21 0 17 210 0.000  
Cameron2 2010 1 W Border 4 In 11 1 17 110 0.091  
Cameron2 2010 1 W Border 4 Out 21 1 32 210 0.048  
Cameron2 2010 1 W Border 5 In 11 2 21 110 0.182  
Cameron2 2010 1 W Border 5 Out 21 3 50 210 0.143  
PeeDee 2010 Cot V Border 1 In 4 0 0 0 0.000  
PeeDee 2010 Cot V Border 1 Out 13 1 0 0 0.077  
PeeDee 2010 Cot V Border 2 In 4 0 5 40 0.000  
PeeDee 2010 Cot V Border 2 Out 13 0 32 130 0.000  
PeeDee 2010 Cot V Border 3 In 4 1 5 40 0.250  
PeeDee 2010 Cot V Border 3 Out 13 4 36 130 0.308  
PeeDee 2010 Cot V Border 4 In 4 1 5 40 0.250  
PeeDee 2010 Cot V Border 4 Out 13 2 21 130 0.154  
PeeDee 2010 Cot V Border 5 In 4 1 8 40 0.250  
PeeDee 2010 Cot V Border 5 Out 13 3 32 130 0.231  
PeeDee 2010 Cot V Border 6 In 4 0 9 40 0.000  
PeeDee 2010 Cot V Border 6 Out 13 1 43 130 0.077  
Tift 2010 LB LB None 1 In 8 0 0 80 0.000  
Tift 2010 LB LB None 1 Out 16 0 0 160 0.000  
Tift 2010 Nash NB Border 1 In 47 1 0 470 0.021  
Tift 2010 Nash NB Border 1 Out 30 1 0 300 0.033  
Tift 2010 Rebecca RB Border 1 In 37 2 0 370 0.054  
Tift 2010 Rebecca RB Border 1 Out 27 1 0 270 0.037  
Tift 2010 LB LB None 2 In 8 3 11 80 0.375  
Tift 2010 LB LB None 2 Out 16 0 21 160 0.000  
Tift 2010 Nash NB Border 2 In 47 2 14 470 0.043  
Tift 2010 Nash NB Border 2 Out 30 0 8 300 0.000  
Tift 2010 Rebecca RB Border 2 In 37 1 38 370 0.027  
Tift 2010 Rebecca RB Border 2 Out 27 1 18 270 0.037

Tift 2010 LB LB None 3 In 8 1 16 80 0.125  
Tift 2010 LB LB None 3 Out 16 7 49 160 0.438  
Tift 2010 Nash NB Border 3 In 47 0 56 470 0.000  
Tift 2010 Nash NB Border 3 Out 30 0 38 300 0.000  
Tift 2010 Rebecca RB Border 3 In 37 3 59 370 0.081  
Tift 2010 Rebecca RB Border 3 Out 27 2 36 270 0.074  
Tift 2010 LB LB None 4 In 8 1 14 80 0.125  
Tift 2010 LB LB None 4 Out 16 3 27 160 0.188  
Tift 2010 Nash NB Border 4 In 47 2 77 470 0.043  
Tift 2010 Nash NB Border 4 Out 30 9 37 300 0.300  
Tift 2010 Rebecca RB Border 4 In 37 4 50 370 0.108  
Tift 2010 Rebecca RB Border 4 Out 27 3 40 270 0.111  
Tift 2010 LB LB None 5 In 8 1 1 80 0.125  
Tift 2010 LB LB None 5 Out 16 8 18 160 0.500  
Tift 2010 Nash NB Border 5 In 47 0 62 470 0.000  
Tift 2010 Nash NB Border 5 Out 30 23 113 300 0.767  
Tift 2010 Rebecca RB Border 5 In 37 1 39 370 0.027  
Tift 2010 Rebecca RB Border 5 Out 27 1 37 270 0.037  
Tift 2010 LB LB None 6 In 8 1 31 80 0.125  
Tift 2010 LB LB None 6 Out 16 8 98 160 0.500  
Tift 2010 Nash NS Spray 6 In 47 2 69 470 0.043  
Tift 2010 Nash NS Spray 6 Out 30 16 78 300 0.533  
Tift 2010 Rebecca RB Border 6 In 37 0 0 370 0.000  
Tift 2010 Rebecca RB Border 6 Out 27 0 0 270 0.000  
Tift 2010 LB LB None 7 In 8 0 21 80 0.000  
Tift 2010 LB LB None 7 Out 16 8 79 160 0.500  
Tift 2010 Rebecca RB Border 7 In 37 2 94 370 0.054  
Tift 2010 Rebecca RB Border 7 Out 27 5 94 270 0.185  
PeeDee 2011 Cot X Border 1 In 4 0 0 40 0.000  
PeeDee 2011 Cot X Border 1 Out 13 0 0 130 0.000  
PeeDee 2011 Cot X Border 2 In 4 3 0 40 0.750  
PeeDee 2011 Cot X Border 2 Out 13 3 4 130 0.231  
PeeDee 2011 Cot X Border 3 In 4 0 0 40 0.000  
PeeDee 2011 Cot X Border 3 Out 13 1 1 130 0.077  
PeeDee 2011 Cot X Border 4 In 4 1 0 40 0.250  
PeeDee 2011 Cot X Border 4 Out 13 3 2 130 0.231  
PeeDee 2011 Cot X Border 5 In 4 0 0 0 0.000  
PeeDee 2011 Cot X Border 5 Out 13 1 0 0 0.077  
PeeDee 2011 Cot X Border 6 In 4 0 0 0 0.000  
PeeDee 2011 Cot X Border 6 Out 13 0 0 0 0.000  
Tift 2011 Nashv NV Border 1 In 32 0 0 320 0.000  
Tift 2011 Nashv NV Border 1 Out 26 2 0 260 0.077  
Tift 2011 Nashv NV Border 2 In 32 0 2 320 0.000  
Tift 2011 Nashv NV Border 2 Out 26 0 2 260 0.000

```

Tift 2011 Reb Reb Spray 2 In 24 2 2 240 0.083
Tift 2011 Reb Reb Spray 2 Out 29 1 6 290 0.034
Tift 2011 Nashv NV Border 3 In 32 1 9 320 0.031
Tift 2011 Nashv NV Border 3 Out 26 0 2 260 0.000
Tift 2011 Reb Reb Spray 3 In 24 1 9 240 0.042
Tift 2011 Reb Reb Spray 3 Out 29 0 17 290 0.000
Tift 2011 Nashv NV Border 4 In 32 0 16 320 0.000
Tift 2011 Nashv NV Border 4 Out 26 0 10 260 0.000
Tift 2011 Reb Reb Spray 4 In 24 0 8 240 0.000
Tift 2011 Reb Reb Spray 4 Out 29 2 21 290 0.069
Tift 2011 Nashv NV Border 5 In 32 1 7 320 0.031
Tift 2011 Nashv NV Border 5 Out 26 0 13 260 0.000
Tift 2011 Reb Reb Spray 5 In 24 0 16 240 0.000
Tift 2011 Reb Reb Spray 5 Out 29 6 48 290 0.207
Tift 2011 Nashv NV Border 6 In 32 1 15 320 0.031
Tift 2011 Nashv NV Border 6 Out 26 3 10 260 0.115
Tift 2011 Reb Reb Spray 6 In 24 0 11 240 0.000
Tift 2011 Reb Reb Spray 6 Out 29 3 47 290 0.103
Tift 2011 Nashv NV Border 7 In 32 0 32 320 0.000
Tift 2011 Nashv NV Border 7 Out 26 2 21 260 0.077
;
PROC GLIMMIX data=InOut nobound;
CLASS Flags ID WOB Loc Year trt;
MODEL *Variable* = trt loc loc*trt wob trt*wob loc*wob trt*loc*wob;
RANDOM ID(trt) Loc*id(trt) ;
LSMEANS Loc trt wob / pdiff cl adjust=tukey lines;
RUN;QUIT;

```

## Appendix C

### SAS Code for Chapter IV

Program 5: Mortality effects for type of glue and gender.

```
Data Deaths;
INPUT Glue$ Gen$ Trial$ Death N;
DATALINES;
C M 1 1 5
CCm M 1 4 5
FA M 1 2 5
SG M 1 3 5
LS M 1 3 5
GG M 1 4 5
C F 1 2 5
CCm F 1 3 5
FA F 1 3 5
SG F 1 2 5
LS F 1 3 5
GG F 1 0 5
C M 2 3 5
CCm M 2 5 5
FA M 2 1 5
SG M 2 5 5
LS M 2 2 5
GG M 2 2 5
C F 2 2 5
CCm F 2 5 5
FA F 2 4 5
SG F 2 2 5
LS F 2 2 5
GG F 2 2 5;
PROC GLIMMIX;
CLASS Glue Gen N;
MODEL Death/N = Glue Gen Glue*Gen / dist = bin link = logit;
RANDOM Trial;
LSMEANS Glue Gen Glue*Gen / PDIFF;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
RUN;
%include 'c:/pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.01,sort=yes);
RUN;QUIT;
```



Program 6: Mortality effects for type of glue.

```
Data Deaths;
INPUT Glue$ Gen$ Trial$ Death N;
DT = drops/20;
DATALINES;
C M 1 1 5
CCm M 1 2 5
FA M 1 4 5
SG M 1 3 5
LS M 1 3 5
GG M 1 3 5
C F 1 3 5
CCm F 1 4 5
FA F 1 4 5
SG F 1 4 5
LS F 1 3 5
GG F 1 3 5
C M 2 3 5
CCm M 2 2 5
FA M 2 1 5
SG M 2 2 5
LS M 2 3 5
GG M 2 3 5
C F 2 2 5
CCm F 2 3 5
FA F 2 2 5
SG F 2 2 5
LS F 2 3 5
GG F 2 0 5
;
PROC GLIMMIX;
CLASS Glue Gen N;
MODEL Death/N = Glue Gen Glue*Gen / dist = bin link = logit;
RANDOM Trial;
LSMEANS Glue Gen Glue*Gen / PDIFF;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
RUN;
%include 'c:/pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
RUN;QUIT;
```

Program 7: Mortality effects for glue with tag and gender.

```
Data Deaths;
INPUT Glue$ Gen$ Trial$ Death N;
DT = drops/20;
DATALINES;
C M 1 1 5
CCm M 1 2 5
FA M 1 4 5
SG M 1 3 5
LS M 1 3 5
GG M 1 3 5
C F 1 3 5
CCm F 1 4 5
FA F 1 4 5
SG F 1 4 5
LS F 1 3 5
GG F 1 3 5
C M 2 3 5
CCm M 2 2 5
FA M 2 1 5
SG M 2 2 5
LS M 2 3 5
GG M 2 3 5
C F 2 2 5
CCm F 2 3 5
FA F 2 2 5
SG F 2 2 5
LS F 2 3 5
GG F 2 0 5
;
PROC GLIMMIX;
CLASS Glue Gen N;
MODEL Death/N = Glue Gen Glue*Gen / dist = bin link = logit;
LSMEANS Glue Gen Glue*Gen / PDIFF;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
RUN;
%include 'c:/pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
RUN;QUIT;
```

Program 8: Program for testing tagged and untagged walking mobility.

Data SGSBmob;

INPUT Release \$ Gender \$ Treatment \$ Length Mass Dist;

DATALINES;

1 F C 12 0.098 62  
1 F C 14 0.15 154  
1 F C 17 0.226 27  
1 F C 16 0.144 81  
1 F C 17 0.232 197  
1 M C 12 0.118 87  
1 M C 14 0.129 89  
1 M C 13 0.114 104  
1 M C 13 0.139 107  
1 M C 13 0.152 155  
1 F GG 14 0.116 53  
1 F GG 16 0.18 120  
1 F GG 15 0.183 50  
1 F GG 16 0.206 90  
1 F GG 16 0.153 100  
1 M GG 15 0.194 72  
1 M GG 14 0.172 34  
1 M GG 14 0.14 50  
1 M GG 14 0.161 78  
1 M GG 14 0.176 80  
1 F Gtag 17 0.3 60  
1 F Gtag 17 0.204 110  
1 F Gtag 17 0.195 110  
1 F Gtag 14 0.117 105  
1 F Gtag 16 0.164 90  
1 M Gtag 14 0.141 70  
1 M Gtag 14 0.167 75  
1 M Gtag 13 0.128 65  
1 M Gtag 11 0.093 50  
1 M Gtag 14 0.138 50  
2 F C 17 0.237 132  
2 F C 17 0.246 120  
2 F C 17 0.265 80  
2 F C 16 0.22 119  
2 F C 15 0.247 79  
2 M C 15 0.155 86  
2 M C 14 0.14 60  
2 M C 12 0.083 71  
2 M C 15 0.114 54  
2 M C 15 0.145 75

```

2 F GG 17 0.22 138
2 F GG 17 0.25 90
2 F GG 17 0.264 95
2 F GG 17 0.26 120
2 F GG 14 0.23 135
2 M GG 15 0.155 82
2 M GG 14 0.145 79
2 M GG 15 0.158 75
2 M GG 14 0.144 65
2 M GG 14 0.15 50
2 F Gtag 17 0.249 72
2 F Gtag 15 0.19 65
2 F Gtag 15 0.26 110
2 F Gtag 16 0.233 122
2 F Gtag 15 0.251 134
2 M Gtag 15 0.155 55
2 M Gtag 14 0.127 70
2 M Gtag 15 0.173 81
2 M Gtag 12 0.084 52
2 M Gtag 13 0.144 77
;
PROC MIXED;
CLASS Release Gender Treatment Length Mass;
MODEL Dist=Gender Treatment Gender*Treatment / DDFM=SATTERTH;
RANDOM Release;
LSMEANS Gender Treatment Gender*Treatment / PDIFF;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
RUN;
%include 'c:/pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
RUN;QUIT;

```

Program 9: Program for testing tagged and untagged flying mobility.

Data SGSBFly;

INPUT Release \$ Gender \$ Treatment \$ Length Mass Dist;

DATALINES;

1 F C 14 0.179 33.528  
1 F C 14 0.195 47.244  
1 F C 17 0.154 45.72  
1 F C 15 0.091 14.6304  
1 F C 14 0.174 0  
1 F C 16 0.189 33.2232  
1 F C 17 0.192 12.4968  
1 F C 14 0.16 11.2776  
1 F C 14 0.147 60.6552  
1 F C 14 0.138 3.048  
2 F C 18 0.279 9.144  
2 F C 17 0.18 6.4008  
2 F C 17 0.15 20.7264  
2 F C 18 0.215 16.4592  
2 F C 14 0.2 12.4968  
2 F C 15 0.121 9.144  
2 F C 16 0.174 6.7056  
2 F C 15 0.16 8.8392  
2 F C 14 0.115 6.7056  
2 F C 15 0.119 30.48  
1 F GG 15 0.159 33.2232  
1 F GG 15 0.235 6.7056  
1 F GG 15 0.195 5.4864  
1 F GG 15 0.176 12.192  
1 F GG 17 0.171 58.8264  
1 F GG 15 0.208 106.0704  
1 F GG 17 0.205 1.2192  
1 F GG 15 0.251 35.6616  
1 F GG 18 0.2 24.384  
1 F GG 14 0.128 1.524  
2 F GG 16 0.173 26.2128  
2 F GG 15 0.169 27.432  
2 F GG 17 0.214 31.3944  
2 F GG 17 0.184 33.8328  
2 F GG 18 0.179 12.4968  
2 F GG 15 0.142 8.2296  
2 F GG 16 0.197 14.3256  
2 F GG 16 0.191 29.5656  
2 F GG 17 0.21 36.2712  
2 F GG 16 0.195 22.86

1 F Gtag 17 0.267 16.4592  
1 F Gtag 16 0.204 20.7264  
1 F Gtag 16 0.23 10.3632  
1 F Gtag 17 0.197 22.86  
1 F Gtag 13 0.119 16.4592  
1 F Gtag 17 0.271 21.336  
1 F Gtag 16 0.186 29.8704  
1 F Gtag 16 0.256 39.624  
1 F Gtag 16 0.217 5.1816  
1 F Gtag 16 0.214 60.96  
2 F Gtag 15 0.177 8.5344  
2 F Gtag 15 0.173 106.68  
2 F Gtag 17 0.205 6.7056  
2 F Gtag 17 0.236 4.572  
2 F Gtag 12 0.122 11.2776  
2 F Gtag 13 0.141 28.0416  
2 F Gtag 13 0.142 2.7432  
2 F Gtag 15 0.117 21.9456  
2 F Gtag 15 0.129 26.2128  
2 F Gtag 12 0.094 33.8328  
1 M C 13 0.126 2.1336  
1 M C 14 0.156 18.5928  
1 M C 14 0.176 60.0456  
1 M C 14 0.15 5.7912  
1 M C 14 0.164 1.524  
1 M C 12 0.14 29.5656  
1 M C 14 0.138 36.8808  
1 M C 14 0.198 11.2776  
1 M C 14 0.153 6.096  
1 M C 15 0.184 13.1064  
2 M C 14 0.113 22.86  
2 M C 15 0.164 9.144  
2 M C 14 0.123 6.096  
2 M C 15 0.153 16.4592  
2 M C 15 0.175 3.3528  
2 M C 14 0.155 8.2296  
2 M C 14 0.139 10.9728  
2 M C 15 0.136 11.5824  
2 M C 13 0.163 3.048  
2 M C 13 0.115 10.9728  
1 M GG 13 0.168 49.3776  
1 M GG 14 0.182 1.8288  
1 M GG 12 0.078 17.0688  
1 M GG 13 0.145 6.096

```

1 M GG 14 0.143 2.1336
1 M GG 13 0.12 0
1 M GG 13 0.128 11.2776
1 M GG 12 0.103 7.62
1 M GG 13 0.128 10.9728
1 M GG 14 0.154 30.48
2 M GG 14 0.127 4.2672
2 M GG 14 0.132 4.2672
2 M GG 15 0.16 10.0584
2 M GG 13 0.128 10.3632
2 M GG 14 0.129 14.3256
2 M GG 12 0.117 10.9728
2 M GG 14 0.152 9.7536
2 M GG 12 0.101 0
2 M GG 15 0.151 53.0352
2 M GG 14 0.152 14.6304
1 M Gtag 14 0.178 10.668
1 M Gtag 11 0.098 5.1816
1 M Gtag 13 0.157 8.5344
1 M Gtag 14 0.157 7.3152
1 M Gtag 13 0.103 7.3152
1 M Gtag 14 0.144 4.2672
1 M Gtag 14 0.162 3.3528
1 M Gtag 15 0.128 10.9728
1 M Gtag 14 0.11 52.1208
1 M Gtag 14 0.175 8.2296
2 M Gtag 11 0.115 8.2296
2 M Gtag 12 0.146 73.7616
2 M Gtag 13 0.074 14.3256
2 M Gtag 14 0.141 32.9184
2 M Gtag 14 0.144 10.3632
2 M Gtag 13 0.095 9.7536
2 M Gtag 11 0.162 10.3632
2 M Gtag 14 0.168 7.0104
2 M Gtag 14 0.162 12.192
2 M Gtag 11 0.143 6.4008
;
PROC MIXED;
CLASS Release Gender Treatment Length Mass;
MODEL Dist=Gender Treatment Gender*Treatment / DDFM=SATTERTH;
RANDOM Release;
LSMEANS Gender Treatment Gender*Treatment / PDIFF;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;

```

```
RUN;  
%include 'c:/pdmix800.sas';  
%pdmix800(ppp,mmm,alpha=.05,sort=yes);  
RUN;QUIT;
```



Program 10: Program for testing tagged and untagged field mobility.

Data SGSBmob;

INPUT Release \$ ID \$ Sex\$ Length Mass Crop \$ Treatment \$ Hour\$ X Y Dist;

DATALINES;

2 41 F 12 0.098 Fallow Tag 1 0 0.3048 0.3048  
2 41 F 12 0.098 Fallow Tag 24 0 0.3048 0.3048  
2 42 F 14 0.15 Fallow Tag 1 0.3048 0.9144 1.31210304  
2 44 F 16 0.144 Fallow Tag 1 0.3048 0.3048 0.6096  
2 44 F 16 0.144 Fallow Tag 24 0.3048 0.6096 0.9144  
2 45 F 17 0.232 Fallow Tag 1 0 0 0  
2 47 F 16 0.18 Fallow Tag 1 -0.3048 -0.9144 1.2192  
2 49 F 16 0.206 Fallow Tag 1 0.3048 0.3048 0.6096  
2 49 F 16 0.206 Fallow Tag 24 0.3048 0.6096 0.9144  
2 52 M 15 0.178 Fallow Tag 24 -1.2192 0.6096 1.8288  
2 52 M 15 0.178 Fallow Tag 1 0.3048 0.3048 0.6096  
2 54 M 12 0.142 Fallow Tag 1 0.3048 0.9144 1.2192  
2 57 M 12 0.114 Fallow Tag 1 0.3048 0.3048 0.6096  
2 61 F 17 0.3 Fallow Mark 1 -0.3048 0 0.3048  
2 73 M 14 0.13 Fallow Mark 1 0 0.3048 0.3048  
1 81 F 17 0.245 Cotton Tag 1 0.3048 0 0.3048  
1 81 F 17 0.245 Cotton Tag 24 0.6096 0.9144 1.524  
1 82 F 17 0.161 Cotton Tag 1 0.3048 0 0.3048  
1 83 F 15 0.177 Cotton Tag 1 0 232.8672 232.8672  
1 87 F 13 0.155 Cotton Tag 1 0.3048 0 0.3048  
1 87 F 13 0.155 Cotton Tag 24 0.3048 0 0.3048  
1 89 F 18 0.194 Cotton Tag 1 -0.3048 0 0.3048  
1 89 F 18 0.194 Cotton Tag 24 0.3048 0 0.3048  
1 90 F 17 0.257 Cotton Tag 1 -0.3048 0 0.3048  
1 94 M 13 0.139 Cotton Tag 1 0 0 0  
1 94 M 13 0.139 Cotton Tag 24 0.6096 0 0.6096  
1 95 M 13 0.152 Cotton Tag 1 -0.3048 0 0.3048  
1 95 M 13 0.152 Cotton Tag 24 -0.3048 0 0.3048  
1 96 M 15 0.194 Cotton Tag 24 -1.8288 -0.3048 2.1336  
1 96 M 15 0.194 Cotton Tag 1 0 0 0  
1 97 M 14 0.172 Cotton Tag 1 -0.3048 0 0.3048  
1 100 M 14 0.176 Cotton Tag 24 0 -11.5824 11.5824  
1 100 M 14 0.176 Cotton Tag 1 0.3048 0 0.3048  
1 101 F 15 0.184 Cotton Mark 1 0.3048 0 0.3048  
1 102 F 14 0.094 Cotton Mark 1 0.3048 0 0.3048  
1 102 F 14 0.094 Cotton Mark 24 0.3048 0 0.3048  
1 105 F 16 0.196 Cotton Mark 24 -0.6096 0 0.6096  
1 105 F 16 0.196 Cotton Mark 1 0 0 0  
1 108 F 17 0.258 Cotton Mark 1 0.3048 0 0.3048  
1 108 F 17 0.258 Cotton Mark 24 0.3048 0 0.3048

1 110 F 16 0.155 Cotton Mark 1 0 0 0  
1 110 F 16 0.155 Cotton Mark 24 0 0 0  
1 111 M 13 0.153 Cotton Mark 1 0.6096 0 0.6096  
1 111 M 13 0.153 Cotton Mark 24 0.9144 0 0.9144  
1 112 M 14 0.136 Cotton Mark 1 0 0 0  
1 113 M 13 0.127 Cotton Mark 1 0.3048 0 0.3048  
1 118 M 12 0.13 Cotton Mark 1 0.3048 0 0.3048  
1 118 M 12 0.13 Cotton Mark 24 0.3048 0 0.3048  
1 121 F 17 0.291 Fallow Tag 1 0.9144 0 0.9144  
2 121 F 17 0.291 Cotton Tag 1 0.9144 0 0.9144  
1 122 F 18 0.206 Fallow Tag 1 -0.9144 -0.6096 1.524  
2 122 F 18 0.206 Cotton Tag 1 -0.9144 -0.6096 1.524  
1 122 F 18 0.206 Fallow Tag 24 -0.3048 -0.3048 0.6096  
1 123 F 17 0.209 Fallow Tag 1 0.3048 0 0.3048  
2 123 F 17 0.209 Cotton Tag 1 0.3048 0 0.3048  
2 123 F 17 0.209 Cotton Tag 24 0.3048 0 0.3048  
1 124 F 16 0.162 Fallow Tag 1 -0.3048 0 0.3048  
2 124 F 16 0.162 Cotton Tag 1 -0.3048 0 0.3048  
2 124 F 16 0.162 Cotton Tag 24 -0.3048 0 0.3048  
1 125 F 14 0.15 Fallow Tag 1 0.3048 0 0.3048  
2 125 F 14 0.15 Cotton Tag 1 0.3048 0 0.3048  
2 125 F 14 0.15 Cotton Tag 24 0.3048 0 0.3048  
1 126 F 16 0.164 Fallow Tag 1 0 0 0  
2 126 F 16 0.164 Cotton Tag 1 0 0 0  
1 129 F 16 0.185 Fallow Tag 1 0 0 0  
1 129 F 16 0.185 Fallow Tag 24 0 0.3048 0.3048  
2 129 F 16 0.185 Cotton Tag 1 0 0 0  
1 130 F 17 0.212 Fallow Tag 1 0 0 0  
2 130 F 17 0.212 Cotton Tag 1 0 0 0  
2 130 F 17 0.212 Cotton Tag 24 0 0.6096 0.6096  
1 132 M 14 0.163 Fallow Tag 24 0 0.9144 0.9144  
1 132 M 14 0.163 Fallow Tag 1 0.6096 0 0.6096  
2 132 M 14 0.163 Cotton Tag 1 0.6096 0 0.6096  
1 133 M 14 0.165 Fallow Tag 1 0.3048 0 0.3048  
2 133 M 14 0.165 Cotton Tag 1 0.3048 0 0.3048  
1 133 M 14 0.165 Fallow Tag 24 0.9144 0 0.9144  
1 134 M 14 0.178 Fallow Tag 1 0 0 0  
2 134 M 14 0.178 Cotton Tag 1 0 0 0  
2 134 M 14 0.178 Cotton Tag 24 0 0 0  
1 135 M 13 0.105 Fallow Tag 1 0 0 0  
2 135 M 13 0.105 Cotton Tag 1 0 0 0  
1 135 M 13 0.105 Fallow Tag 24 0.6096 0 0.6096  
1 136 M 11 0.082 Fallow Tag 1 0.3048 0 0.3048  
2 136 M 11 0.082 Cotton Tag 1 0.3048 0 0.3048

```

1 139 M 13 0.128 Fallow Tag 1 1.8288 0 1.8288
2 139 M 13 0.128 Cotton Tag 1 1.8288 0 1.8288
1 140 M 12 0.129 Fallow Tag 1 0 0 0
2 140 M 12 0.129 Cotton Tag 1 0 0 0
2 141 F 15 0.206 Cotton Mark 1 0 0 0
2 142 F 17 0.209 Cotton Mark 1 -0.3048 0 0.3048
2 143 F 16 0.167 Cotton Mark 1 -0.3048 0 0.3048
2 143 F 16 0.167 Cotton Mark 24 -0.3048 0 0.3048
2 144 F 17 0.24 Cotton Mark 24 -0.3048 0 0.3048
2 144 F 17 0.24 Cotton Mark 1 0 0 0
2 147 F 17 0.209 Cotton Mark 1 0 6.096 6.096
2 148 F 15 0.173 Cotton Mark 1 0.3048 0 0.3048
2 150 F 16 0.185 Cotton Mark 1 0 0.6096 0.6096
2 150 F 16 0.185 Cotton Mark 24 0.6096 0.6096 1.2192
2 152 M 14 0.167 Cotton Mark 1 0 0 0
2 153 M 13 0.128 Cotton Mark 1 0 0 0
2 153 M 13 0.128 Cotton Mark 24 0.3048 0 0.3048
2 159 M 14 0.15 Cotton Mark 1 0 0 0
2 160 M 14 0.191 Cotton Mark 1 0.6096 0 0.6096
2 160 M 14 0.191 Cotton Mark 24 0.6096 0.3048 0.9144

```

```

;
PROC MIXED;
CLASS Release ID Sex Length Mass Crop Treatment Hour;
MODEL Dist=Crop|Treatment|Sex / DDFM=SATTERTH;
RANDOM Release;
LSMEANS Crop|Treatment|Sex / PDIFF;
ods output diffs=ppp lsmeans=mmm;
ods listing exclude diffs lsmeans;
RUN;
%include 'c:/pdmix800.sas';
%pdmix800(ppp,mmm,alpha=.05,sort=yes);
RUN;QUIT;

```

Appendix D

Letter of permission for Chapter V

Dr. Gardner,

Attached is the revised copy of my article 'Host Preference of the Parasitoid *Trichopoda pennipes* (Diptera: Tachinidae) with *Euschostus servus* and *Nezara viridula* (Nemiptera: Pentatomidae).' As this paper forms part of my doctoral dissertation, may I have a letter giving permission to reprint it as part of that dissertation?

-Grant Pilkay  
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You have permission to reprint this manuscript in your dissertation.

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Appendix E

Letter of permission for Chapter VI

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Sincerely,  
Mike

--

Michael Gates, Co-Editor  
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