ALTERNATE MANAGEMENT STRATEGIES FOR MANAGING PHYTOPHAGOUS STINK BUGS (HEMIPTERA: PENTATOMIDAE) IN COTTON IN THE SOUTHEASTERN USA

by

ISHAKH PULAKKATU-THODI

(Under the Direction of Michael D. Toews)

ABSTRACT

Practitioners of integrated pest management in cotton have witnessed significant changes in the last several decades. Eradication of the boll weevil, widespread use of selective insecticides, and introduction of genetically modified cotton in the mid-1990s are major factors that have influenced pest management in cotton. Phytophagous stink bugs, previously controlled coincidently with broad-spectrum insecticides, are now an economically important group of pests in cotton production. The green stink bug, the southern green stink bug, and the brown stink bug infest cotton fields during the reproductive stages; symptoms of stink bug injury to cotton include boll abscission, lint staining, yield loss, and reduced fiber quality. Published research on the management of stink bugs has focused on developing sampling procedures, understanding spatial dynamics, and assessing the damage inflicted by the pest.

Complementary to the ongoing research on stink bug management at the University of Georgia, the author investigated effects of planting date manipulation on stink bug density and associated boll injury. Results show that cotton planted in May suffered significantly less boll injury than cotton planted during June. Furthermore, percent boll injury exceeded the Extension recommended treatment threshold more frequently in June-planted cotton than in May-planted cotton. Finally, lint yield and color were deleteriously affected in June-planted cotton.

Within-field distribution of boll injury was investigated to understand the spatial dynamics of the pest complex in commercial cotton fields. Using IDW interpolation, variogram analysis, and Moran's *I*, the spatial variability of stink bug injury within fields was demonstrated. Stink bug injury was found to be spatially associated at distances ranging from ~75m to 275m, with an average distance of ~150m. Significant spatial association was observed in 3 out 5 fields.

Efficacy of whole-field insecticide treatments in commercial fields was compared with a partial treatment of applying insecticides in alternating strips. Both treatments reduced boll injury significantly compared with the level of damage before treatments. In strip-treated fields, a significant decline in boll injury was observed in untreated strips as well. Based on variogram analysis of boll injury before and after treatments, it was shown that both treatments disrupted spatial aggregation of stink bugs.

INDEX WORDS: Stink bug, planting date, skip spray application, variogram analysis, inverse distance interpolation, IPM, cotton pest management

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ISHAKH PULAKKATU-THODI

B.S., Kerala Agricultural University, Kerala, India, 2004

M.S., Mississippi State University, Mississippi, USA, 2010

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ISHAKH PULAKKATU-THODI

Major Professor: Michael D. Toews

Committee: George Vellidis
John R. Ruberson
Jeremy K. Greene
Phillip M. Roberts

Electronic Version Approved:

Maureen Grasso
Dean of the Graduate School
The University of Georgia
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DEDICATION

I dedicate this work to my parents Mohammed Pulakkatu thodi and Ayisha Kalaparambil, my wife Sabeetha Kaladi palliyalil, and my sons Ihsan Pulakkatu thodi and Farhan Pulakkatu thodi.

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Long field trips to sample stink bugs in cotton fields and processing of innumerous cotton bolls were really challenging. Support from Annie Horak, Ta-I Huang, Jamal Hunter, Barry Luke and Miguel Sorria made it fun-filled entertainment; I acknowledge their efforts with sincere gratitude.

Earning a Ph.D. from a US university is definitely a great achievement for a student from a rural part of India. I started my academic journey from humble surroundings and I feel proud to finish it off with an honorable degree from a prestigious US university. I remember all the blessings and prayers from my parents and teachers and encouragement and support from my brothers and sister, without which this journey would not have been possible.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Phytophagous stink bugs, often referred to as the stink bug complex, commonly include the green stink bug, *Chinavia hilaris* (Say) (formerly/congruently known as *Acrosternum hilare*), the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euschistus servus* (Say). These species infest cotton during critical growth stages (Reay-Jones et al. 2009, Hopkins et al. 2010). A recent estimate of cotton yield losses due to arthropods placed stink bugs as the fourth most damaging pest or group of pests across the USA (Williams 2009). Thrips and tarnished plant bug, *Lygus lineolaris* (Palisot), occupied the first and second positions, respectively, while the bollworm/budworm complex *Helicoverpa zea* (Boddie) and *Heliothis virescens* (Fabricius), respectively, was third most damaging (Williams 2009). The trend was similar during 2010, 2011 and 2012 (Williams 2010, 2011, 2012). However, regional disparity exists in the current pest scenario and crop losses based on the geographical distribution of cotton agro-ecosystems across the Cotton Belt. Cotton suffered greater injury from stink bugs in Georgia, Florida, and the Carolinas than from any other pest/complex in 2009, while in the Mid-South, stink bugs caused considerably less yield loss compared with tarnished plant bug. The western tarnished plant bug, *Lygus hesperus* Knight, is predominant in western areas of the Cotton Belt, while thrips are the major concern in terms of yield loss in Texas (Williams 2009).

Stink bugs were once suppressed coincidently by the application of broad-spectrum insecticides targeting the boll weevil, *Anthonomus grandis grandis* Boheman. Eradication of boll weevil and widespread adoption of cotton containing transgenes from *Bacillus thuringiensis* (*Bt*) resulted in a dramatic decrease in the number of insecticide applications and have consequently resulted in an increase in pest pressure from stink bugs (Greene et al. 2001). Cotton varieties with single-*Bt*-gene insertions (i.e., Bollgard) were replaced by varieties with dual-gene insertions (i.e., Bollgard II, WideStrike, and TwinLink), which offered better protection from the bollworm/budworm complex (*Helicoverpa zea* (Boddie) and *Heliothis virescens* (Fabricius), respectively). The true bugs, which include plant bugs and the stink bugs, have been a major concern in the second generation of genetically *Bt*-modified cotton varieties because of enhanced control of and reduced insecticide use for bollworm (Greene et al. 2008). Millions of dollars are spent on management costs for stink bugs, and tens of thousands of bales are lost due to damage caused by these pests during boll development (Williams 2009). Current management practices to manage phytophagous stink bugs primarily consist of whole-field treatments of insecticide upon detection of stink bug populations or boll injury above the Extension recommended treatment thresholds. However, based on recent advances elucidating the ecology and life history of stink bugs, there is potential to improve current management strategies, while preserving yield and maintaining fiber quality.

1.1 History of Cotton Production

Cotton belongs to the genus *Gossypium* in the Malvaceae plant family. The genus has about 50 known species globally. *Gossypium hirsutum* L., *G. barbadense* L., *G. arboreum* L., and *G. herbaceum* L. are 4 major species of commercial interest grown for textile fiber (Fryxell 1992). The place of origin of the genus *Gossypium* remains largely unknown; however, the primary centers of diversity are west-central and southern Mexico, north-east Africa and Arabia, and Australia. Phylogenetic studies with existing *Gossypium* species suggested that the genus arose about 10-20 million years ago (Wendel and Albert 1992; Seelanan et al. 1997). Archeological excavations from the Indus Valley (presently in Pakistan) suggested that cotton fibers were used in fabric as early as 3000 B.C. (Gulati and Turner 1928). *Gossypium hirsutum* and *G. barbadense* are the 2 most common commercially grown species, but *G. hirsutum* comprises approximately 90% of world plantings.

The history of cotton in the Americas dates back to the beginning of the 16th century. It is believed that cotton seed was planted in Florida around 1556. The colonists started growing cotton along the James River in Virginia during the 1600s (Haney et al. 2009). Cotton farming grew with the industrial revolution in England during the 1700s and invention of the cotton gin in America by Eli Whitney in 1793. Currently, cotton is one of the most important textile fibers in the world, accounting for approximately 35% of total world fiber use. The US, China, and India are the major producers, providing approximately 66% of the world's cotton. In the US, cotton production is confined to 17 southern states, with Texas and Georgia producing the major share. American upland cotton accounts for about 97% of the annual cotton crop in the US. Cotton production peaked during 2005, with 23.9 million bales, and has since declined. Increased acreage of corn and soybean, due to higher demands and prices, has resulted in declining cotton acreage since 2005 (Fig. 1.1).

Cotton is an important cash crop. The US cotton industry sustains about 440,000 jobs directly associated with cotton, producing revenues exceeding $120 billion (A.G. Jordan 2004, National Cotton Council). Cotton production in the southeastern states (including Alabama, Florida, Georgia, North Carolina, South Carolina, and Virginia) comprised about 25% of the US crop and had a direct farm-gate value of $802 million in 2009 (USDA-NASS 2010). Better yields, prices, and international demand for cotton were reflected in recent cotton statistics, with lint trading above $2.2 per kg ($1 per pound) on the spot market in the spring of 2011.

1.2 Biology of Cotton

Cotton is native to the tropics but has been adapted for commercial cultivation in subtropical climates. Cotton is a perennial by growth habit, but it is grown as an annual crop across the US Cotton Belt. It can attain a height of 1-2 meters or more if not limited by moisture and temperature. Growth becomes very limited below 15.5^{o} C and temperature above 38^{o} C is detrimental if it persists several days without adequate moisture in the soil. Due to the extensive taproot system, cotton is generally considered a drought tolerant crop. Growth of cotton seedlings starts with germination of seed and is dependent on the availability of soil moisture, an optimal range of temperature, and gas exchange.

Fruiting on a cotton plant lasts for an extended period of time. The first true cotton leaf appears 10-12 days after emergence. The first flower-bud appears on the lowest fruiting branch approximately 35-45 days after emergence, depending upon temperature, and additional flower buds follow at regular intervals. The time interval between the appearance of first flower bud and opening of the flower is generally about 25-30 days. Vegetative growth is reduced during the peak period of flowering, but may increase after the rate of flowering declines. Duration of flowering is reduced by late planting, strong plant competition and environmental stress. The duration of annual cotton growth lasts approximately 140 days. In most varieties, boll opening begins 120 days after shoot emergence.

1.3 Pest Management in Cotton

Two significant events have tremendously altered pesticide use and pest complex importance in cotton. Prior to the 1990s, major pests were boll weevil and caterpillars, such as *Heliothis virescens* (Fabricius) and *Helicoverpa zea* (Boddie). In Georgia, the average number of insecticidal sprays prior to initiation of boll weevil eradication in 1987 was 15 (Fig. 1.2). Eradication of the boll weevil was achieved by aggressive control strategies throughout the fruiting period of crop production in a formal eradication program. The challenge from most fruit and foliage feeding caterpillars was overcome by the introduction of transgenic *Bacillus thuringiensis* (Bt) cotton. Widespread deployment (>95%) of transgenic Bt cotton technology has greatly reduced problems and resulting insecticide applications for lepidopteran pests. As a result of these changes, the number of insecticide applications for all insects in southeastern cotton production has dropped from weekly sprays to about 2 or 3 total applications per hectare per year (Williams 2009).

Reduced insecticide use created a favorable environment for secondary pests such as stink bugs and they emerged as the key insect pest complex in southeastern cotton. Yield losses resulting from stink bug feeding on cotton bolls have been documented in various studies (Cassidy and Barber 1939, Toscano and Stern 1976, Barbour et al. 1990, Greene et al. 2001, Toews and Shurley 2009, Willrich et al. 2004a, 2004b) and have become a major concern in recent years. This situation has led researchers to investigate better ways to mitigate the problem. New approaches with further reduction in pesticide use are needed to address the stink-bug problem.

1.4 Stink Bug Biology

Stink bugs are highly polyphagous and can feed on a wide range of cultivated and non-cultivated plants. They are reported to feed on more than 200 cultivated and non-cultivated hosts and tend to be highly aggregated (Todd and Herzog 1980, McPherson and McPherson 2000, Reay-Jones 2010a). Besides cotton, they are an economic pest in row crops such as corn, *Zea mays* (L.) (Negron and Riley 1987), soybean, *Glycine max* (L.) (McPherson and McPherson 2000); fruit crops such as peach, *Prunus persica* (L.) and apple, *Malus domestica* (Borkh) (Leskey and Hogmire 2005); small grains such as wheat, *Triticum aestivum* (L.), (Viator et al. 1983) and grain sorghum, *Sorghum bicolor* (L.) (Hall and Teetes 1982); and vegetables such as tomato, *Lycopersicon esculentum* (Mill) (Lye et al. 1988). Stink bugs generally prefer crops or weeds bearing immature pods or fruits. Southern green stink bugs oviposit under leaves or pods in the upper portion of crops (Todd 1989). Barrel-shaped eggs are laid in clusters of 30 to 50 individual eggs and may hatch within 7 to 12 days. Development rate is temperature dependent. On average, 35-37 days are required for development from egg to adult during the growing season. Optimal developmental temperature for *N. viridula* is reported to be 30^{o}C (Todd 1989) Crop phenology and other abiotic factors may influence clutch size (Todd 1989, Panizzi et al. 2004). First and second instars of southern green stink bug stay around the egg mass and readily disperse as third instars. First instars do not need to feed, but second instars clearly feed on plant tissues. Small nymphs often feed on vegetative portions of plants, but larger nymphs and adults prefer to feed on developing seeds (Todd 1989, Hirose et al. 2006). There are approximately 4 to 5 generations per year for *N. viridula* (Todd 1989).

Brown stink bug and green stink bug complete 2 generations per year (McPherson and Mohlenbrock 1976, McPherson and McPherson 2000). Brown stink bug can feed on variety of hosts including many vegetables, fruits such as pecan and peach, field crops such as soybean, corn and cotton, and many weed species (McPherson and McPherson 2000, Rolston and Kendric 1961). Varying feeding preferences are observed among different species; for example, southern green stink bug feeds primarily on herbaceous annuals, while the green stink bug prefers woody shrubs and trees (Jones and Sullivan 1982, McPherson 1982). In North America, stink bugs emerge in early spring, feed on early season spring hosts, such as clovers or wheat, and later move to other preferred hosts such as corn, soybean, and peanuts that are in a reproductive developmental stage. Cotton fields that are located near large plantings of corn, soybeans, or peanuts suffer significant losses due to invasion by stink bugs.

Failure to identify stink bug aggregation in cotton at the proper time can result in significant loss of yield unless fields are treated with recommended insecticides at appropriate timings. Because pheromones produced by stink bugs facilitate their movements and eventual interactions, their distribution in the field could be clumped or aggregated and may cause pest managers to overestimate pest abundance. Many scientists have reported an edge effect where stink bugs were much more abundant on the edges of fields that shared a common boundary with some other host, such as corn, early-maturing soybeans, or peanuts.

1.5 Stink Bug Damage in Cotton

Stink bugs are observed in cotton fields from seedling emergence until harvest; however, cotton seedlings or flower buds (squares) are generally not injured by these pests (Willrich et al. 2004a). The infestations that occur during flowering can result in significant boll injury. Stink bug damage to cotton bolls is characterized by rough, warty growths on the inner carpel wall and lint staining (Wene and Sheets 1964). Stink bug feeding causes abscission of small bolls and reductions in yield, lint quality, and seed germination (Barbour et al. 1990, Willrich et al. 2004c, Toews and Shurley 2009). Apart from the feeding damage, some species, such as southern green stink bug, are capable of transmitting cotton seed and boll-rotting bacteria, such as *Pantoea agglomerans*. Infections by the strain Sc 1-R of *P. agglomerans* can cause rot of an entire locule that suffered feeding injury from a stink bug (Medrano et al. 2007).

1.6 Stink Bug Sampling in Cotton

Traditional methods of sampling for stink bugs in cotton fields using sweep nets and drop cloths have disadvantages. Dense canopies, thick branches, and the presence of numerous fruiting forms (pre-floral buds, blooms and bolls) make using sweep nets and drop cloths difficult for estimating numbers of stink bugs. Moreover, scouting of large commercial cotton fields using traditional methods is time consuming and laborious. Other impeding factors that affect efficient sampling and critical decision making on when pesticides are to be applied are the broad host range, strong flight ability, and within-field distribution of stink bugs (Willrich et al. 2003). An alternate method for assessing stink bug damage by examining immature cotton bolls (2.4 to 2.7 cm in diameter) suggested that treatments could be initiated when 20-25% bolls had internal lesions (Greene et al. 2001). They defined the stink bug damage by the presence or absence of at least 1 warty growth on the inner carpel wall with or without associated stained lint. Considering the time and labor required to process the cotton bolls and assess internal injury, Toews et al. (2009) evaluated enumeration of external feeding lesions on samples of 10, 15, 20, or 25 bolls per sample as an improvised procedure. This method was less precise than internal boll injury for detecting and classifying boll injury induced by stink bugs.

Dissecting bolls to observe internal feeding symptoms is an effective monitoring tool for stink bugs (Greene and Herzog 1999, Bundy et al. 2000, Toews et al. 2009). The presence of wart-like callus tissue on internal carpel walls, with or without stained lint is characteristic of stink bug damage. Stink bug monitoring using internal lesions is 10-fold more accurate to estimate stink bug abundance compared with sweep-net or drop-cloth sampling (Toews et al. 2008).

A novel method for detecting stink bugs by sensing volatile compounds emitted by them was explored by Henderson et al. (2010). They used a commercially available electronic nose (Cyranose 320) comprising an array of 32 carbon-black composite sensors to detect volatile compounds such as trans-2-decenal and trans-2-octenal. Under lab conditions, they were successful in predicting boll damage caused by stink bugs and in detecting the presence of stink bugs using this device (Henderson et al. 2010).

It has been demonstrated that stink bugs can be attracted using commercially available pheromones. Tillman et al. (2010) showed that *N. virdula* and *E. servus* can be attracted and trapped using aggregation pheromones *trans*- to *cis*-(*Z*)-α-bisabolene epoxide blend and methyl (*E*,*Z*)-2,4-decadienoate, respectively. The efficiency of the trap improved when a higher concentration of *N. viridula* pheromone was used. The native species *C. hilaris* failed to show any attraction to its reported male-produced pheromone; however, it was cross-attracted to *Plautia stali* pheromone [methyl (*E*,*E*,*Z*)-2,4,6-decatrienoate]. The number of stink bugs caught in traps generally declined when cotton started setting bolls, indicating that pheromone traps were more effective when used during early stages of crop growth (Tillman et al. 2010).

1.7 Stink Bug Dispersal

Proximity of some crops, such as peanut and soybean, and presence of forest patches near cotton fields have considerable influence on population dynamics of stink bugs. Late instars and adults of *N. viridula* and *E. servus* migrate from peanuts into adjacent cotton fields when cotton bolls become available for feeding (Tillman et al. 2009). The proximity of crops such as soybean and peanut negatively influenced seedcotton yield, gin turnout, lint color and lint value of cotton centrally located among corn, soybean, and peanuts (Toews and Shurley 2009). A similar study conducted in commercial cotton fields showed a significantly higher number of injured bolls on cotton plants adjacent to soybean and peanut (Reeves et al. 2010). A higher density of stink bug nymphs was also observed at the peanut-cotton border. Densities of both adults and nymphs were higher on rows immediately adjacent to other crops (Reeves et al. 2010). Stink bugs were observed aggregating on field borders near peanut fields and caused more feeding damage on bordering cotton plants, often referred to as an edge effect (Reay-Jones et al. 2010a). The sequential movement of stink bugs from early-season hosts to crops like peanut, soybean, corn, and cotton, based on crop availability during crop growing season, has prompted researchers to look into spatial dynamics of stink bugs.

Using computer programs integrated with geographic information system (GIS) and spatial statistics, it is possible to visually examine the pattern of dispersal of stink bugs. A number of such studies have been conducted in recent years (Tillman et al. 2009, Reay-Jones et al. 2010a). Ability to predict the dispersal of insect pests is of great interest as it would enable growers to apply insecticides to only the areas of the field where it is most required. The mapping of pest populations has become much easier due to the availability of GIS software and handheld GPS devices with high spatial resolution. Classical examples of mapping mobile insects using GIS approaches include monitoring of grasshoppers and locusts (Tappan et al. 1991) and gypsy moth (Liebhold et al. 1996, Liebhold et al. 1998, Yang et al. 1998).

1.8 Stink Bug Management in Cotton

A chronological analysis of published research on stink bug management in cotton reveals that most of the recent research has focused on developing thresholds (Greene et al. 2001), assessing damage (Willrich et al. 2004a,b,c, Siebert et al. 2005, Bauer et al. 2006), developing effective sampling plans (Reay-Jones et al. 2009, Toews et al. 2009, Hopkins et al. 2010, Reay-Jones et al. 2010b), and studying spatial dynamics (Tillman et al. 2009, Toews and Shurley 2009, Reay-Jones et al. 2010a, Reeves et al. 2010).

Organophosphate insecticides or pyrethroids are used for the control of stink bugs, after scouting and detection of populations above threshold levels. Insecticides are applied as whole-field sprays without considering variation in spatial aggregation of stink bugs in the field. Injudicious use of insecticides compromises the IPM philosophy and may lead to the development of insecticide resistance, secondary pest outbreaks and loss of biological control. Recent research has suggested that brown stink bugs have developed moderate tolerance against pyrethroid insecticides (Willrich et al. 2003).

Stink bug damage is most critical during the third, fourth, and fifth week of the cotton bloom cycle. Southeastern entomologists recommend use of a dynamic treatment threshold for stink bugs whereby the treatment threshold is set lowest during this period (Greene et al. 2008, Bacheler 2009). In Georgia, the treatment threshold is set at 20% internal boll injury during the second week of bloom, 10-15% internal boll injury during the third through fifth weeks of bloom, 20% during the sixth week of bloom, and 30% during the seventh week of bloom.

Due to a better understanding of stink bug biology and life history (Todd 1989, McPherson and McPherson 2000, Reay-Jones et al. 2010), cultural practices, such as adjusting date of planting to mitigate peak pest pressure, could be used to manage the stink bug complex. Manipulation of planting dates may allow the crop to escape in time from damaging pest populations and has been found to be effective in many cropping systems. Availability of multiple crops as potential hosts in the same farmscape and the ability to survive and reproduce on a wide array of weed hosts imposes a great challenge to managing stink bugs. A universal practice that farmers use upon detection of stink bugs above threshold level is to treat the entire field with an insecticide. This practice fails to recognize some important recent findings, such as spatial aggregation of stink bugs on field borders (Tillman et al. 2009, Reay-Jones et al. 2010a). Because good management practices include the use of broad-spectrum insecticides, it is highly likely that these applications eliminate key natural enemies such as *Trichopoda pennipes* (Ruberson and Wickings 2008).

Mitigating the development of insecticide resistance is important because few insecticide classes provide effective control of stink bugs. Partial field application of insecticides might be a useful and a cost effective tactic, if it were demonstrated to be efficacious in commercial fields. There are several studies that demonstrate efficacy of partial application of insecticides in other model systems. For example, insecticide treatments by spraying alternate rows or alternate pair of rows was effective against leafhoppers and aphids in cotton (Surulivelu and Kumaraswami

1989). Similar findings with site-specific treatments are reported in studies in wheat (Karimzadeh et al. 2011) and potato (Weisz et al. 1996). Rangeland grasshoppers were managed effectively in a Reduced Agent and Area Treatment (RAAT) program by applying insecticides in intermittent swaths at a reduced rate. The program was economical because of a reduction in insecticide use up to 50% and preservation of beneficial non-target arthropods (Lockwood et al. 2000). Similar tactics have been used to manage *Oothecca mutabilis* Sahlberg, a highly mobile insect pest infesting cowpeas, *Vigna unguiculata* Walpers (Ward et al. 2002a). Reductions in pest infestation were similar when 50 or 75% percent of fields were treated with insecticide (Ward et al. 2002b). Many cotton professionals have observed a decline in stink bug population in untreated plots located adjacent to treated plots. These observations suggest that spraying less than the entire field may yield similar results as the traditional approach.

The first objective of this study, which is described in Chapter 2, was to investigate the influence of planting date on stink bug effects on boll injury, yield, fiber quality, and economic returns in Georgia cotton. The study included 4 planting dates ranging from early May to late June across 3 locations and 2 years. The second objective, which is described in Chapter 3, was to investigate within-field spatial distribution of stink bug induced boll injury in commercial cotton fields. Knowledge about the spatial behavior of the pest can augment current management practices and aid critical decision making on insecticide use. In the third objective, described in detail in Chapter 4, we studied the feasibility of a partial insecticide use plan by adopting a skip-spray strategy to reduce insecticide input in commercial cotton fields.

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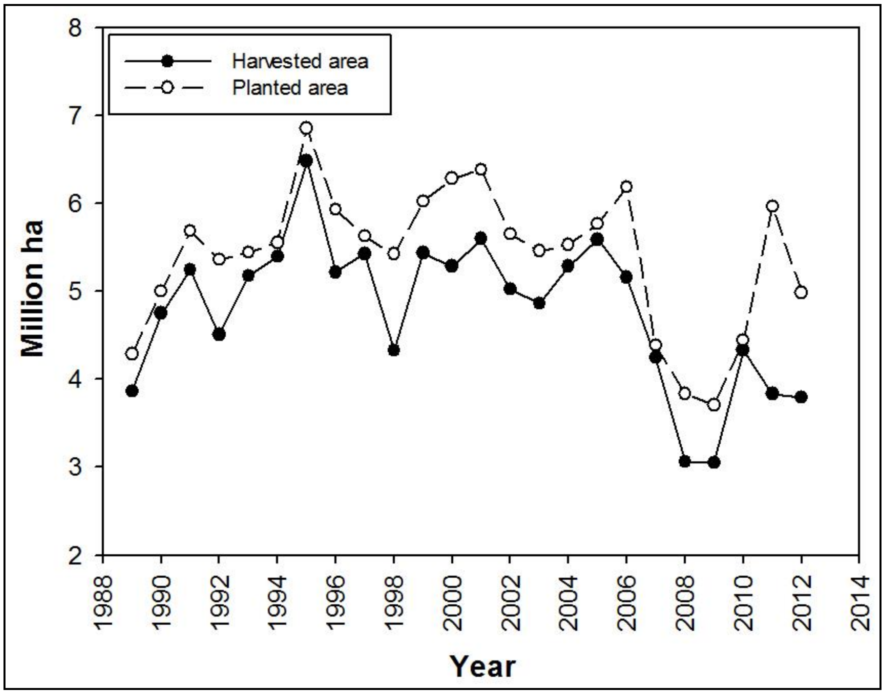

Fig. 1.1. Planted and harvested hectares of US cotton (1989-2012) (USDA-NASS 2013).

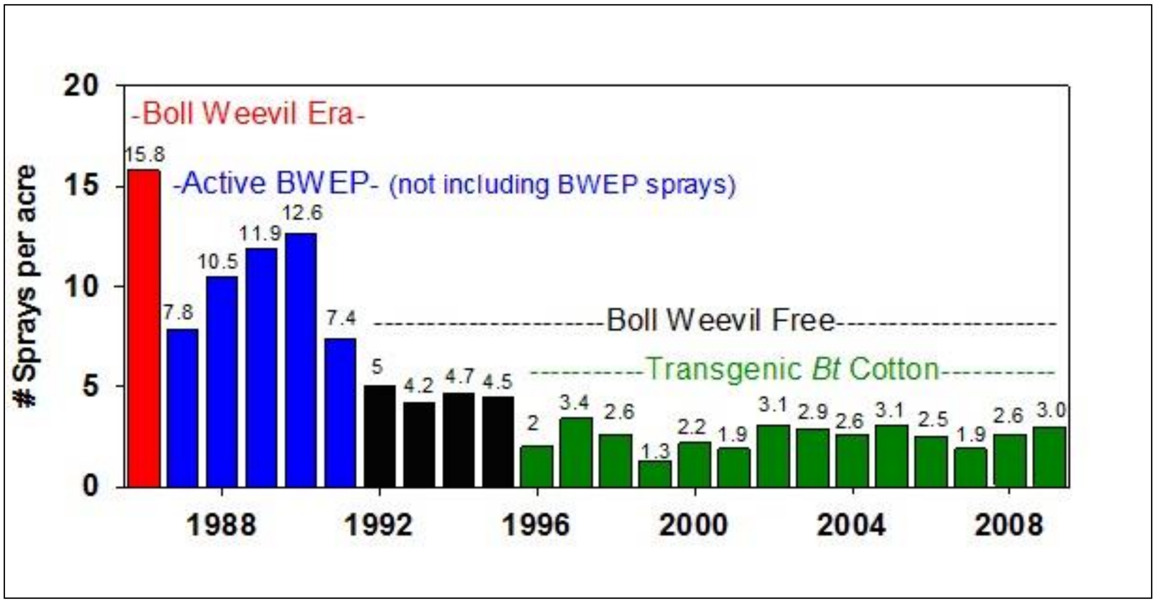

Fig. 1.2. Average number of insecticide applications (all insect pests) per acre to cotton in Georgia before, during, and after eradication of the boll weevil (Source: Beltwide Cotton Conferences).

CHAPTER 2

INFLUENCE OF PLANTING DATE ON STINK BUG INJURY, YIELD, FIBER QUALITY, AND ECONOMIC RETURNS IN GEORGIA COTTON

Pulakkatu-thodi I., D. Shurley, and M. D. Toews. Submitted to *Journal of Economic Entomology*, 09/11/2013.

ABSTRACT: Phytophagous stink bugs are economically important pests of annual and perennial crops in the southeastern US. Due to insecticide resistance and risk of secondary pest outbreaks, there is interest in identifying cultural practices that could lead to reduced insecticide applications. The objective of this project was to assess the importance of planting date on stink bug damage in cotton. Unsprayed plots of cotton with fortnightly planting dates were established at 3 locations in southern Georgia in each of 2 crop years. During the bloom cycle, boll injury induced by stink bugs was estimated weekly in each plot. Plots were subsequently defoliated, mechanically harvested, and ginned to assess differences in fiber quality attributable to injury from stink bugs. Results show that the rate of boll damage increased more rapidly through the bloom cycle for planting dates in June compared with May planting dates. Similarly, mean lint yield from May planting dates was significantly greater than June planting dates. Mean HVI color +b, a measure of fiber yellowness, was greater in cotton planted in June. Finally, economic analyses strongly suggested that cotton planted in May will confer several tangible advantages to cotton growers.

KEY WORDS: cultural pest management, integrated pest management, cotton fiber quality, piercing-sucking pests

Introduction

Management of cotton pests in the southeastern US has shifted from traditional production systems that rely heavily on broad-spectrum insecticides to integrated systems that utilize pest-resistant cultivars and selective insecticides (Summy and King 1992, Greene et al. 2001). Eradication of the boll weevil and widespread adoption of transgenic cotton varieties targeting caterpillars are generally responsible for the significant reduction in pesticide use. However, these factors also contributed to the emergence of the stink bug complex as an economically important group of pests in cotton (Greene and Herzog 1999, Greene et al. 2001). Preferential feeding by stink bugs on young developing cotton bolls causes abscission of young bolls or a loss of yield and lint quality when larger bolls are damaged (Barbour et al. 1990, Willrich et al. 2004a). Of several species of stink bugs that are encountered in cotton fields, the green stink bug, *Chinavia hilaris* (Say) [formerly known as *Acrosternum hilare* (Say)], the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euschitus Euschistus servus* (Say), are the most common (Turnipseed et al. 1995, Reay-Jones et al. 2009). Apart from direct feeding damage, stink bugs are capable of transmitting cotton boll-rotting bacteria such as *Pantoea agglomerans*. Infections by the strain Sc 1-R of *P. agglomerans* can cause rotting of an entire locule that suffered feeding wounds (Medrano et al. 2007). Stink bugs have been consistently ranked among the most damaging insect pests of cotton in southeastern states in recent years (Williams 2008, 2009, 2011). Approximately 0.53 million ha of cotton in Georgia were infested with stink bugs in 2011, and those infestations required insecticide treatment of approximately 0.4 million ha; at an average of 2 applications per season (Williams 2011).

Stink bugs are reported to feed on more than 200 cultivated and non-cultivated host species (Todd and Herzog 1980, McPherson and McPherson 2000, Reay-Jones et al. 2010). Besides cotton, they are an economic pest in row crops such as corn, *Zea mays* L. (Negron and Riley 1987) and soybean, *Glycine max* (L.) (McPherson and McPherson 2000); fruit crops such as peach, *Prunus persica* (L.), and apple, *Malus domestica* (Borkh) (Leskey and Hogmire 2005); small grains, such as wheat, *Triticum aestivum* L. (Viator et al. 1983), and grain sorghum, *Sorghum bicolor* (L.); (Hall and Teetes 1982) and vegetables such as tomato, *Lycopersicon esculentum* Mill. (Lye et al. 1988). Polyphagous pests often exploit diverse habitats of cultivated and non-cultivated hosts for food and colonization (Kennedy and Storer 2000). Because polyphagous pests are often highly mobile, they trigger insecticide application decisions in varied cropping systems (Carrière et al. 2012). All species of North American pentatomids overwinter as adults that emerge in early spring and begin feeding on seed bearing plants, such as wheat, clovers, and various weeds and build up populations by producing several generations per year (Todd 1989). At the landscape level, fields of soybean in reproductive growth stages are believed to act as sinks for populations of stink bugs (Olson et al. 2011). Cotton grown in close proximity to alternate hosts, for example soybean and peanut, is deleteriously affected by stink bug feeding as quantified by seedcotton yield, gin turnout, lint color, and lint value (Toews and Shurley 2009).

Due to a better understanding of the biology and life history of stink bugs (Todd 1989, McPherson and McPherson 2000, Reay-Jones et al. 2010), cultural practices, such as adjusting date of planting to mitigate peak pest pressure, could be used to manage the stink bug complex. Damage caused by stink bugs is most critical during the third, fourth and fifth week of the bloom in cotton (Greene et al. 2009, Bacheler 2009). Therefore, entomologists recommend the use of a dynamic treatment threshold for stink bugs whereby the treatment threshold is set lowest during this period (Greene et al. 2009, Bacheler 2009). In Georgia, the treatment threshold is set at 20% internal boll injury during the second week of bloom, 15% internal boll injury during the third through fifth weeks of bloom, 20% during the sixth week of bloom, and 30% during the seventh week of bloom. Manipulation of planting dates can shift the "window" of susceptibility of a crop away from peak populations of a pest and has been found to be effective in many cropping systems. For example, planting date affected abundance of stink bugs in early- and late-planted corn with early-planted corn having significantly lower numbers of southern green and brown stink bugs (Tillman 2010). Furthermore, uniform delayed planting was recommended in the rolling plains of Texas to manage boll weevil in cotton by exploiting predictable patterns of beetle's diapause and overwintering survivorship and post-diapause quiescence (Slosser 1978). Soybean cultivars planted early in the mid-southern states have experienced fewer lepidopteran defoliators but harbored more stink bugs (Baur et al. 2000).

Cotton planting in the southeastern US generally starts in late April and continues until early June (NASS-USDA 1997). It is desirable to identify a safer timeframe of planting cotton where damage due to stink bug could be minimized. The objective of this study was to quantify stink bug damage in terms of boll injury, yield, lint quality, and economic value of cotton planted at 4 different dates spanning over the typical southeastern cotton planting season.

Materials and Methods

Study Locations: This experiment was conducted in 2011 and 2012 on experiment farms operated by the University of Georgia or USDA. In 2011, trials were conducted near Tifton

(31°30'44.159"N 83°32'53.8296"W), Midville (32°52'20.536"N 82°12'52.9704"W), and Plains (32°2'12.174"N 84°22'2.8236"W). Trials were repeated in 2012 near Tifton (31°30'28.0814"N 83°33'22.0129"W) and Plains. During both years at all locations, ‘DP 0912 B2RF,’ containing Cry1Ac and Cry2Ab proteins for resistance to lepidopteran caterpillars, was planted. Overhead irrigation was provided at all locations, and Extension recommended agronomic practices for cotton grown on conventional tillage were followed.

Plot Layout: At each location, plots were arranged in a randomized complete block design with 3-5 replicates. In 2011, planting dates at Tifton were 5/12, 5/26, 6/9, and 6/23, and, at Midville, they were 5/10, 5/24, 6/7, and 6/21. Planting dates at Plains were 5/9, 5/23, 6/6, and 6/20. All plots were 8 rows wide and 15.24m long, except in Midville, where the plots were 30.48m long. In 2012, planting at Tifton was conducted on 5/10, 5/24, 6/7, and 6/21, whereas planting at Plains occurred on 5/10, 5/24/, 6/7, and 6/21. Plots at Tifton were 8 rows wide and 12.19m long, and plots in Plains were 4 rows wide and 15.24m. Regardless of planting date or location, all plots were planted using seed from the same bag. The same pneumatic planter and planting depth was utilized for all plots.

Sampling: Sweep-net sampling for stink bugs and collection of immature cotton bolls for assessing stink bug injury were done weekly. Samples of seedcotton for yield estimation and fiber quality assessment were taken from each plot. Sampling for stink bugs and bolls was commenced from the second week of bloom in each plot and included a sweep-net (38.1 cm diameter) sample of 20 sweeps from a single row and collection of 20 quarter sized (2.4 to 2.7 cm in diameter) soft bolls. Bolls were collected randomly from the rows, and no more than 1 boll was collected from a given plant. Consistent boll size was assured with use of a scouting decision aid (NCSU/CU/UGA Extension 2010) comprised of a stiff plastic card with 2 holes

(2.25 cm and 2.8 cm); appropriate-sized bolls fit through the large hole but not through the smaller hole. Collected stink bugs and boll samples from each plot were held in labeled plastic bags for processing in the laboratory. Boll samples were examined in the laboratory for internal damage that, included feeding punctures, warty growths on the inner carpel wall, stained lint or rotten locks (Greene and Herzog 1999, locules (Toews et al. 2009); stink bugs were identified to species and life stage.

At the end of the season, cotton was chemically defoliated, and the center 2 rows from each plot were picked with a 2-row spindle picker modified to collect seedcotton into bags. The resulting seedcotton was weighed and ginned at the UGA Microgin (Tifton, GA), which handles research quantities of seedcotton. Representative ginned fiber samples from each plot were sent to the USDA classing office located at Macon, GA, for official grading. Cotton lint classification followed USDA's official grade standards for American upland cotton (USDA-AMS 2001). Lint characteristics such as color grade, leaf grade, staple length, micronaire, strength, color Rd (a measure of fiber brightness), and color +b (a measure of fiber yellowness) were determined using the Uster High Volume Instrument (HVI).

Economic analyses were conducted on lint yield/ha and resulting fiber quality based on the December 2011 and December 2012 average Georgia cash (spot) prices received for base quality (Color- 41, Leaf- 4, Staple- 34) published by AMS-USDA (2011-2012). The average cash prices for December 2011 and December 2012 were $1.92 and $1.47/kg (87.14 and 72.67 cents/lb), respectively. Baseline prices receive an incentive or discount based on the quality characteristics determined by grade standards. Fiber quality characteristics considered for analysis included color, leaf, staple (CLS), micronaire, strength, and uniformity.

Data Analysis: Planting dates in 2011 differed by up to 2 calendar days with that of 2012. To avoid confusion and for the ease of analysis, those dates were synchronized with the 2012 dates. Percentage boll damage data were analyzed using linear regression methods because data were collected weekly throughout 6 weeks of the bloom cycle. Simple linear curve models were fitted using the PROC REG procedure in SAS 9.3 (SAS Institute 2012), with weeks of bloom on the *x*-axis as the independent variable and mean percentage of boll injury on the *y*-axis as the dependent variable. Fit of the regression model was evaluated using pattern of residuals and *F* tests for lack of fit. Comparisons among individual slopes were made possible by testing slopes of 2 planting dates at a time. Boll damage data collected from week 2 through week 6 were considered for the analysis, and data collected from remaining weeks (for example, there were soft bolls available at 1site during the seventh week of bloom) were omitted from analyses. The PROC MEANS procedure was used to extract sums, means, and standard errors. The PROC GLIMMIX procedure and LSMEANS statement were used to compare lint yield, seedcotton yield, and fiber quality parameters among the 4 planting dates. Data from the 3 locations were pooled together for analysis. In 2012 only, yield data from the 2 June planting dates at Tifton were omitted from the analysis due to poor stand establishment. Because insect density was low, stink bug captures were summed across planting dates and weeks of bloom to illustrate the stink bug species composition.

Results

Boll Damage: Mean percentages of boll damage due to stink bug feeding in plots planted on 10 May, 24 May, 6 June and 21 June in 2011 were 6.5 ± 1.0, 9.7 ± 1.2, 15.6 ± 1.9, and 18.8 ± 2.4, respectively. There was a positive linear trend from early-planted to late-planted cotton.

The earliest sampling date in 2011 was 14 July, and, in 2012, it was 16 July. In 2012, for the respective planting dates, mean percentages of boll damage were 11.8 ± 1.6, 13.6 ± 2.1, 22.8 ± 3.6, and 21.7 ± 3.9. Overall, boll damage was numerically greater in 2012 (17.3 ± 1.5) compared with 2011 (12.6 ± 0.9). Linear increases in percentage boll damage allowed fitting a regression line through the temporal data and comparison of linear slopes of line graphs of 4 planting dates. In 2011, regression lines for planting date were described by 'mean percentage of boll damage (y) = 2.3 ± 1.0 * week of bloom(x) - 2.53 ± 4.3' for 10 May, 'Y=3.5±1.0 * X – 4.0 ± 4.3' for 24 May, 'Y=6.0±1.0 * X – 8.6 ± 4.4' for 6 June, and 'Y=7.5 ± 1.0 * X – 10.9 ± 4.2' for 21 June. The overall model for 2011 showed significant differences in slopes (F=56.43; df= 8, 240; P<0.01), with an adjusted r^2 value of 0.64. Statistical comparison of the linear slopes showed significant increase in the amount of damage in June planting dates in 2011 (Fig. 2.1a.). Corresponding regression lines in 2012 were (y)=2.5 ± 1.8x - 2.0 ± 7.7 for 10 May, 5.4 ± 1.8 * X-8.1 ± 7.6 for 24 May, Y=7.2 ± 2.0 * X – 6.6 ± 8.3 for 6 June and Y= 8.0 ± 1.9 * X – 9.3 ± 7.8 for 21 June. Only the late June planting date showed significantly greater damage, but the overall trend remained the same (F=29.61; df= 8, 126; P<0.01 and adjusted r^2 = 0.63) (Fig. 2.1b). Mean percentages of boll damage by calendar week exhibited an increase in damage after August in both years (Table 2.1).

Percent boll injury in plots with June planting dates exceeded the Extension recommended treatment threshold much more frequently than those with May planting dates. In 2011, the percent boll injury for plants in both the 10 and 24 May planting dates never exceeded the threshold. However, damage to plants from both June planting dates exceeded the threshold on 3 of the dates. In 2012, percent boll injury for both May-planting dates exceeded the

Extension recommended threshold twice, while boll injury exceeded the threshold 3 times with June plantings.

Yield and Gin Turnout: Mean seedcotton yield (kg/ha) and mean lint yield (kg/ha) differed significantly as a function of planting date in 2011 (F = 18.35; df = 3, 36; P<0.01, F = 23.43; df = 3, 36; P<0.01). Both planting dates in May exhibited statistically comparable seedcotton yield, which was significantly greater than yield from both June planting dates (Table 2.2). Analysis of mean lint yield showed that cotton planted in early and late June yielded significantly lower lint compared with cotton planted in May (Fig. 2.2a), with late June planted cotton yielding the least lint. Percentage gin turnout was marginally non-significant among planting dates (F= 2.75; df = 3, 36; P = 0.06). In 2012, mean seedcotton and lint yield (kg/ha) was significantly highest for the 10 May planting date (F = 8.20-7.56; df = 3, 10; P<0.01) (Fig. 2.2b). Also in 2012, gin turnout was significantly higher for both May planting dates compared with that from June planting dates (F = 13.08, df = 3, 18 P<0.01) (Table 2.2).

Fiber Quality: In 2011, planting date significantly affected fiber yellowness and brightness as indicated by HVI color +b value (F = 68.23; df = 3, 36; P<0.01) and HVI color Rd value (F = 19.18; df = 3, 36; P<0.01), respectively (Table 2.2, Fig. 2.3a). HVI color +b and HVI color Rd values together determine the color grade of cotton fiber. May planting dates exhibited statistically comparable color +b values, which were significantly less yellow than June planted cotton (Fig. 2.3a). Conversely, HVI color Rd value indicated brighter lint in the late June planting date (Table 2.2). Planting date was not a factor in defining variability in staple length (F = 2.19; df = 3, 36; P=0.10). However, planting date was a factor in determining variability in staple strength (F=4.78; df=3, 36; P<0.01) and uniformity (F = 5.16; df = 3, 36; P<0.01). Interestingly, these values indicated better quality for late-planted cotton.

Fiber samples were also available from all sites in 2012. HVI color +b value was significantly better for cotton planted on 10 May (F = 11.79; df = 3, 18; P<0.01), than for cotton planted during late May or in June (Fig. 2.3b). Fiber uniformity was significantly affected by planting date (F = 4.01; df = 3, 18; P = 0.02) in 2012, indicating better values towards late planting dates. Other quality parameters which showed significant variability in 2012 were staple length (F=6.60; df=3, 18; P<0.01) and staple strength (F=6.19; df=3, 24; P<0.01), but LSMEAN values for these parameters showed that all the planting dates, except the late June planting date, had comparable values. Fiber reflectance (HVI color Rd) did not vary significantly based on planting date in 2012 (F=2.74; df=3, 18; P=0.07).

Stink Bug Capture: Number of stink bugs captured by the sweep net was generally very low in both years. In 2011, 287 samples (20 sweeps per sample) were conducted, and only 14 stink bugs were captured. Of these, 42.8% were brown stink bug, and 57.1% were green stink bugs. No southern green stink bugs were captured. Much greater pressure from stink bugs was observed in 2012. From 166 sweep net observations, a total of 39 stink bugs were captured, with 92.3% of them being southern green stink bugs and the remainder being brown stink bugs. Statistical comparisons were not attempted on stink bug captures due to low numbers.

Economic Analysis: Lint value based on average Georgia cash prices (December 2011 and 2012), adjusted for fiber quality, differed significantly due to planting date in 2011 (F = 21.33; df = 3, 36; P<0.01) and 2012 (F = 6.27; df = 3, 10; P=0.01). Both May planting dates had statistically similar lint values that were significantly greater than both June planting dates in 2011. Cotton planted in late June exhibited the least lint value (Fig. 2.4a). Cotton planted in early May had the significantly highest lint value in 2012 (Fig. 2.4b) compared with the remaining planting dates. Economic returns appear to be primarily driven by lint yield (Fig. 2.2a and b, Fig.

2.4a and b). Statistical comparison of adjusted base prices did not indicate significant differences in both years (2011: F = 1.93; df = 3, 36; P=0.14; 2012: F = 1.93; df = 3, 18; P=0.19).

Discussion

These studies demonstrated that cotton planted in June was generally at a higher risk of being damaged by stink bug populations than cotton planted during May in Georgia. Mean percentages of boll damage were consistently high in the later planted cotton plots. Mean percentage of boll damage by calendar weeks showed that the numerically greatest percentage damage was recorded during the third week of September in 2011 (42.9%) and the second week of September in 2012 (38.0%) (Table 2.1). On the contrary, boll damage was at safer levels (below 10%) starting from early July through early August in both years. Use of internal symptoms of boll damage as an accurate estimation of the presence of stink bugs and the correlation with other sampling methods has been established in previous research (Greene and Herzog 1999, Greene et al. 2001, Toews et al. 2008, Greene et al. 2009). Our study implies that planting cotton early in the planting window will allow growers to escape peak stink bug pressure and, thereby, possibly eliminate or minimally reduce the number of sprays required to manage them.

The optimal planting window for cotton is when the soil temperature at a 5-cm depth approaches 18°C and the weather forecast is favorable for crop development (Silvertooth et al. 1999). Previous research showed that the planting season for optimal cotton lint yield was between 20 April and 10 May in midsouthern and southeastern states (Aguillard et al. 1980, Waddle 1984). However, the typical planting season in Georgia usually lasts from 20 April to 5 June (NASS-USDA 1997). In general, early planting is thought to improve yields (Pettigrew

2002, Boquet and Clawson 2009) and moderately late planting, 10 to 15 days after the recommended planting window ends, does not always reduce cotton yield (Slosser 1993, Bauer 1998). Out of the 4 planting dates we selected, the first 2 plantings were well within the typical planting window in Georgia. The third planting date was around the boundary of the typical planting window, and the fourth planting date would likely occur as a result of replanting or double-crop situations. In our study, yield reduction was consistent in both years in late-planted cotton, and, based on the amount of injury suffered by late planted cotton, there is strong reason to believe that stink bugs contributed to at least a portion of the observed yield loss. Lint yield was reduced up to 36% in 2011 in cotton planted during late June when compared with lint yield of cotton planted in early May. Damage from stink bugs was numerically higher during August in 2012 compared with the same period in 2011. An increase in pressure from stink bugs in 2012 possibly contributed to higher lint loss that season (Fig. 2.2b). The potential of stink bugs to reduce yield in cotton has been demonstrated previously (Barbour et al. 1990, Willrich et al. 2004b).

Loss of quality in cotton fibers due to feeding by stink bugs can be manifested in several ways. Injury from feeding affects physical properties of cotton fiber such as lint color, micronaire (a measure of fiber maturity), uniformity, staple length, and strength (Cassidy and Barber 1939, Barbour et al. 1990, Bommireddy 2007). Toews and Shurley (2008) showed that quality of cotton fibers harvested from the edges of cotton fields was negatively affected due to higher concentration of bugs in borders shared with other crops. In this study, values of HVI color +b were lowest in cotton planted in May in 2011 and in May 10-planted cotton in 2012. Fiber reflectance, as measured by the HVI color Rd value, indicated better quality in late planted cotton in 2011, but that was not evident in 2012. Other quality parameters, such as staple length, staple strength, and uniformity, indicated better quality in June-planted cotton during one year, but that was not consistent in both years. In general, the influence of stink bug damage was not evident in quality parameters, except HVI color +b values. Along with genetics, various environmental factors, primarily weather, are known to affect cotton fiber quality (Bradow 2000). It is possible that changes in weather factors due to delayed planting masked the influence of stink bugs on these quality parameters.

Economic returns were consistently greater in cotton planted earlier in May. Statistically, lint yield was the most important factor that influenced economic returns, as returns followed nearly the exact same trend as those exhibited by lint yield (Fig. 2.2 and Fig. 2.4). Previous research (Toews and Shurley 2008) showed that damage from stink bugs can affect the economic value of lint. Although there were documented statistical differences among planting dates, the remaining quality parameters were not sufficiently different to affect economic returns. Differences in HVI color Rd may reflect changing environmental conditions, such as rainfall, after the boll opened. Similarly, HVI strength and length vary as a function of weather when fibers are developing and likely have nothing to do with stink bug feeding. Considering that the optimal planting window starts in late April, there may be potential for further improvement in yield and fiber quality by planting earlier than May 10.

Decreased stink bug capture in the sweep net does not necessarily suggest less pressure from stink bugs. An observed lack of efficiency in sampling stink bugs using sweep nets is well documented (Toews et al. 2008). Conversely, assessment of internal boll-feeding injury has been shown to be much more sensitive to changes in stink bug density in the field. Here, gradual increases in percent boll damage with characteristic stink bug feeding injuries established that a greater density of stink bugs was present in the crop later in the season.

A better understanding of the population dynamics, movement, and alternate host usage of stink bugs will contribute to improved cultural control practices for stink bugs. Previous research by Carrière et al. (2012) demonstrated that landscape factors, such as adjacent habitats of alternate hosts of a pest, can influence population dynamics of insect pests in cotton fields. Toews and Shurley (2009) showed that cotton planted adjacent to peanut or soybean was more susceptible to injury from stink bugs than cotton adjacent to corn. That study included only 1planting date for each crop; because stink bugs prefer to feed on flowering plants, it is likely that the temporal sequence of adjacent flowering hosts affected movement of stink bugs among crops. Data presented here strongly suggest that manipulations of planting date for cotton are effective at curtailing injury from stink bugs. Although cultural control practices, such as manipulations of planting date, may not completely eliminate the need for insecticide applications, concurrent use of several tactics supports sound IPM practices.

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Table 2.1. Mean percentages of boll injury (±SEM) caused by stink bugs by calendar weeks in Georgia during 2011 and 2012.

Year	Month	Week	N	Boll injury % ± SEM
2011	July	2	4	0.00 ± 0.00
		3	13	3.85 ± 1.15
		4	26	4.07 ± 1.41
	August	1	26	7.12 ± 1.36
		2	39	6.73 ± 1.29
		3	39	11.28 ± 1.55
		4	57	12.73 ± 1.67
	September	1	8	29.38 ± 5.13
		2	35	21.42 ± 3.04
		3	13	42.89 ± 4.22
2012	July	3	7	5.00 ± 2.67
		4	16	5.94 ± 1.53
	August	1	6	14.17 ± 3.52
		2	17	9.71 ± 1.84
		3	19	16.05 ± 1.86
		4	33	18.50 ± 2.45
	September	1	11	30.91 ± 6.56
		2	10	38.00 ± 10.09
		3	7	34.29 ± 9.09
		4	4	6.25 ± 2.39
	October	1	4	10.00 ± 4.56

Table 2.2. Mean ± SEM of various parameters evaluated for cotton planted at 4 different planting dates in Georgia in 2011 and 2012. Means followed by same letter are not significantly different.

Parameters	Planting date	2011	2012
		Mean ± SEM	**Mean ± SEM**
Seedcotton yield (kg/ha)	5/10	3126.01^{a} ± 178.19	2494.78^{a} ± 116.43
	5/24	3026.75^{a} ± 175.38	1979.18^{b} ± 253.43
	6/07	2472.80^{b} ± 141.70	1051.68^{b} ± 60.52
	6/21	2053.87^{c} ± 124.89	1208.07^{b} ± 150.49
Percent gin turnout	5/10	38.53^{a} ± 0.26	38.00^{a} ± 0.30
	5/24	38.23^{a} ± 0.30	38.42^{a} ± 0.29
	6/07	38.69^{a} ± 0.42	36.14^{b} ± 0.45
	6/21	37.38^{b} ± 0.72	34.71^{c} ± 0.71
HVI color Rd	5/10	72.54^{d} ± 0.86	74.09^{a} ± 0.38
	5/24	73.65^{c} ± 0.68	76.10^{b} ± 0.67
	6/07	74.75^{b} ± 0.62	75.37^{ab} ± 0.26
	6/21	76.41^{a} ± 0.52	75.53^{ab} ± 0.62
HVI strength index	5/10	29.91^{a} ± 0.22	29.43^{a} ± 0.27
	5/24	30.28^{ab} ± 0.42	29.41^{a} ± 0.30
	6/07	30.94^{bc} ± 0.19	29.84^{a} ± 0.48
	6/21	31.41^{c} ± 0.38	31.67^{b} ± 0.59
HVI uniformity index	5/10	82.96^{a} ± 0.16	82.00^{a} ± 0.25
	5/24	83.08^{a} ± 0.20	82.49^{ab} ± 0.19
	6/07	83.04^{a} ± 0.26	82.99^{bc} ± 0.66
	6/21	83.74^{b} ± 0.11	83.43^{c} ± 0.23
HVI length (Inch)	5/10	1.11^{ab} ± 0.01	1.09^{a} ± 0.01
	5/24	1.11^{b} ± 0.00	1.08^{a} ± 0.01
	6/07	1.11^{ab} ± 0.00	1.11^{ab} ± 0.02
	6/21	1.13^{a} ± 0.01	1.14^{b} ± 0.01

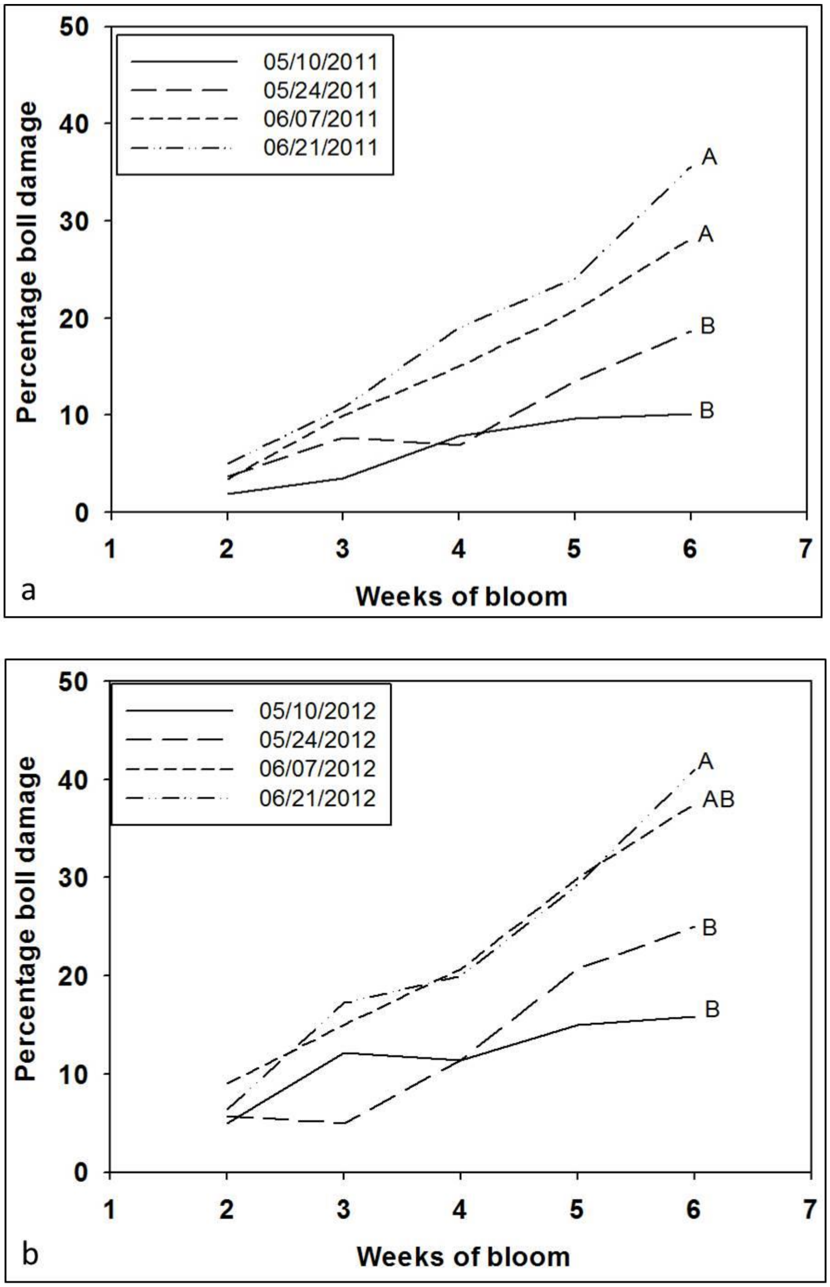

Fig. 2.1. Mean percentages of boll damage by weeks of bloom by planting date in 2011 (a) and 2012 (b). Lines denoted by same letters are not significantly different.

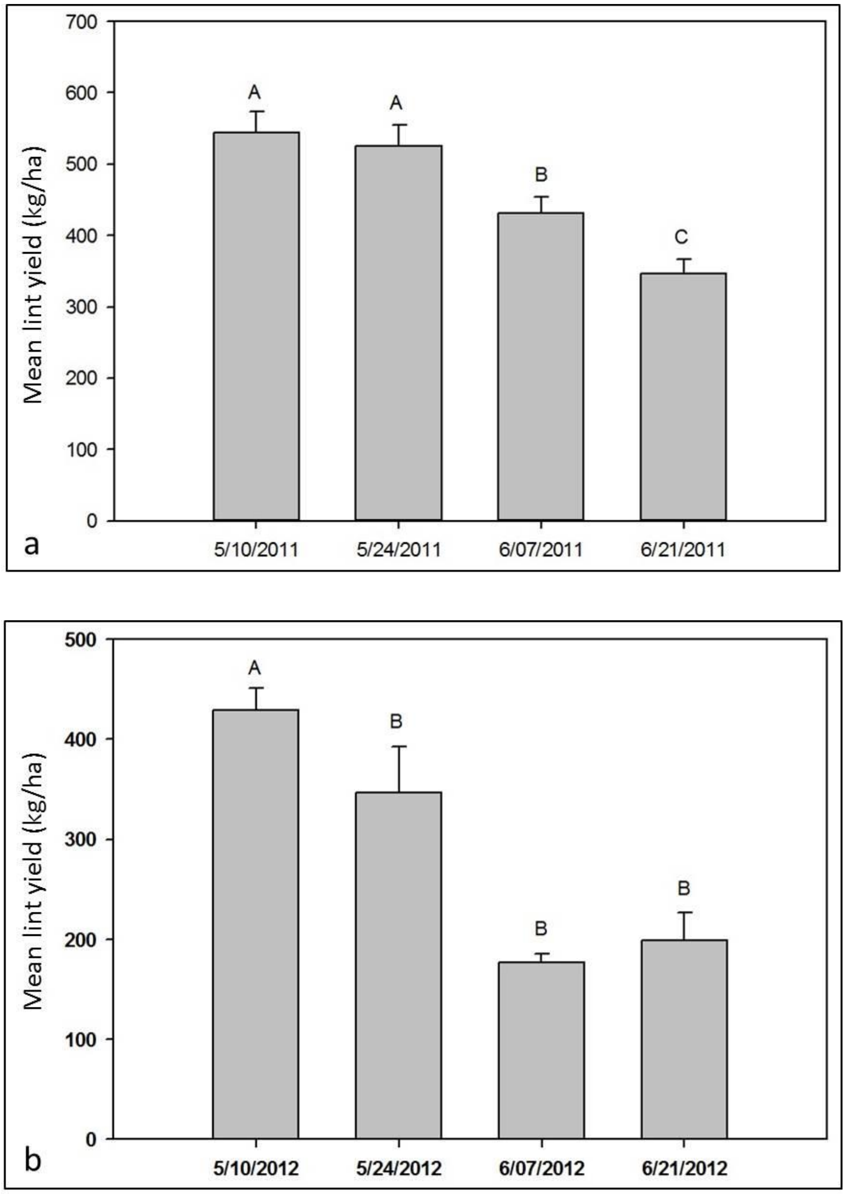

Fig. 2.2. Mean lint yield (kg/ha) ± SEM for planting dates in 2011 (a) and 2012 (b). Bars denoted by same letters are not significantly different.

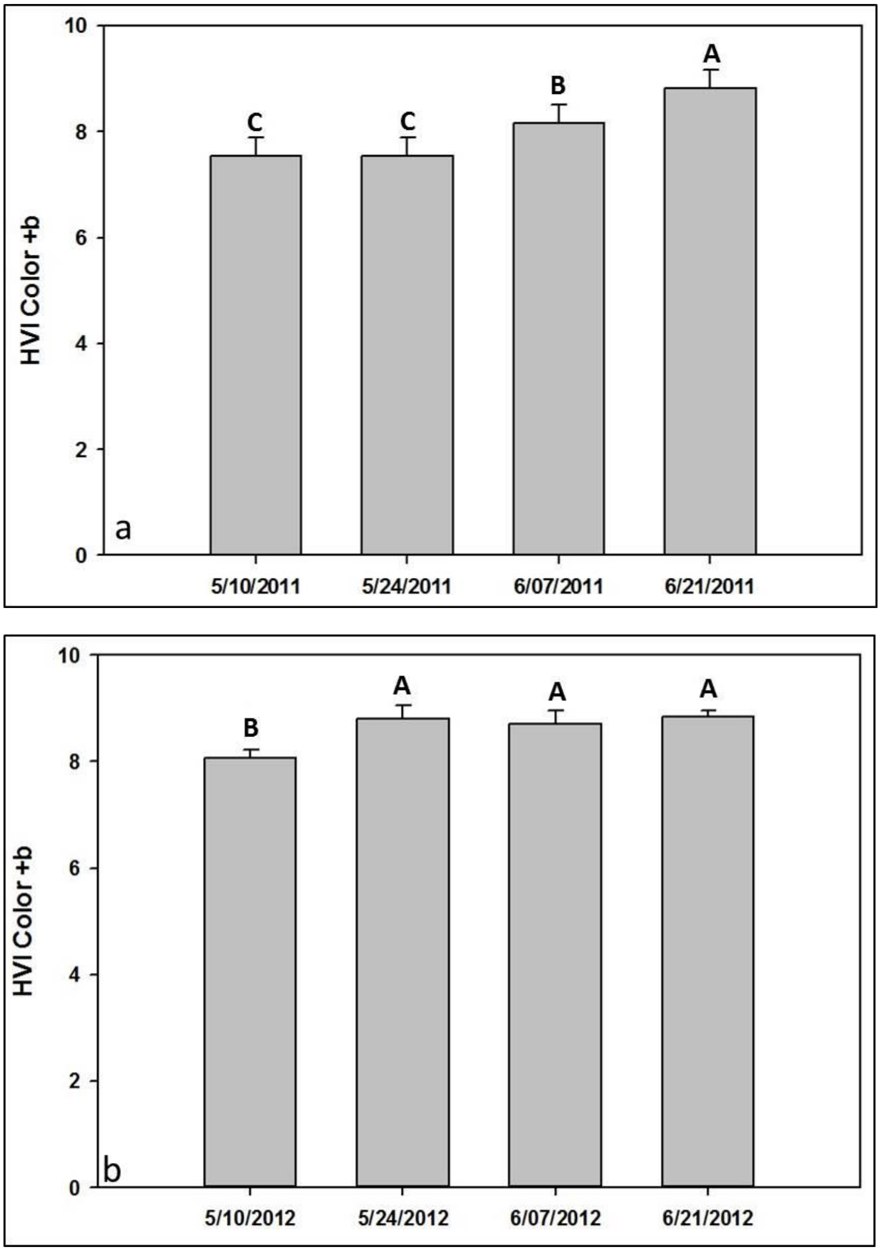

Fig. 2.3. Mean HVI color +b value ± SEM for lint harvested by planting dates in 2011 (a) and 2012 (b). Bars denoted by same letters are not significantly different.

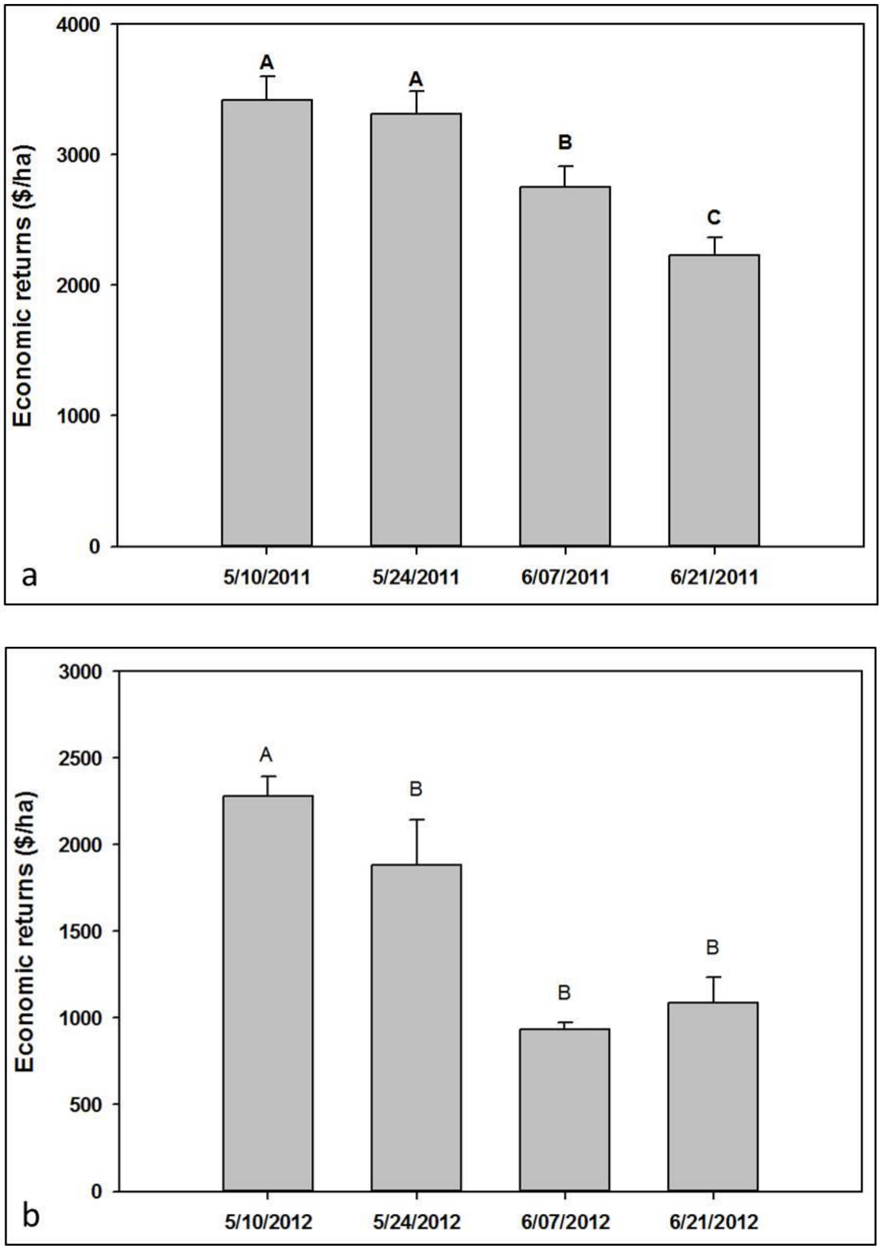

Fig. 2.4. Economic returns per ha by planting date for 2011 (a) and 2012 (b). Bars denoted by same letters are not significantly different.

CHAPTER 3

WITHIN-FIELD SPATIAL DISTRIBUTION OF STINK BUG INDUCED BOLL INJURY IN COMMERCIAL COTTON FIELDS OF THE SOUTHEASTERN USA

Pulakkatu-thodi, I., D. Reisig, J. K. Greene, F. P. F. Reay-Jones, and M.D. Toews. Submitted to *Environmental Entomology*, 12/04/2013

ABSTRACT: Spatial distribution of boll injury induced by stink bugs was studied in 5 commercial cotton fields (~22 ha each) in 2011 and 2012 to understand variability in stink bug dynamics within the field. Cotton bolls and stink bugs were collected weekly from a geo-referenced grid of sampling points (1 sample per 0.40 ha) in each field. The inverse distance weighted interpolation, variogram analysis and Moran's Index *I* were used to describe spatial variability of stink bug damage within the fields. Boll injury was found to be spatially associated at distances ranging from ~75 m to 275 m with an average distance ~150 m. An exponential variogram model was selected as the best fitting model to describe the spatial association in 4 out of 5 fields. Spatial association was significant in 3 out 5 fields, as indicated by Moran's Index *I*. The spread of boll injury from stink bugs was gradual in most fields, and an insecticide treatment was required during the fourth or fifth week of bloom. Capture of stink bugs using a sweep net was inefficient, and boll injury was found to be a better predictor of activity by the pest complex.

KEY WORDS: Spatial statistics, variogram, stink bug, Moran's I, spatial association

Introduction

Phytophagous stink bugs are economic pests of cotton, *Gossypium hirsutum* L., in the southeastern US (Greene et al. 2001, Reay-Jones et al. 2009). The stink bug complex in cotton includes 3 principal species: green stink bug, *Chinavia hilaris* (Say); southern green stink bug, *Nezara viridula* L.*;* and brown stink bug, *Euschistus servus* (Say). Feeding by these pests causes direct injury to developing cotton bolls, resulting in abscission of immature bolls, lint staining, general loss of fiber quality, and reduced yield (Barbour et al. 1990, Bundy et al. 2000, Willrich 2004, Toews and Shurley 2009). Based on recent estimates of crop loss in cotton, stink bugs are consistently among the most damaging pests in southeastern states (Williams 2008, 2009, 2011). Approximately 0.5 out of 0.6 million hectares of cotton in Georgia were infested with stink bugs in 2011, and those infestations required insecticide treatment on approximately 0.4 million hectares at an average of 2 applications per season (Williams 2011). Management costs for foliar treatment of stink bugs averaged $19.47- $37.06 per hectare during 2009-2011, with most fields being treated multiple times (Williams 2009, 2010, 2011). Both brown stink bugs and southern green stink bugs can transmit the boll-rotting bacterium *Pantoea agglomerans*. Infections by the strain Sc 1-R of *P. agglomerans* can cause rotting of the entire locule that suffered feeding wounds (Medrano et al. 2007). The reduced need for applications of broad-spectrum insecticides, due to eradication of the boll weevil, *Anthonomus grandis* Boheman, and widespread adoption of transgenic cotton varieties to manage lepidopterans, is believed to be the major reason for increased incidence of stink bugs in southeastern farmscapes (Bundy et al. 2000, Greene et al. 2001).

The polyphagous nature of stink bugs (Todd 1989, McPherson and McPherson 2000, Reay-Jones et al. 2010) and sequential availability of suitable hosts in southeastern farmscapes have prompted researchers to examine spatial behavior and variability in distribution of stink bugs in crop fields (Tillman et al. 2009, Reay-Jones et al. 2010, Tillman 2011). Clumped or aggregated patterns have been reported previously for species such as *N. viridula* (Todd and Herzog 1980), and spatial variability and aggregation in densities of stink bugs and their damage has been observed in cotton fields, especially at field borders (Tillman et al. 2009, Reay-Jones et al. 2010). Dispersal of stink bugs from crops, such as corn and peanuts, to cotton is thought to be driven by availability of suitable hosts in time and space (Tillman et al. 2009), but their aggregation behavior could be influenced by pheromones or clumped egg-laying behavior. Ecology of many cosmopolitan stink bugs, such as *N. viridula* and *E. servus*, is well studied (Rolston 1961, Todd 1989, McPherson and McPherson 2000, Herbert and Toews 2010, Herbert and Toews 2011), but understanding their spatial behavior in commercially scaled cotton fields could augment current management efforts. Previous data show that assessment of stink bug pressure in cotton fields is done more efficiently by sampling boll injury than by estimating actual insect density using a sweep net (Toews et al. 2008). Furthermore, current Extension recommended treatment thresholds are based on percentage boll injury levels during the flowering cycle (Greene et al. 2008, Bacheler et al. 2009). Reay-Jones et al. (2010) suggested that the permanent nature of boll injury relative to the temporal presence of stink bugs is one reason why damage is a better metric to study spatial characteristics of stink bug activity in cotton.

Traditional methods of evaluating insect populations from independent samples include indices such as the variance to mean ratio, Taylor's power law (Taylor 1961), Lloyd's mean crowding index (Lloyd 1967), Wald sequential probabilities ratio (Wald 1947), and Iwao's patchiness regression (Iwao 1968). Those methods were developed for use on independent samples from a given population. Conversely, spatial statistics are used to explore data points that are expected to exhibit spatial dependence (Issaks and Srivastava 1989, Liebhold et al. 1993). Geospatial techniques, such as interpolation using Inverse Distance Weighted (IDW) and Spatial Analysis by Distance Indices (SADIE), have been used to describe stink bug populations or damage (Tillman et al. 2009) at the interface of cotton and peanut and for cotton fields ranging from 4 to 12 hectares (Reay-Jones et al. 2010). IDW is a deterministic method which uses a mathematical function to estimate values across unsampled locations. Variogram analysis, developed for geology and mining, has more recently been adapted for environmental and ecological studies.

The variogram measures the spatial dependence in sample data by evaluating the variance as a function of distance and direction between paired observations (Cressie 1991). The semivariance γ for lag distance h is shown in Equation 1.

$$\gamma(\mathrm{h}) = \frac{1}{2\mathrm{N}(\mathrm{h})}\sum_{\mathrm{i}=1}^{\mathrm{N}(\mathrm{h})}\left[\mathrm{z}(\mathrm{x_i})\text{-}\mathrm{z}(\mathrm{x_i}+\mathrm{h})\right]^2 \qquad \text{(Eq. 1)}$$

In Equation 1, $z(x_i)$ is the sampled variable at point x_i, $z(x_i + h)$ is the sampled variable at point $x_i + h$, and $N(h)$ is the number of pairs separated by lag h. Variogram parameters, including the sill, nugget, and range, give valuable information about the spatial structure of the response variable (Issaks and Srivastava 1989). Typically, the semivariance increases with increasing lag distance, until reaching an asymptote. The distance at which the variogram quits increasing is called the range (A), and the semivariance value where it levels off is the sill (C). The nugget (Co) is a spatially independent component of variance due to scales less than the minimum sampling distance or due to sampling error, and it intersects the y-axis above the origin. Data collected from points separated by distance less than ‘the range’ are spatially autocorrelated, while data collected from points beyond the range are uncorrelated. Statistical hypothesis testing to identify significant spatial aggregation at specified distances is possible using Moran’s Index *I* (Issaks and Srivastava 1989, Perry et al. 2002) shown in Equation 2.

$$I = \frac{n}{S_o} \frac{\sum_{i=1}^{n} \sum_{i=j}^{n} w_{ij} (x_i - \bar{x})(x_j - \bar{x})}{\sum_{i=1}^{n} (x_i - \bar{x})^2} \qquad \text{(Eq. 2)}$$

Where n is the total number of observations, x_i and x_j are the observations at locations i and j, $\bar{x}$ is the mean of observations, w_{ij} is a spatial weight between observations i and j, and S_o is the sum of all w_{ij}’s. Variograms have been used to describe spatial structure of the western tarnished plant bug, *Lygus hesperus* (Knight), in cotton (Carriere 2006); sunflower midge, *Contarinia shulzi* (Gagne) in sunflower (Hodgson 2004); and the coffee berry borer, *Hypothenemus hampei* (Ferrari); and leafminer, *Leucoptera coffeella* (Guérin-Méneville) in coffee (Alves et al. 2011). Spatially interpolated maps of insect counts based on the variogram provide linear statistical estimates of values at unsampled locations (Myers 1991, Liebhold et al. 1993). Moran’s *I* has been previously used to describe spatial dynamics of *Homalodisca coagulata* (Say) by Park (2006). Predictability in pest distribution can facilitate site-specific management by suggesting application of insecticides to areas where needed and, in turn, helping to reduce management costs (Weisz et al. 1995, Bacheler et al. 1998) and preserve refuges of natural enemies and parasitoids in untreated areas of the field (Weisz et al. 1996).

The objective of this project was to monitor within-field distribution of stink bugs and boll damage in commercial cotton fields (~22 ha) and then model the resulting spatial relationships using geostatistical techniques.

Materials and Methods

Study Locations. The study was conducted over a 2-yr period in 5 commercial cotton fields (2 in 2011 and 3 in 2012) with an area ranging from 17 to 28 ha and an average area of 22.14 ha. In 2011, fields were located near Midville, GA (Burke County, 32°52′12.5″N 82°13′24.5″W), and Pantego, NC (Beaufort County 35°37′09.1″N 76°44′07.8″W). In 2012, one of the fields was located near Plains, GA (Summer County 32°02′36.7″N 84°22′07.2″W), and 2 remaining fields were located near Nashville, GA (31°17′02.4″N 83°21′04.7″W and 31°16′31.4″N 83°21′01.7″W). Fields at Midville and Nashville were planted with DP 1050 B2RF cotton. The field at Plains was planted using both cotton varieties DP 1048 B2RF and 1050 B2RF. The Pantego, NC, location was planted with DP 1028 B2RF, and the second field at Nashville, GA, was planted with cotton variety PHY 499 WRF. Overhead irrigation was used on all fields, and regionally appropriate Extension recommended agronomic practices were followed. All of the fields, except the one in Pantego, NC, had at least 1 border shared with an adjacent field of peanuts. Field boundaries and arrangement of sampling points are shown in Fig. 3.1, while more specific details of the fields are shown in Table 3.1.

Field Layout. Prior to first flowering, fields were spatially mapped using a GPS receiver and mapping software (Arcmap10, ESRI 2010). Uniformly spaced sampling points were assigned using an approximately 60-meter grid in a rectangular pattern. Sample points in each field were marked using 2.4-m tall flags labeled with a unique number. This density of sample points provided an approximate density of 1 sample per 0.4 ha. To avoid known bias from sampling field edges, all sample locations near the field edges were no closer than 30 meters from the field perimeter. Weekly sampling, including both sweep net and internal boll damage estimates, commenced during the second week of bloom. At each sample point, stink bugs were sampled using 2 sets of 25 sweeps (single row) with a 38.1-cm sweep net. For estimating boll injury, 10 soft bolls were collected from each sample point and pooled. The selection of correct boll size was aided by the scouting tool developed by North Carolina State University/Clemson University/University of Georgia Extension, which consists of a stiff plastic card with small and large holes sized at diameter 2.4 and 2.7 cm, respectively (Bacheler et al. 2010). Only those bolls which could pass through the large hole, but not through the small hole, were collected. Actual sample location relative to the sample flag varied by a predetermined number of rows (e.g., 2 rows east of flag in week 1 followed by 1row west of flag in week 2) to avoid sampling the exact same plants in consecutive weeks.

Sampled bolls were transported in an ice chest to the lab and then dissected to assess internal injury to bolls based on symptoms of feeding by stink bugs (Barbour et al. 1990, Bundy et al. 2000, Willrich 2004, Toews and Shurley 2009). Each individual boll was scored on a binomial scale, and a composite score of all bolls from that location was recorded. A mean percentage of field-wide boll injury was calculated each week, and insecticide application was initiated when boll damage exceeded the dynamic treatment threshold (Bacheler et al. 2009). Briefly, the dynamic treatment threshold for stink bugs in cotton is a widely used procedure in which the threshold for insecticide treatment changes based on the number of susceptible bolls in a particular week of bloom**.** Insecticide treatment is recommended when the percentage of boll injury exceeds 10% during the third, fourth, or fifth week of bloom, of flowering, while a higher threshold (30-50%) is used for the remaining weeks (Bacheler et al. 2009). Only those sample data collected prior to insecticide application were considered for this analysis to avoid obvious bias in estimating dispersal of stink bugs.

Data Analyses. Linear descriptive statistics were used for assessing weekly percentage of boll injury. Due to low boll injury in initial weeks and the high number of zeros in data sets, mean percentage of boll injury (MPBI) was averaged across weeks up to the week of threshold injury to get an overall mean percentage of boll injury (OMPBI). For exploratory spatial analysis and estimating variability of stink bug injury across each field, OMPBI was interpolated using the Inverse Distance Weighted (IDW) method in ArcMap (ESRI 2011). The reliability of IDW maps was evaluated by cross-validation using the software tool GS+ (Gamma Design Software, Plainwell, MI), which involves temporary removal of each measured datum one at a time and estimation of its value by all other available data in the spatial domain (Issaks and Srivastava 1989). This process, repeated for all data points in a given spatial domain, enables construction of a graph with observed and estimated data from which linear regression coefficients could be calculated.

Geostatistical Analysis. Stink bug injury was modeled using variogram analysis in GS+. Raw, fractional data from each sample point were arcsine transformed to get a nearly normal distribution (Zar 1999). Data were tested for any obvious surface trend by visual analysis of quantile map in GS+ (Bohling 2011). Omnidirectional or isotropic variogram was calculated for 2 reasons: 1) no obvious surface trend was observed in any data set and 2) in variogram analysis, at least 30 data pairs per lag distance are required to estimate variogram adequately (Issaks and Srivastava 1989), and this criterion was difficult to meet for anisotropic (directional) variogram due to limited numbers of samples in each field (range = 41 to 72 samples per field). Variogram model fitting involves fitting a theoretical model to the empirical or experimental variogram using a non-linear weighted least square regression, and model evaluations are done by comparing residual sum of squares (Cressie and Hawkins 1980). The software GS+ provides variogram model fitting based on 4 functions: linear, spherical, exponential, and gaussian. Hypothesis testing on the presence of spatial aggregation was performed using Moran's *I* and associated z-scores and *P* values. A positive *I* indicated positive autocorrelation, zero indicated a random pattern, and a negative value indicated a dispersed pattern. A z-score of 1.96 and above indicated significant spatial aggregation at the 0.05 level (Issaks and Srivastava 1989).

There are inconsistent reports on the minimum number of samples required for constructing a reliable empirical variogram (Pardo-Iguzquiza 1998, Webster and Oliver 2001, Olea 2006). According to Webster and Oliver (2001), a minimum of 100 data measurements were needed for a given spatial domain, while Olea (2006) suggested at least 50 measurements were needed for variogram analysis. Pardo-Iguzquiza (1998) indicated that "a few dozen data may suffice" when transformed data are used. In this study, all the fields had fewer than 100 data points and 2 fields had fewer than 50. For relatively small samples, the key is to strike a balance between the numbers of lag classes and pair counts in each lag classes by specifying proper lag, h.

Results

Insect Density. The total number of stink bugs (adults and nymphs) captured with the sweep-net method from the 2 fields in 2011 was 49 (sum of 3 wk of sampling in Field A and 4 wk of sampling in Field B). Of these, 39 stink bugs were captured from Field A and the remainder from Field B. The majority were brown stink bugs (69.3%), and the remainder were green stink bugs; no southern green stink bugs were captured during this period. In 2012, 7 stink bugs (6 southern green stink bugs and 1 brown stink bug) were captured from 3 fields during the study. Because the total number of stink bugs was low, statistical analyses were not merited.

Boll Injury. Mean percentages of boll injury (MPBI) during the second and third weeks of bloom were 7.0 ± 1.0 and 6.5 ± 1.0%, respectively, and exceeded the Extension recommended dynamic treatment threshold during the fourth week of bloom with an MPBI of 14.1 ± 1.3% in Field A. In Field B, the MPBI increased gradually from 2.2 ± 0.8% during the second week of bloom to 6.1 ± 1.3% during the fourth week of bloom. MPBI exceeded the dynamic threshold during the fifth week of bloom (12.7 ± 1.8%). MPBIs of 1.6 ± 0.6, 8.4 ± 1.7, and 12.5 ± 2.1%, during the second, third, and fourth week of bloom, respectively, were recorded in Field C, while Field D yielded MPBIs of 7.0 ± 2.3, 4.6 ± 0.9, 5.6 ± 1.0, and 9.6 ± 1.2%, during the second, third, fourth, and fifth week of bloom, respectively. Field E had a high infestation in the second week of bloom (MPBI of 15.7 ± 1.6%), but that dropped to 10.4 ± 1.3% by the third week. By calendar dates, Fields A and B exceeded threshold by the fourth week of July and second week of August, respectively, in 2011. In 2012, Fields C and D exceeded threshold by the first week of August and Field E by the third week of July. Overall mean percentage of boll injury (OMPBI) averaged over weeks up to the week of threshold injury was 9.3 ± 0.7, 6.8 ± 0.9, 7.5 ± 1.0, 5.6 ± 0.5, and 13.1 ± 1.0 for fields A, B, C, D, and E, respectively.

Spatial Analyses. Interpolated maps of OMPBI indicated spatial variability in distribution of stink bug injury across fields, with apparent clustering of boll injury in some fields (Fig. 3.2). Increased injury towards the periphery is evident in Fields A, B, C, and E. The reliability of IDW maps was tested by cross-validation (Issaks and Srivastava 1989) (Table 3.2). A representative cross-validation graph was made for Field A (Fig. 3.3). In general, the regression coefficient was relatively high, indicating that interpolation was reliable in estimating variability of damage caused by stink bugs.

Isotropic or ominidirectional variogram analysis of arcsine-transformed data showed that boll injury in each field was spatially correlated (Fig. 3.4). Variograms were calculated for an active lag distance (search radius) of about 50% of broadest extend of field as a standard procedure in all fields, except for Field C where it was about 80% of the broadest extend. This approach gave a sufficient number of lag classes for calculating empirical variograms in each data set. The lag distance h was based on average sampling distance and was rounded to include a minimum of 30 pairs of sample points in each lag class. Therefore, each point in the empirical variogram is the average of 30 or more data pairs. Parameters used for variogram analysis are given in Table 3.1 and model parameters with fit statistics are given in Table 3.3. Based on residual sums of squares, the exponential model was selected having best fit for Fields A, B, C, and D, while a Gaussian model was selected for Field E (Table 3.3). All variograms reached the sill (C) indicating that spatial autocorrelation was present. 'Proportion' is a characteristic that measures the degree of spatial dependence. Values close to 1 indicate substantial presence of spatial dependence. A variogram with no nugget variance (where the curve passes through the origin) will have a proportion value of 1, and it will be 0 where there is no spatially dependent variation (pure nugget model). Apart from the model with best fit, parameters from 2 other models were also evaluated (Table 3.3).

The range, defined as the distance above which spatial dependence of the measured variable ceases to exist, varied between 74.3 and 274.8 m, with an average range of 154.8 m in models with the best fit. For other models, the range varied between 76.7 and 271.7 m, with an average range of 115.8 m. Nugget variance, which indicates a spatially independent component, was lower compared with the sill, the spatially dependent component, for all models. These data indicated that variability arising from measurement error or sampling scale was generally low.

Generally speaking, the range indicates the upper bound of neighborhood where spatial autocorrelation is present. This, in turn, indicates that spatial association will be higher when samples are inferred from shorter distances than the range. A relatively larger 'range' in 4 exponential models compared with a shorter range in the Guassian model suggested presence of low-density clusters of injury by stink bugs in the field.

Based on Moran's Index *I*, significant spatial aggregation was detected in Field A (*I*= 0.05; Z-score 2.25; *P*=0.02), Field B (*I*= 0.10; Z-score 2.08; *P*=0.03), and Field D (*I*= 0.09; Z-score 2.12; *P*=0.03). Conversely, a random pattern was detected in Field C (*I*= -0.01; Z-score 0.94; *P*=0.94) and Field E (*I*= 0.01; Z-score 0.70; *P*=0.48). The weight factor was based on inverse distance weighting, and several distances were attempted at multiples of 50 m. Significant aggregation was noted at a distances of 250, 150, and 150 m for fields A, B, and D, respectively. The Z-score deteriorated at distances above or below this range for respective fields. It should be noted that these distances were similar to the range parameter estimated using variogram analysis.

Discussion

Thorough sampling and subsequent characterizing of boll injury in commercial cotton fields are laborious tasks, but necessary as part of improving future pest management in the crop. Most arthropod pests, including stink bugs (Taylor et al. 1978, Wilson and Room 1983, Reay-Jones et al. 2010), exhibit an aggregated distribution in crop fields. A better understanding of this spatial and temporal variation can facilitate better management strategies. Data shown here were based on boll injury, a sensitive and persistent measure of stink bug feeding in cotton.

Reay-Jones et al. (2010) and Tillman (2009) used IDW maps and SADIE analyses to study the spatial dynamics of stink bugs in cotton and cotton-peanut interfaces and were able to show spatial variability in distribution of stink bugs. Additionally, they identified significant spatial aggregation in some fields. Interpolated maps using the IDW method are suitable for initial exploratory analysis of spatial data (Perry et al. 2002), and SADIE analyses have been successfully used in many systems to quantify spatial characteristics of arthropod pests (Holland et al. 1999, Ferguson et al. 2000, Reay-Jones 2010). However, variogram analysis is able to quantify the extent (distance) of spatial association.

Our study using variogram analysis of stink bug injury showed that feeding injury from stink bugs was spatially associated up to an average range of 154.8 m based on best fitted variogram models. The biological significance of the parameter ‘range’ is that it can be considered as a neighborhood where sampled data are related to one another. For example, a variogram of a low-density, dispersed population will have higher range, whereas a short range is characteristic of high-density population (Jung-Joon et al. 2011). In this study, Fields A and E had comparable areas and numbers of data points. Due to natural variation in soil fertility observed in Field E, cotton plants showed differences in maturity; for example, cotton plants in some parts of the field started flowering while most of plants in the field were still in vegetative stages. This early availability of cotton bolls might have attracted stink bugs to those specific areas of the field and resulted in a greater density of stink bugs during initial weeks. Variogram data for field A were averaged over 3 weeks, of which 2 initial weeks had lower percentage injury. Spatial behavior of insects can be erratic, and their distributions are usually best explained by exponential or spherical models (Issaks and Srivastava 1989). The exponential model was the best fitting theoretical variogram in 4 out of 5 candidate fields. The results indicated by Moran's *I* statistic are in line with the findings of variogram analysis in 3 out of 5 fields.

Variogram analysis is computed based on the assumption that field observations are a random function Z(x), where x denotes the location of the observation. Spatial data often violate this assumption, which results in spatial autocorrelation of the data. If sampled data are to be used for linear statistical analyses (ex. ANOVA), only those samples which are farther apart than the range of the variogram are truly independent. If data are to be used for spatial statistics, spatial autocorrelation is an opportunity to understand the underlying spatial process such as factors contributing to the aggregation of the data. Clearly, these data showed that autocorrelation among neighboring points was common even though the samples were taken more than 50 m apart. The implication is that treating only small areas of the field, for example, just a few square meters, is unlikely to confer benefit. Conversely, the data support that precision targeting of insecticides for stink bugs would need to include fairly large areas, such as several thousand square meters.

Sampling using percent boll injury was superior to sampling using the sweep net. The number of stink bugs captured using sweep nets in our study was insufficient for statistical comparison, a result previously shown by Toews et al. (2008). Moreover, the semi-permanent nature of boll injury, compared with the mobile nature of bugs, suggests that boll injury is a better response variable for sampling stink bugs in cotton. A previous study showed that the spatial distribution of stink bugs in cotton fields does not always coincide with stink bug injury (Reay-Jones et al. 2010). Sampling with a sweep net is hindered when the cotton is very tall. It can be difficult for scouts to reach the top of the plant, and the total proportion of the plant being sampled is smaller on tall plants compared with shorter plants. Additionally, late in the bloom cycle there will be several large bolls present near the top of the plant that interfere with an efficient sweep-net stroke. Other factors, including direct sunlight and time of day, might also influence sampling stink bugs with a sweep net. Stink bugs are reported to move actively on individual cotton plants during daytime in search of food (Huang and Toews 2009). Similarly, intercrop movement of stink bugs occur between cotton and other fields crops (Tillman 2009, Toews and Shurley 2009) based on the maturity of the crops.

Based on the findings of this and previous studies by Tillman (2009) and Toews and Shurley (2009), which showed considerable effects at the interface of cotton and adjacent crops, the authors observed that boll damage attributed to stink bugs is generally greater in edge samples (first 50m from the edge of the field). For large commercial fields, this information suggests that treating the edges of the field might be a viable technique in lieu of treating the entire field. It is important to note that all spatial analyses are scale dependent. Variogram analysis fails to identify spatial aggregation at distances shorter than sampling distance, and the scale of association might be different for different pests. Prior knowledge about the biology of the organism is critical for understanding the parameters of spatial analysis. Our study used an approximate 60-m grid for establishing sampling points. A sampling plan with twice the resolution of this plan would be a desirable next step.

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Table 3.1. Details of commercial cotton fields selected for weekly sampling of stink bugs and associated boll injury in Georgia and North Carolina in 2011 and 2012

Field ID	Location	Area, ha	No. of sampling points	Average sampling distance, m	Broadest extent, M	Date of first sampling
A	Pantego, NC	28.0	72	52.7	854	7/14/2011
B	Midville, GA	17.0	41	58.5	631	7/19/2011
C	Plains, GA	18.2	44	58.8	480	7/11/2012
D	Nashville, GA	21.1	56	54.4	606	7/12/2012
E	Nashville, GA	26.4	68	56.4	804	7/12/2012

Table 3.2. Fit statistics of cross-validation of Inversed Distance Weighted interpolation of mean percentage of cotton boll injury. Regression coefficients indicate the level of precision achieved in predicting temporarily discarded data based on an optimum number of neighboring data

Field ID	Location	No. of maximum neighbors	Regression coefficient	Standard Error
A	Pantego, NC	12	0.59	0.30
B	Midville, GA	9	0.63	0.47
C	Plains, GA	12	0.73	0.47
D	Nashville, GA	4	0.29	0.26
E	Nashville, GA	16	0.40	0.49

Table 3.3. Details of variogram model parameters of mean percentage of boll injury (arcsine transformed), induced by stink bugs in cotton, with fit statistics in 5 fields sampled in Georgia and North Carolina in 2011 and 2012. Bolded models indicate the statistically appropriate model for that field

Field	Location	Lag, h (m)	Active Lag, (m)	Model	Nugget variance (Co)	Sill (C)	Structural variance (Co+C)	Range, m (A)	RSS	Proportion (C/Co+C)
A	Pantego, NC	55	400	**Exponential**	**0.0030**	**0.0175**	**0.0205**	**274.8**	**5.782E-06**	**0.869**
				Spherical	0.0003	0.0183	0.0186	140.5	1.452E-05	0.986
				Gaussian	0.0101	0.0103	0.0204	271.7	9.287E-06	0.502
B	Midville, GA	65	300	**Exponential**	**0.0014**	0.0169	**0.0183**	**123.3**	**3.753E-06**	**0.923**
				Spherical	0.0000	0.0176	0.0176	88.1	7.188E-06	0.999
				Gaussian	0.0009	0.0168	0.0177	76.7	6.867E-06	0.946
C	Plains, GA	55	400	**Exponential**	**0.0020**	**0.0131**	**0.0151**	**149.4**	**2.799E-05**	**0.871**
				Spherical	0.0004	0.0144	0.01476	113.8	2.861E-05	0.971
				Gaussian	0.0013	0.0134	0.01478	96.6	2.863E-05	0.909
D	Nashville, GA	55	300	**Exponential**	**0.0003**	0.0059	**0.0062**	**152.1**	**4.853E-07**	**0.950**
				Spherical	0.0002	0.0057	0.0059	108.1	6.032E-07	0.969
				Gaussian	0.0002	0.0057	0.0059	86.4	6.086E-07	0.971
E	Nashville GA	60	400	**Gaussian**	**0.003**	0.0200	**0.023**	**74.3**	**1.614E-05**	**0.878**
				Spherical	0.000	0.0228	0.0228	85.9	1.618E-05	0.999
				Exponential	0.0019	0.0211	0.0230	90.3	1.751E-05	0.918

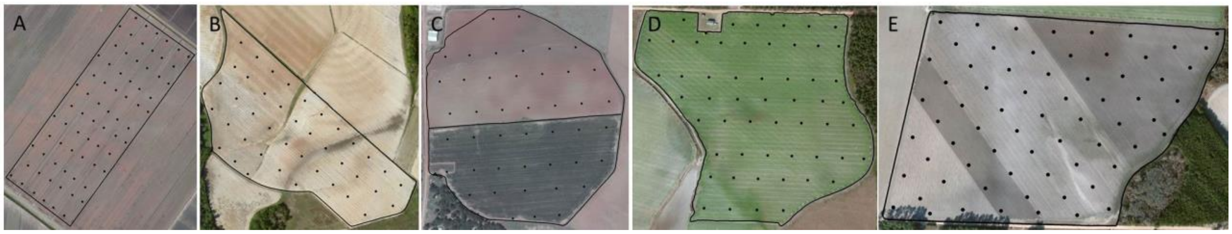

Fig. 3.1. Field geometry and layout of sampling points of 5 fields selected for sampling stink bugs and associated boll injury to cotton in Georgia and North Carolina in 2011 and 2012. The black polygon indicates the field boundary of each study area and black dots indicate sampling points. A: Pantego; B: Midville; C: Plains; D: Nashville-1; C: Nashville-2.

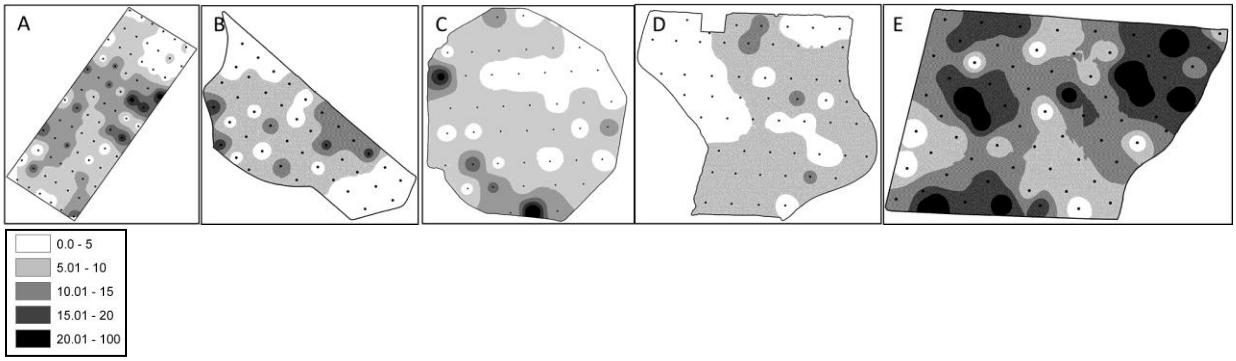

Fig. 3.2. Inverse distance weighted maps of overall mean percentage of boll injury to cotton in 5 fields sampled for stink bug damage in Georgia and North Carolina during 2011 and 2012. A: Pantego; B: Midville; C: Plains; D: Nashville-1; C: Nashville-2. White to dark coloration represent an incremental increase in with white areas having least and dark areas having most damage.

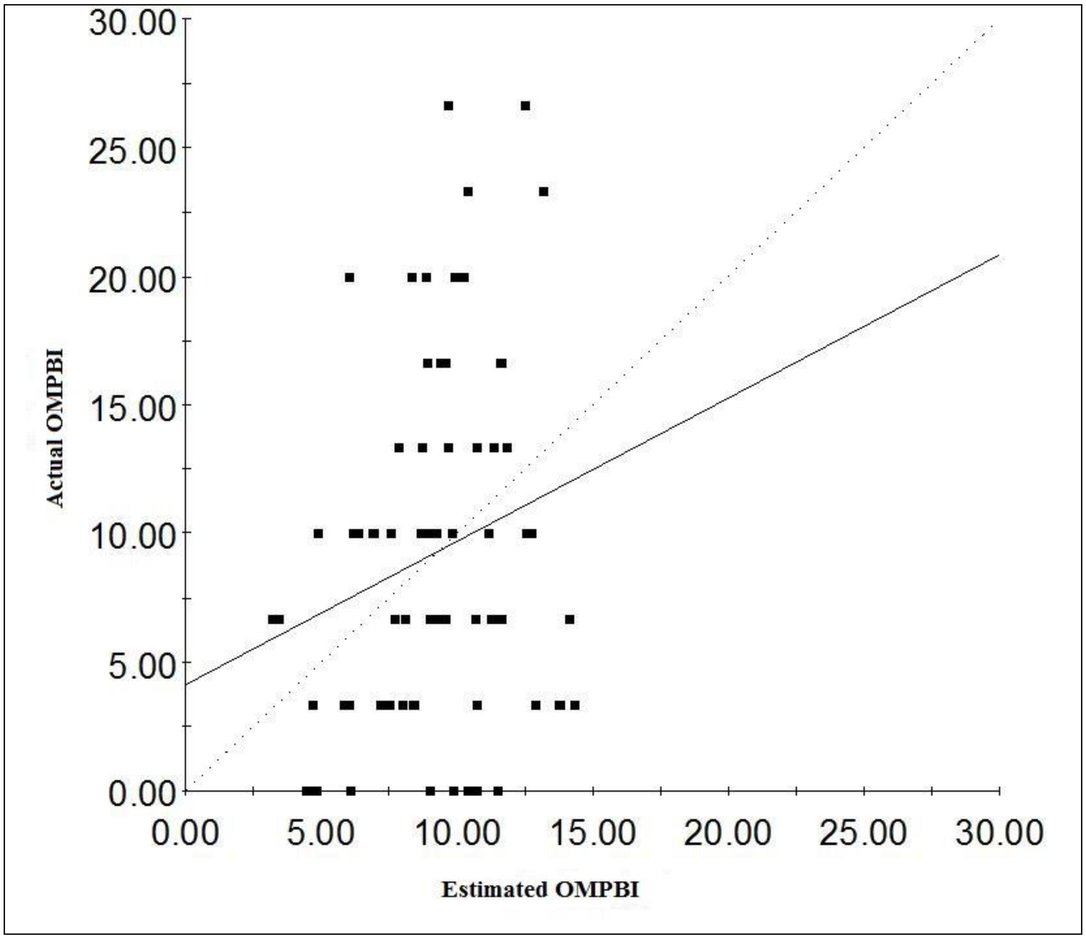

Fig. 3.3. Representative cross-validation graph of estimated and actual overall mean percentage of boll injury of Field A (Pantego-NC, 2011). Each point in the graph indicates a spatial location for which an actual and estimated value is available. Fit statistics are shown in Table 3.2. The dotted line represents actual values and the solid line represents estimated values.

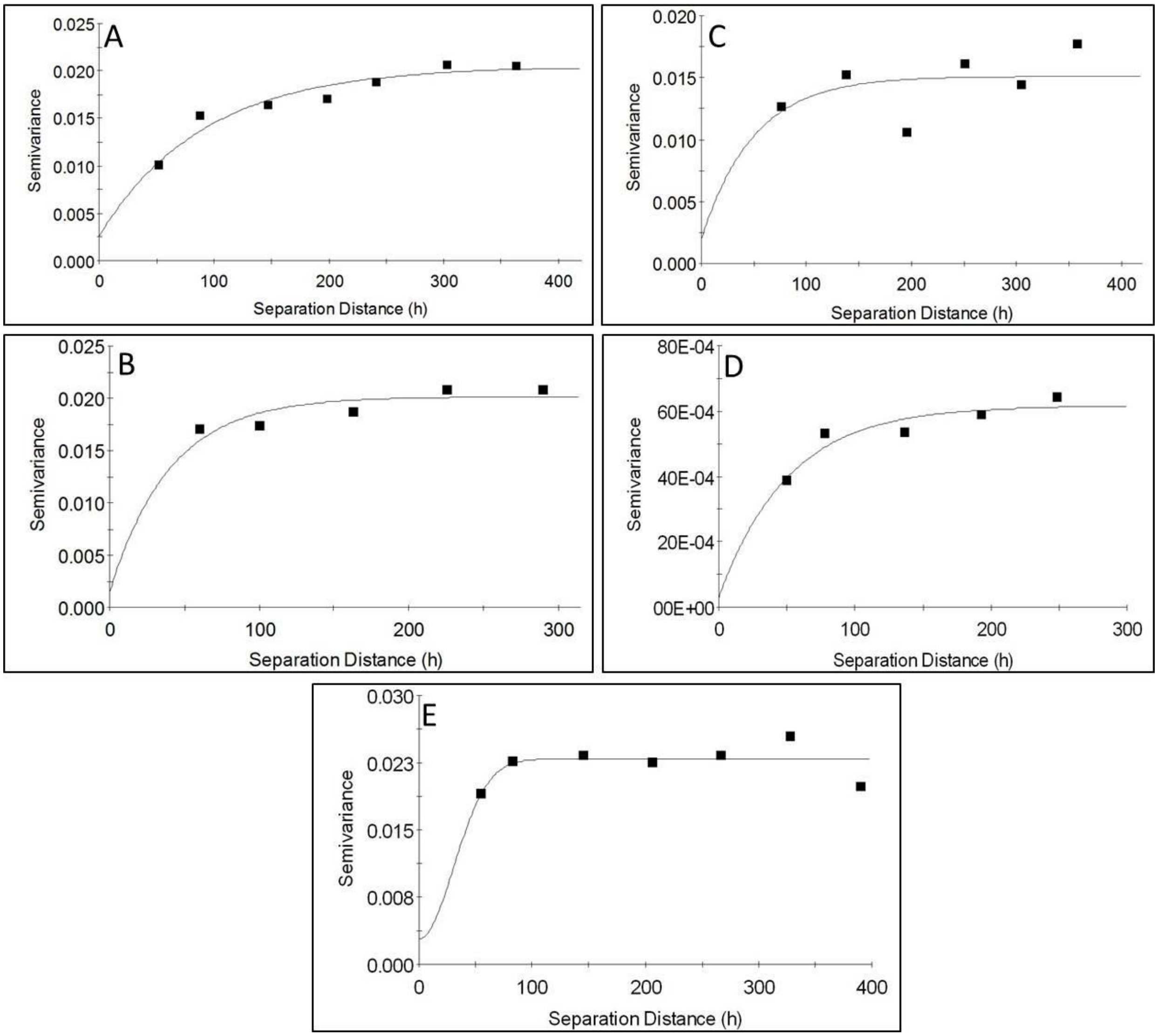

Fig. 3.4. Variogram analysis of arcsine transformed data of overall mean percentage of boll injury in 5 commercial cotton fields sampled for stink bug injury in Georgia and North Carolina in 2011 and 2012. A: Pantego; B: Midville; C: Plains; D: Nashville-1; C: Nashville-2. Each point in a variogram is an average of 30 or more pairs of data. Details on the best fitting models and fit statistics are shown in Table 3.3.

CHAPTER 4

EFFICACY OF ALTERNATING INSECTICIDE PASS APPLICATIONS FOR MANAGING PHYTOPHAGOUS STINK BUGS IN COMMERCIAL COTTON FIELDS

Pulakkatu-thodi, I., D. Reisig, J. K. Greene, F. P. F. Reay-Jones, and M.D. Toews. To be submitted to *Environmental Entomology*.

ABSTRACT: Phytophagous stink bugs damage cotton by preferentially feeding on developing seeds inside cotton bolls using piercing-sucking mouth parts. If the pest complex is not controlled properly, feeding injury from stink bugs can result in significant loss of yield and fiber quality. Stink bugs are currently managed in southeastern cotton fields by organophosphate or pyrethroid insecticides applied to entire individual fields. Previous research has shown that density of stink bugs and associated boll injury are variable and often clustered across the fields. The objective of this study was to compare the efficacy of insecticide treatment when applied in alternating passes with a sprayer in a skip-spray approach with the conventional approach of broadcasting insecticides over the whole field. The study was conducted in 12 commercial cotton fields in Georgia and North Carolina. Stink bugs and boll injury were sampled weekly throughout the cotton bloom cycle at evenly spaced and geo-referenced sampling points in each field. Results demonstrated that the skip-spray approach significantly reduced boll injury similar to that observed with conventional whole-field treatments. A single skip-spray application reduced boll injury below the Extension recommended threshold in 3 out of 6 fields studied compared with the conventional treatment which reduced boll injury below the threshold in 5 out of 6 fields. Interestingly, boll injury in unsprayed strips of the skip-sprayed fields was also reduced. Spatial analyses by Inverse Distance Weighted (IDW) interpolation and variogram analysis suggested that damage from stink bugs appeared in clusters, and both approaches of insecticide treatment disrupted these clusters.

KEY WORDS: skip application, stink bug, variogram, spatial statistics

Phytophagous stink bugs (Pentatomidae) have become important economic group of pests of cotton, *Gossypium hirsutum* (L.), in the southeastern US. These piercing-sucking pests preferentially feed on developing seeds inside cotton bolls, which detrimentally affects lint yield and quality. The southern green stink bug, *Nezara viridula* (L.), brown stink bug, *Euschistus servus* (Say), and green stink bug, *Chinavia hilaris* (Say),) formerly known as *Acrosternum hilare* (Say), are the most commonly encountered pentatomids in this production system (Greene et al. 1999, Reay-Jones et al. 2009). Both adults and immatures feed on developing cotton bolls and cause boll abscission or stained and inferior quality lint (Barbour et al. 1990, Willrich et al. 2004). Some species, such as southern green stink bug, transmit boll-rotting bacteria, such as *Pantoea agglomerans,* that cause rotting of locules (Medrano et al. 2007). Stink bugs have been recently ranked among the most damaging insect pests in southeastern cotton production (Williams 2008, 2009, 2011).

Approximately 0.5 out of 0.6 million hectares of cotton in Georgia were infested with stink bugs in 2011, and those infestations required insecticide treatment of approximately 0.4 million hectares; at an average of 2 applications per season (Williams 2011). The upsurge of stink bugs as an economic pest group is believed to be due to several factors, most notably due to eradication of the boll weevil, *Anthonomus grandis grandis* Boheman, and widespread adoption of transgenic cotton varieties for managing lepidopterans; both of which curtailed heavy use of broad-spectrum insecticides that are believed to have suppressed the stink bugs previously (Summy and King 1992, Greene and Herzog 1999, Greene et al. 2001). Stink bugs are reported to feed on hundreds of cultivated and non-cultivated hosts (Todd and Herzog 1980, McPherson and McPherson 2000, Reay-Jones et al. 2010). These polyphagous bugs are economic pests in row crops, such as corn, *Zea mays* L. (Negron and Riley 1987), soybean, *Glycine max* (L.)

(McPherson and McPherson 2000); fruit crops, such as peach, *Prunus persica* (L.), and apple, *Malus domestica* (Borkh) (Leskey and Hogmire 2005); and small grains, such as wheat, *Triticum aestivum* L. (Viator et al. 1983),) and grain sorghum, *Sorghum bicolor* (L.) (Hall and Teetes 1982),); and vegetables, such as tomato, *Lycopersicon esculentum* Mill. (Lye et al. 1988).

A high degree of variability in distribution of stink bugs and associated boll injury within cotton fields has been reported previously (Reay-Jones et al. 2010, Reeves et al. 2010). For example, field borders adjacent to other field crops, such as peanut, soybean, or corn, harbor greater numbers of stink bugs and suffer proportionally more associated boll injury (Tillman et al. 2009, Toews and Shurley 2009, Reeves et al. 2010). Similarly, yield and fiber quality of cotton grown adjacent to soybean and peanut are deleteriously affected by stink bug feeding as quantified by seedcotton yield, gin turnout, lint color, and lint value (Toews and Shurley 2009). Strong dispersal and polyphagous nature enable stink bugs to exploit diverse habitats of cultivated and non-cultivated hosts in a sequential manner (Jones and Sullivan 1982, Kennedy and Storer 2000). Cotton, a relatively late-maturing crop compared with corn and wheat (NASS-USDA 1997), is at greater risk of being infested by stink bugs later in the season.

Current management practices for stink bugs do not account for spatial variability of the pest complex and boll injury within cotton fields. Extension guidelines for treating fields for stink bugs stipulate that growers adopt a dynamic treatment threshold based on the number of bolls susceptible to feeding injury present during a given week of bloom (Bacheler et al. 2009, Greene et al. 2009). For example, during weeks 3-5 of bloom, when there are numerous susceptible bolls present, the treatment threshold is lowered to 10-15% injured bolls. Conversely, when there are fewer susceptible bolls present in weeks 1 and 2 and 6-8 of bloom, the treatment threshold is 20-50% injured bolls (Greene et al. 2009, Bacheler et al. 2009). Alternatively, insecticide application can be initiated when there is 1 stink bug per 6 row feet. Regardless of sampling method, growers apply organophosphate or pyrethroid insecticides to the entire field when the threshold is exceeded. Whole-field application of insecticides reduces populations of natural enemies, and repeated applications can lead to outbreaks of secondary pests, such as aphids, whiteflies, and spider mites (Gross and Rosenheim 2011).

One way to avoid eliminating natural enemies and reduce the potential for secondary pest outbreaks is to apply insecticides in alternating strips of treated and untreated crop, also referred to as a skip-spray strategy. Using this methodology, the applicator sprays the entire border pass of the field and then alternates between spraying and not spraying (for example, spray 20 rows and then leave the next 20 rows unsprayed, and so forth) across the remainder of the field. Alternating strips of treated and untreated crop might be sufficient to mitigate economically damaging levels of stink bugs and resulting boll injury, while substantially reducing active ingredient applied and application costs. Variations of this approach have been successful in other cropping systems. Insecticide treatments in alternating rows or pairs of rows were equally effective against leafhoppers and aphids in cotton compared with conventional sprays (Surulivelu and Kumaraswami 1989). This skip-spray approach also resulted in larger populations of green lacewings and coccinellid beetles compared with conventional treatments (Surulivelu and Kumaraswami 1989). Similar findings were reported in studies of site-specific treatment in wheat (Karimzadeh et al. 2011) and potato (Weisz et al. 1996), with documented reduction of overall insecticide input. Rangeland grasshoppers were managed effectively by using the Reduced Agent and Area Treatment (RAAT) program, which involved applying insecticides in intermittent swaths at a reduced rate. The program was economical and successful because of reduction in insecticide use up to 50% and improved conservation of beneficial non-target arthropods (Lockwood et al. 2000).

The objective of this study was to compare efficacy, assessed using boll injury, of a skip-spray strategy with the whole--field approach of applying insecticides for stink bugs in cotton. Additionally, spatial dynamics of stink bug activity were evaluated before and after the treatment using geostatistical tools to better understand potential constraints of this method.

Materials and Methods

Study Locations. The study was conducted in 12 commercial cotton fields each ranging from 13.8 to 26.4 ha (average of 16.8 ha) located in Georgia and North Carolina during 2011 and 2012 (Fig. 4.1). In Georgia, there were 3 fields in 2011 located at Tifton (Tift County, 31°31′49.9″N 83°33′39″W), Midville (Burke County, 32°52′12.5″N 82°13′24.5″W), and Plains (Summer County, 32°02′44.4″N 84°22′40.2″W), respectively. Five fields were selected in 2012, including 2 fields at Nashville (Berrien County, 31°17′02.4″N 83°21′04.7″W, 31°16′31.4″N 83°21′01.7″W), and one each at Plains (32°02′35.9″N 84°22′05.7″W) and Rebecca (Turner County, 31°46′26.8″N 83°29′38.8″W). In North Carolina, 2 fields near Pantego (Beaufort County 35°37′09.1″N 76°44′07.8″W) were selected in both 2011 and 2012. Six fields received a conventional whole-field application of insecticides, and the remaining 6 fields received the skip-spray application. Transgenic cultivars of cotton with resistance to lepidopterans (Bollgard II or Widestrike) were planted at all locations (Table 4.1). Overhead irrigation was available for all fields, and Extension-recommended agronomic practices were followed.

The field at Tifton shared its northern border with fields of peanut and watermelon. On other sides, it was bordered with woodlands and farm buildings. Corn and peanut bordered the Midville field on the north side, and a peanut field was located on the southern side. Other cotton fields and woodlands bordered on the western side, with fallow land on the eastern side. In Plains, peanut was grown on the eastern side of the field and cotton on the southern side. Woodlands and a road bordered the field on the northern and western sides. During 2012, 2 fields near Nashville (Nashville-1 and Nashville-2) had peanuts planted on the western sides. Cotton and woodlands shared the other borders. The third field at Nashville (Nashville-3) was surrounded by cotton, except on the northwestern border, which was bordered by woodlands. The field near Plains (Plains-2) shared its eastern and northeastern border with peanut, while the southern border had fallow land and pine trees; a farm road and pasture were located on the western side and farm buildings on the northwestern side. The field at Rebecca had a bordering peanut field on the northern side and woodlands and roads on other sides. The fields near Pantego shared borders with other cotton fields in both years, but soybeans were planted in nearby fields in 2012 (Fig. 4.2).

Field Layout. Prior to first flowering, fields were spatially mapped using a GPS receiver and mapping software (Arcmap10, ESRI 2010). Uniformly spaced sampling points were assigned using an approximately 60x60-m grid in a hexagonal/rectangular pattern, which provided a sampling density of approximately 1 sample per 0.4 ha (Fig. 4.1). In some cases, rows of sample points in skip-spray fields had to be adjusted (up to 16 rows) to ensure equal numbers of sampling flags in treated and untreated regions of each field. Sample points in each field were delineated using 2.4-m tall fiberglass marking flags labeled with unique numbers. To avoid sampling bias caused by field edges, the outermost sampling points were positioned 30-m from the field edge.

Sampling Procedures. Weekly sampling included both sweep-net and boll-injury procedures. Beginning the second week of bloom and continuing through the sixth week of bloom, technicians sampled for stink bugs by making 2 sets of 25 sweeps each in a single row using a 38.1-cm-diameter sweep net near each sampling point (flag). Internal boll injury was assessed by randomly removing 10 soft bolls (2.4 to 2.7-cm in diameter) near each flag and pooling them into 3.8-liter produce bags. Sampling commenced (with the onset of second week of bloom) on July 19 in Midville and on July 14 in Plains, Pantego-1, and Pantego-2. Selection of correct boll size was aided by the scouting tool developed by North Carolina State University/Clemson University/University of Georgia Extension, which consisted of a stiff plastic card with holes of size 2.4- and 2.7–cm diameter, (Bacheler et al. 2010.). Only those bolls which could pass through the large hole but not through the small hole were collected. Actual sample locations relative to the sample flags varied by quadrant in each week to avoid sampling the same plants in consecutive weeks. Bags of bolls were transported in a cooler to the laboratory where bolls were internally assessed for the presence of previously described stink bug injury (Barbour et al. 1990, Bundy et al. 2000, Willrich 2004, Toews and Shurley 2009). Individual bolls were classified on a binomial scale as 'damaged' or 'non-damaged', and the percentage of damaged bolls in each sample was recorded. Mean boll injury on a field--wide basis was used for initiating insecticide treatments based on Extension recommended thresholds.

Insecticides were applied using 1of the 2 application methods when field-wide mean boll injury exceeded the dynamic treatment threshold (Greene et al. 2009, Bacheler et al. 2009) that varied by week of bloom. During weeks 2 and 6 of bloom, the trigger was 20% boll injury, while the trigger was dropped to 10-15% during weeks 3-5 of bloom when there were more susceptible bolls present. Applicators applied a tank mix of the organophosphate dicrotophos (Bidrin 8®) and the pyrethroid bifenthrin (Discipline 2EC or Brigade 2EC) at maximum labeled rates (0.6 and 0.1 kg AI/ha respectively). In skip-sprayed fields, the width of strips varied between 18-22 rows based on the width of each grower's sprayer boom. Sampling for stink bugs and bolls resumed 1 week after insecticide application. If the treatment threshold was exceeded for a second time in the same field, all border passes were retreated, and strips not receiving insecticide during the earlier treatment were sprayed, leaving the strips treated previously untreated.

Data Analyses. Both parametric and spatial statistics were used to characterize differences among treatments. For purposes of experimental design and decision support, individual fields in a given week were the experimental unit. The decision to make insecticide applications was made by comparing the mean percentage of boll injury (MPBI), calculated by averaging data from all sampling points, with the Extension-recommended treatment threshold. Formal statistical comparisons between treatments utilized ANOVA on boll-injury data that were subjected to arcsine transformation prior to analyses (Zar 1999). Because 5 of the 12 fields required more than one insecticide application, there were a total of 8 replications receiving the skip-spray application and 9 replications receiving whole-field applications. For skip-sprayed fields, comparisons were made between sprayed and unsprayed swaths by considering each sampled swath as an independent experimental unit randomized by location. PROC GLIMMIX in SAS 9.3 (SAS Institute 2011) with LSMEANS statement was used for mean separation.

Captures of stink bugs were expressed as sums for each field and not subjected to statistical analysis due to low capture rates.

For exploratory spatial analysis and estimating variability of stink bug damage before and after the treatments, sample means from each sampling point in each field were interpolated using the Inverse Distance Weighted (IDW) method in ArcMap (ESRI 2011). Inverse Distance Weighted interpolation is a deterministic spatial analytical tool that uses a mathematical function to predict a variable at an unsampled location using sampled variables at known locations using weighted averages based on the distance between sample points. Changes in spatial variability of stink bug damage before and after the treatment were modeled using variogram model fitting in GS+ software (Bohling 2011). Variogram modeling has been used previously to describe spatial structure of western tarnished plant bug, *Lygus hesperus* (Knight), in cotton (Carriere 2006); sunflower midge, *Contarinia shulzi* (Gagne') in sunflower (Hodgson 2004); coffee berry borer, *Hypothenemus hampei* (Ferrari), and leafminer, *Leucoptera coffeella* (Guérin-Méneville), in coffee (Alves et al. 2011); and greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), in cherry tomatoes (Jung-Joon et al. 2011).

The variogram measures the extent of spatial dependence in sample data by evaluating the variance as a function of distance and direction between pairs of observations (Cressie 1991). Variogram model fitting involves fitting a theoretical model to the empirical or experimental variogram using a non-linear weighted least square regression; model evaluations are conducted by comparing residual sum of squares (Cressie and Hawkins 1980). The semivariance γ for lag distance h is given by

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [z(x_i) - z(x_i + h)]^2$$

where $z(x_i)$ is the sampled variable at point x_i x_i, $z(x_i + h)$ is the sampled variable at point, $x_i + h$ and $N(h)$ is the number of pairs separated by lag h. The variogram parameters included the sill, nugget, and range; these parameters give valuable information about the spatial structure of the variable under study (Issaks and Srivastava 1989). The variance typically increases with increasing lag distances before reaching an asymptote. The distance at which the variogram levels off is called the range (A), and the semivariance value where it levels off is the sill (C). The nugget is the height of the jump of the semiovariogram at the discontinuity at the origin. While the sill indicates presence of spatial autocorrelation, the range indicates the distance where sampled variable is spatially correlated.

To calculate a variogram, the raw fractional data from each sampling point were arcsine transformed to get a nearly normal distribution (Zar 1999). The data were tested for any obvious surface trend by visual analysis of quantile map in GS+ (Bohling 2011). In this study, the number of sample points varied between 31 and 68, and constructing a reliable variogram was difficult for fields having less than 38 sampling points. Hence, we modeled the variability of boll injury before and after application of insecticide in 6 fields, with 3 from each treatment. Variograms constructed were omnidirectional (isotropic) because of the absence of any obvious surface trends. The requirement for 30 data pairs per lag class was satisfied by constructing an omnidirectional variogram (Issaks and Srivastava 1989). The software GS+ provides variogram model fitting based on 4 functions: linear, spherical, exponential, and gaussian, and only the best fitted models, based on residual sum of squares, are discussed here.

Results

Insect Density: In 2011 at Midville, only 12 stink bugs (adults and nymphs) were collected in sweep-net samples during the entire year. Of these, 83.3% were green stink bugs, and the remainder were brown stink bugs. At Plains, a total of 13 stink bugs were caught, with 61.5% being green stink bugs and the remainder being brown stink bugs. Only 2 stink bugs (1 green and 1 brown) were captured at Tifton, a field that required 2 whole-field sprays based on boll-injury thresholds. Regardless of location or treatment, no stink bugs were captured the week immediately following the insecticide application. A total of 36 stink bugs were captured from the 2 fields in Pantego, with brown stink bugs (80.5%) comprising the majority; the remainder were all green stink bugs. No southern green stink bugs were captured from any field in 2011. In 2012, fewer stink bugs were captured from fields in Georgia. At Rebecca, 3 green stink bugs and 6 southern green stink bugs were captured, while only 4 stink bugs (2 brown stink bugs and 2 southern green stink bugs) were captured from 3 fields in Nashville. At Pantego, 20 brown stink bugs were captured during the first week of bloom, but none were captured in subsequent weeks.

Boll Injury. Weekly scouting showed that boll injury gradually increased in 8 out of 12 fields (Table 4.2). In 2011, the treatment threshold was exceeded by the fifth week of bloom at Midville and Plains and by the fourth week of bloom in Pantego-1 and Pantego-2. At Tifton, boll injury gradually increased until the fourth week of bloom when a whole-field application was made too early at 7.03 ± 1.34% boll damage; ironically, a follow up application was required 2 weeks later. In 2012, fields at Rebecca and Nashville-1 reached the treatment threshold during the fifth week of bloom, while the field at Plains exceeded the threshold during the fourth week of bloom. Nashville-2, Nashville-3, Pantego-3, and Pantego-4 had high initial infestations and, accordingly, exceeded the treatment threshold during the second or third week of bloom (Table 4.2).

Levels of boll injury in the fields designated for the treatments (skip-spray and whole-field application) before the insecticide application were statistically compared and found to be statistically similar (F = 0.76; df = 1, 15; P = 0.39). Levels of damage were statistically similar after treatment as well (F = 2.66; df = 1, 15; P = 0.12). Statistical comparisons between the week before and after insecticide treatment showed that both systems for delivering insecticides (skip-spray and whole-field application) significantly reduced damage from stink bugs (skip spray: F = 4.51; df = 1, 14; P= 0.05; whole-field spray: F = 11.63; df = 1, 16; P<0.01) (Table 4.3). ‘Percentage reduction’ in mean boll injury did not differ significantly based on treatment (F = 0.45; df = 1, 15; P= 0.51). The range of drop in percentage boll injury was approximately 0.4 to 16.4%, with a mean value of 5.7% for the skip application and 1.8 to 15.8%, with a mean value of 7.4% for conventional application.

Within skip-spray fields, the strips to be sprayed and skipped had statistically similar boll injury before the treatment (F = 2.14; df = 1, 43; P=0.15). The strips that received insecticide showed a marked decrease from the week before to the week after treatment (F = 19.24; df = 1, 43; P<0.01). The untreated strips also had significantly lower damage compared with the level of damage before the treatment (F = 4.26; df = 1, 43; P=0.04), indicating that skip spraying influenced stink bug activity in untreated regions. Levels of boll injury after application of insecticide were comparable in both treated and untreated strips (F = 0.56; df = 1, 43; P=0.45).

Spatial Analyses: Spatially interpolated IDW maps of MPBI (ArcMap-ESRI 2011) demonstrated variability in stink bug dispersal and effectiveness of skip-spray applications in reducing pressure from stink bugs in 2011 (Fig. 4.3a). For example, comparison of IDW maps before and after the treatment in Midville indicated reduction in areas having high incidence of damage. Similarly, in Plains-1, stink bug immigration from the east side of the field before the treatment appears to be diminished after the skip application. Pantego-1 (skip) and Pantego-2 (conventional) fields were surrounded by cotton fields from all sides. Reductions in boll injury were more evident in the field receiving the whole-field application compared with the skip-sprayed field. In the Tifton, there was considerable reduction in interpolated areas having high injury after the whole-field treatment.

Similar results were observed in 2012 (Fig. 4.3b). For example, an overall reduction in interpolated areas exhibiting high boll injury was observed in the Rebecca field after the treatment. At Plains-2 and Pantego-3, boll damage was still above the threshold a week after the skip application; therefore, a second skip application was required to reduce levels of boll injury. The higher concentration of damage in eastern and western areas of the field was effectively reduced by the treatment in Plains-2. In Pantego-3 (skip) and Pantego-4 (conventional), the variability in pest pressure and changes in distribution of damage after the treatment are visible; however, an influence of any adjacent crops was not identifiable in these fields.

Geostatistical Analyses: Isotropic variogram analysis demonstrated that boll injury exhibited spatial autocorrelation before the insecticide treatment (Fig. 4.4a and b) in 6 of the 12 fields tested. All the fields tested had non-zero sill (C) before the treatment, indicating the presence of spatial dependence. Based on least residual sum of squares, an Exponential model was selected as the best fitting model to describe spatial structure of stink bug damage before treatment in 2 fields, the Spherical model in 3 fields, and a Gaussian model for 1 field (Table

4.3). Variogram analysis was performed for an active lag distance (search radius) of 50% of the broadest extent of the field as a standard procedure in all fields, and the lag interval was based on the average sampling distance between sampling points. This approach gave sufficient lag classes for calculating an empirical variogram in each data set. The lag distance h, was adjusted to include a minimum of 30 pairs of sample points in each lag class (Table 4.3). Thus, each point in an empirical variogram is the average of 30 or more data pairs. The range parameter of fields before insecticide treatment varied from 84 to 198 m. After the treatment, 2 variogram models from skip--treated fields showed a pure nugget effect with zero sill suggesting complete absence of spatial dependence (Plains-2 and Rebecca). 'Proportion' (C/Co+C), measures the degree of spatial autocorrelation in the data and value close to 1indicates substantial presence of spatial dependence (Table 4.4). Variogram models from the conventionally treated fields suggested that spatial dependence was present even after the treatment, as indicated by non-zero sill and high proportion values. After the treatment, an Exponential model was selected as the best fitting model for the Tifton field and a Spherical model for Nashville-1 and Nashville-2 fields.

Discussion

Weekly monitoring of stink bug damage demonstrated that a degree of predictability in stink bug pressure existed in fields where initial infestations were low. In general, 3 to 4 weeks after bloom were required in most of the fields for boll injury to reach the treatment threshold. The extent and pattern of increase in infestation was similar. While it was beyond the scope of this study to quantify the influence of local farmscape factors, such as proximity of a preferred host (e.g., peanut or corn), IDW maps suggested that such factors influenced within-field spatial variability of stink bug damage in some fields. For example, increased boll injury nearest an adjacent peanut field was evident at Plains-1 and (2011), Midville during 2011 and at Rebecca (2012), and Plains-2 during 2012. The sampling grid was designed to avoid bias arising from sampling at the edges of the field where stink bug pressure is generally high (Tillman et al. 2009, Reay-Jones et al. 2010, Reeves et al. 2010), with the outermost sampling locations positioned well within the field (up to30 m from edge). By the fourth or fifth week of bloom, boll injury appeared to be distributed across fields in varying levels, and source and direction of infestation was not identifiable in any of the fields. Moreover, similar patterns of infestation were observable in fields surrounded by cotton (Pantego), suggesting high mobility of stink bugs at the farmscape level. Gradual increases in infestation levels suggested that cultural practices, such as planting date, may assist growers with managing stink bugs.

Results from a statistical comparison of boll injury before and after insecticide treatment showed that skip-spray applications could be used to mitigate damage caused by stink bugs. The percentage reduction in stink bug damage in the first attempt was from 0.4 to 9.5%, with a mean reduction of 3.5% in skip-sprayed fields, and the range was 2.3 to 15.8%, with a mean reduction of 5.9% in conventional fields. Results from this study indicated that most fields exceeded the treatment threshold in the fourth or fifth week of bloom, unless there was a high infestation during early anthesis. The skip-spray approach appeared to be adequate for fields that exceeded threshold during the fourth or fifth week of bloom. However, the skip-spray approach might not be protective enough for fields that exceed the treatment threshold during the second or third week of bloom.

After the first skip-spray application, an imminent hurricane prompted technicians to sample the Plains-2 field 3 days ahead of a scheduled date for evaluation. This early sampling probably resulted in a fairly large number of previously injured bolls being counted with the cohort that received the spray application. Additionally, at the Pantego field during 2012, presumably, an unusually high initial infestation of stink bugs caused high boll injury that prompted a second spray. These observations highlight 2 important points for stink bug scouting in cotton: first, a full week should elapse between a spray application and the subsequent scouting visit to ensure sampling of recent injury; second, unusually high boll injury during the second or third week of bloom should be addressed with a whole-field application of insecticide.

During this study on commercially operated fields, decisions for applications of insecticide did not always follow Extension-recommended thresholds. For example, the Tifton field was sprayed when the detected damage level was well below the Extension-recommended threshold (Table 4.2). In another case, insecticide application was delayed due to heavy rains (Nashville-1) after reaching threshold. The second application in another field (Nashville-2) was also unmerited based on the week of bloom and Extension-recommended thresholds.

The variogram analysis indicated that both strip-spray and whole-field treatments either disrupted spatial dependence or reduced the range of spatial dependence. Mean semivariance of sample pairs was more similar after treatments than before treatments (Fig. 4.4a and b), suggesting that both treatment approaches disrupted stink bug activity and reduced boll injury. Re-infestation from adjacent fields, local weather factors, and spatial scale of the field undoubtedly to influence the efficacy of pesticide application and spatial behavior of stink bugs in cotton fields. Future research should focus on understanding spatial dynamics of stink bugs with a higher resolution of sampling and improvising on partial insecticide applications.

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Table 4.1. Details of fields selected to compare efficacy conventional and skip application of insecticide treatments to manage stink bugs in Georgia and North Carolina in 2011 and 2012

Year	State	Field	Sampling points	Area (ha)	Cultivar	Field type	Date of first sampling
2011	Georgia	Midville	41	17.00	DP 1050 B2RF	Skip	7/19/2011
		Plains-1	31	12.23	PHY 375 WRF	Skip	7/14/2011
		Tifton	38	17.35	DP 1050 B2RF	Conventional	7/25/2011
	North Carolina	Pantego-1	36	14.00	DP 1028 B2RF	Skip	7/14/2011
		Pantego-2	36	14.00	DP 1028 B2RF	Conventional	7/14/2011
2012	Georgia	Plains-2	44	18.21	DP 1048/1050 B2RF	Skip	7/11/2012
		Rebecca	47	19.62	PHY 499 WRF	Skip	7/23/2012
		Nashville-1	56	21.12	PHY 499 WRF	Conventional	7/12/2012
		Nashville-2	68	26.40	DP 1050 B2RF	Conventional	7/12/2012
		Nashville-3	32	13.81	DP 1050 B2RF	Conventional	7/24/2012
	North Carolina	Pantego-3	36	14.00	DG 2570 B2RF	Skip	7/27/2012
		Pantego-4	36	14.00	DG 2570 B2RF	Conventional	7/27/2012

Table 4.2. Weekly mean percentage of boll injury (± SEM) induced by stink bugs, starting from second week of bloom in 12 commercial cotton fields observed over two years (2011 and 2012) Figures in bold indicate that it was immediately followed by an insecticide treatment. The figures in parenthesis indicate the Extension recommended threshold for that particular week

Field	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Skip spray						
Midville	3.46 ± 1.23	6.43 ± 1.51	6.09 ± 1.34	**12.68 ± 1.81** (10-15%)	10.65 ± 2.02	-
Plains-1	1.03 ± 0.57	2.85 ± 1.13	8.27 ± 1.57	**11.61 ± 1.74** (10-15%)	8.06 ± 1.56	6.04 ± 1.63
Pantego-1	5.83 ± 1.51	7.77 ± 1.54	**13.71 ± 2.49** (10-15%)	13.33 ± 2.58	-	-
Plains-2	2.12 ± 0.84	8.40 ± 1.71	**12.50 ± 2.15** (10-15%)	**11.81 ±1.87** (10-15%)	3.63 ± 0.86	-
Rebecca	4.34 ± 0.80	1.42 ± 0.60	5.25 ± 0.81	**13.40 ± 1.95** (10-15%)	8.29 ± 1.37	-
Pantego-3	-	**29.72 ± 3.07** (10-15%)	**20.27 ± 1.51** (10-15%)	3.88 ± 1.07	5.55 ± 1.08	7.22 ± 1.41
Conventional spray						
Tifton	1.72 ± 0.86	3.24 ± 1.09	**7.03 ± 1.34** (10-15%)	4.7 ± 1.63	**14.4 ± 2.41** (20%)	3.54 ± 1.14
Pantego-2	8.33 ± 1.35	5.27 ± 1.29	**15.00 ± 2.16** (10-15%)	9.16 ± 1.51	-	-
Nashville-1	7.01 ± 2.36	4.64 ± 0.95	5.63 ± 1.03	9.63 ± 1.21	**9.28 ± 1.27** (20%)	7.20 ± 1.20
Nashville-2	15.97 ± 1.56	**10.77 ± 1.30** (10-15%)	7.83 ± 1.06	11.97 ± 1.44	**11.98 ± 1.19** (20%)	5.88 ± 0.91
Nashville-3	8.38 ± 1.74	**9.35 ± 1.73** (10-15%)	2.81 ± 1.02	6.25 ± 1.53	3.43 ± 0.96	-
Pantego-4	-	**26.66 ± 2.76** (10-15%)	**10.83 ± 1.43** (10-15%)	2.50 ± 0.83	4.44 ± 1.08	6.66 ± 1.54

Table 4.3. Statistical comparison of mean percentage of boll injury (arcsine –transformed) induced by stink bugs in cotton. Back-transformed data in percentages are given for comparison. Means followed by same letters in a row are not significantly different. Means followed by same symbols in a column are not significantly different

Skip vs Conventional				
	Arcsine transformed data		**Percentage data (Back transformed)**	
	Before	**After**	**Before**	**After**
Skip	0.403 ± 0.03a*	0.312 ± 0.03b*	17.13 ± 0.09	10.05 ± 0.09
Conventional	0.369 ± 0.03a*	0.248 ± 0.03b*	14.24 ± 0.09	6.27 ± 0.09
Sprayed vs Unsprayed strip (within skip sprayed fields)				
Sprayed	0.414 ± 0.03a*	0.280 ± 0.03b*	18.14 ± 0.09	8.04 ± 0.09
Unsprayed	0.375 ± 0.03a*	0.308 ± 0.03b*	14.73 ± 0.09	9.79 ± 0.09

Table 4.4. Parameters of geostatistical analysis of mean percentage of boll injury (arcsine transformed) induced by stink bugs before and after the treatments in 6 fields. Midville, Plains-2 and Rebecca are skip treated fields and the rest are conventionally treated

Field	Lag, h (m)	Active Lag, (m)	Response	Model	Nugget variance (Co)	Sill (C)	Structural variance (Co+C)	Range, m (A)	RSS	Proportion (C/Co+C)
Midville	65	300	Before	Exponential	0.0042	0.0478	0.0520	84.0	8.0E-05	0.919
			After	Spherical	0.0001	0.0485	0.0486	60.30	6.9E-05	0.998
Plains-2	65	400	Before	Exponential	0.0064	0.0530	0.0594	97.5	3.6E-05	0.892
			After	Linear	0.0266	**0.000**	0.0266	353.4	6.9E-05	0.000
Rebecca	65	400	Before	Spherical	0.0043	0.0530	0.0573	96.0	1.1E-04	0.925
			After	Linear	0.0422	**0.000**	0.0422	355.3	2.0E-04	0.000
Tifton	65	350	Before	Spherical	0.0363	0.0434	0.0797	198.2	1.7E-04	0.545
			After	Exponential	0.0027	0.0199	0.0226	196.2	2.9E-05	0.882
Nashville-1	60	400	Before	Spherical	0.0008	0.0395	0.0403	90.4	8.1E-06	0.980
			After	Spherical	0.0011	0.0408	0.0419	85.1	1.6E-05	0.974
Nashville-2	60	400	Before	Gaussian	0.0001	0.0461	0.0462	87.4	1.0E-04	0.998
			After	Spherical	0.0046	0.0370	0.0416	42.0	1.2E-05	0.889

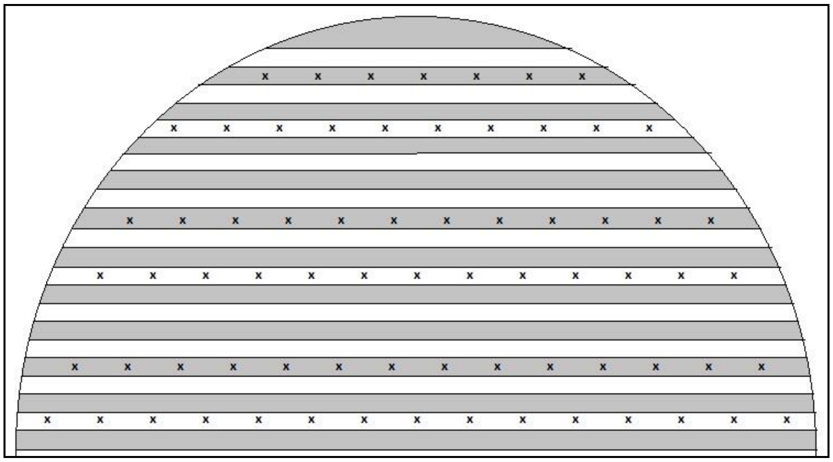

Fig. 4.1. Schematic for a 28 ha irrigated cotton field showing regions of field to be sprayed (shaded) and skipped. Sampling points (x's) with 1 sample point every 0.4 ha are arranged

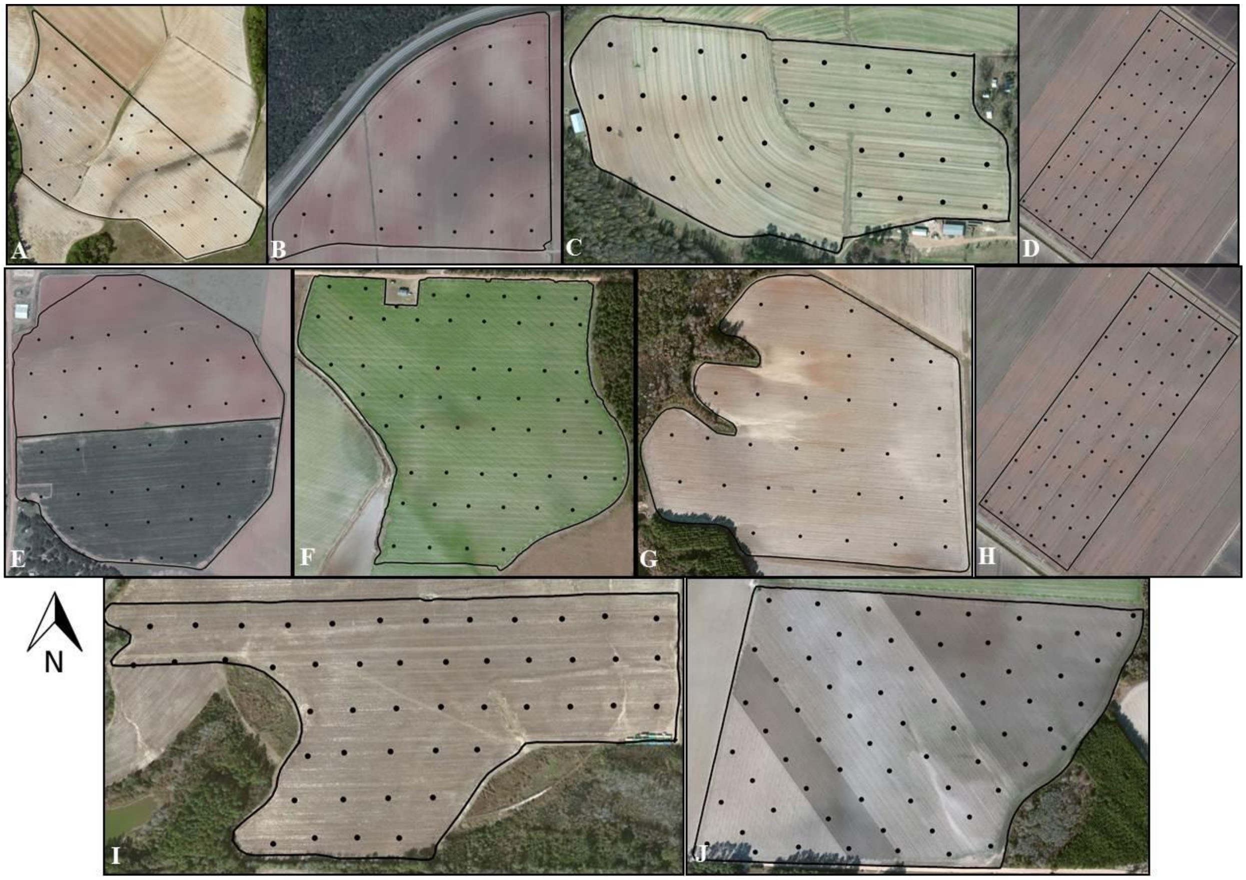

Fig. 4.2. Imagery of fields selected for sampling stink bugs and cotton bolls for assessing stink bug injury in 2011 (top row) and 2012 (middle and bottom rows) with field boundary and sampling points. A. Midville; B. Plains-1; C. Tifton; D. Pantego; E. Plains-2; F. Nashville-1; G. Nashvill-3, H. Pantego; I. Rebecca; and J. Nashville-2. D and H were further divided in to upper (conventional) and lower (skip) fields.

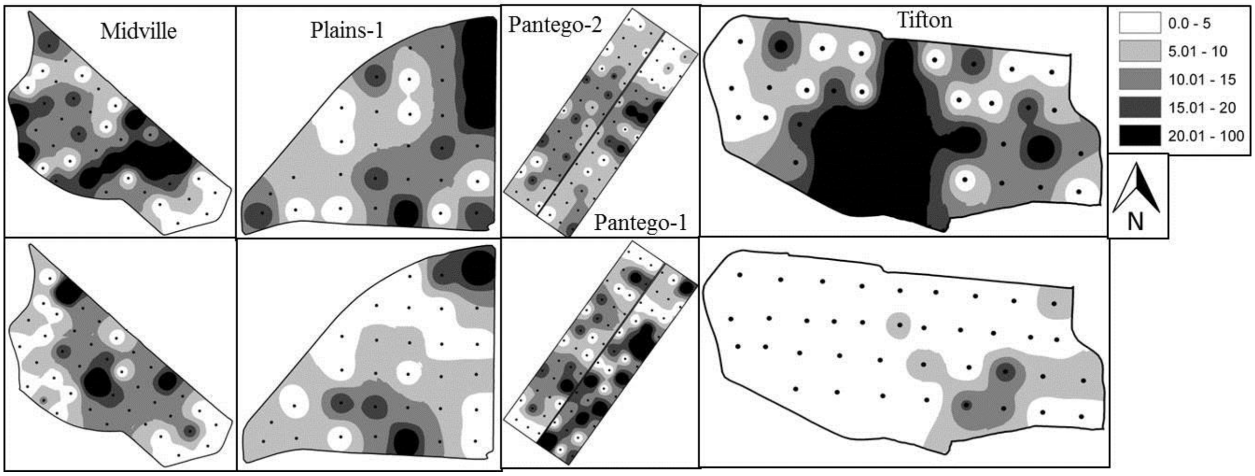

Fig. 4.3a. Inverse distance weighted interpolation maps of mean percentage of boll injury induced by stink bugs before (top row) and after (bottom row) the treatments in 2011. Midville, Plains and Pantego-1 were treated by skip-spray approach and Pantego-2 and Tifton were treated by whole-field application of insecticides. Areas having least damage are white and areas having most damage are dark. Maps are drawn to scale but not projected to the scale for the ease of layout.

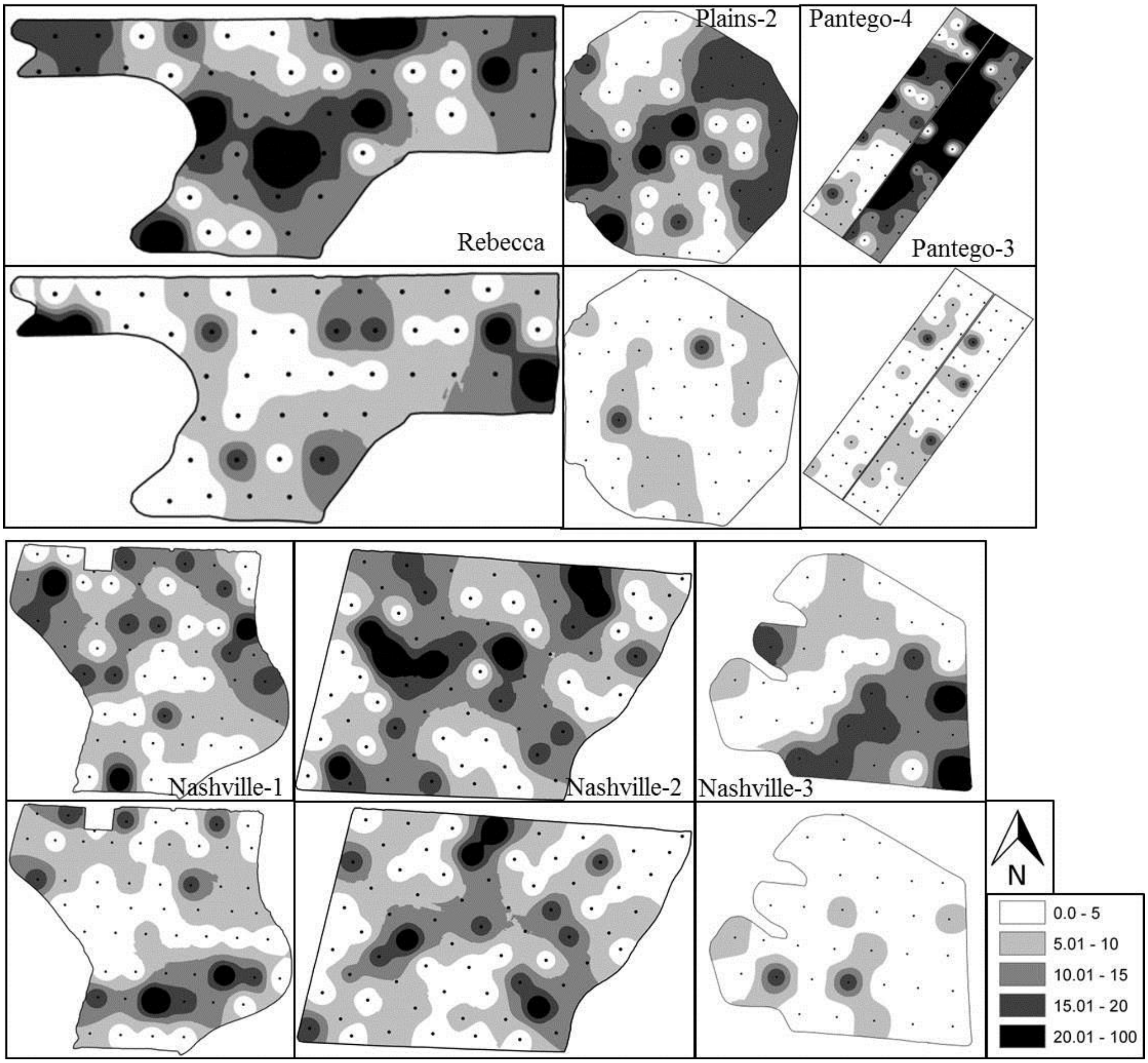

Fig. 4.3b. Inverse distance weighted interpolation maps of mean percentage of boll injury induced by stink bugs before (top row) and after (bottom row) the treatments in 2012. Rebecca, Plains-2 and Pantego-3 were treated by skip-spray approach and Pantego-4 and Nasville-1, 2, and 3 were treated by whole-field application of insecticides. Areas having least damage are white and areas having most damage are dark. Maps are drawn to scale but not projected to the scale for the ease of layout.

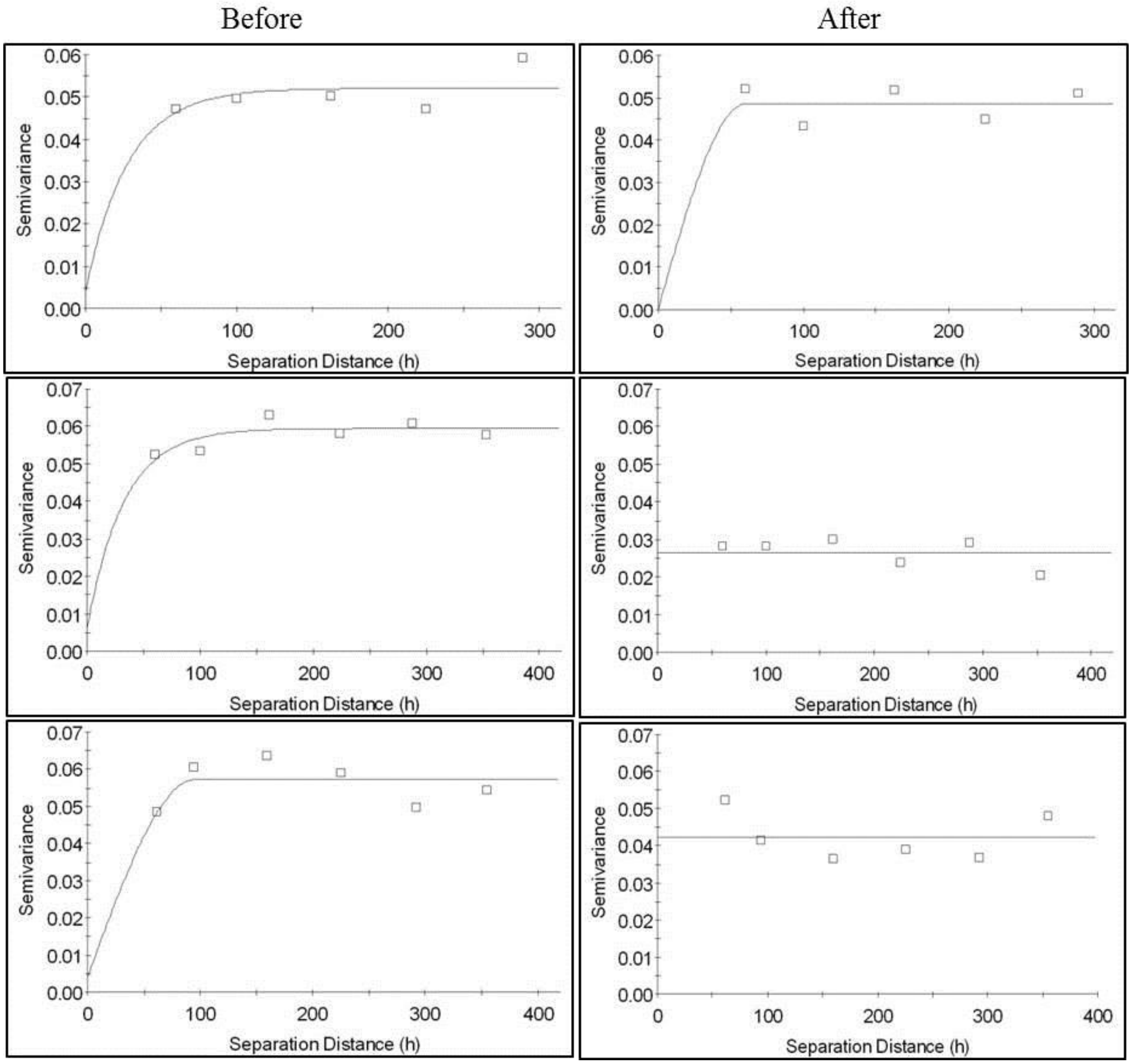

Fig. 4.4a. Variogram analysis of mean percentage of boll injury (arcsine transformed) induced by stink bugs, before and after the treatment in 3 skip-treated fields: first row- Midville; second row-Plains; third row- Rebecca. Variogram parameters are given in Table 4. 4.

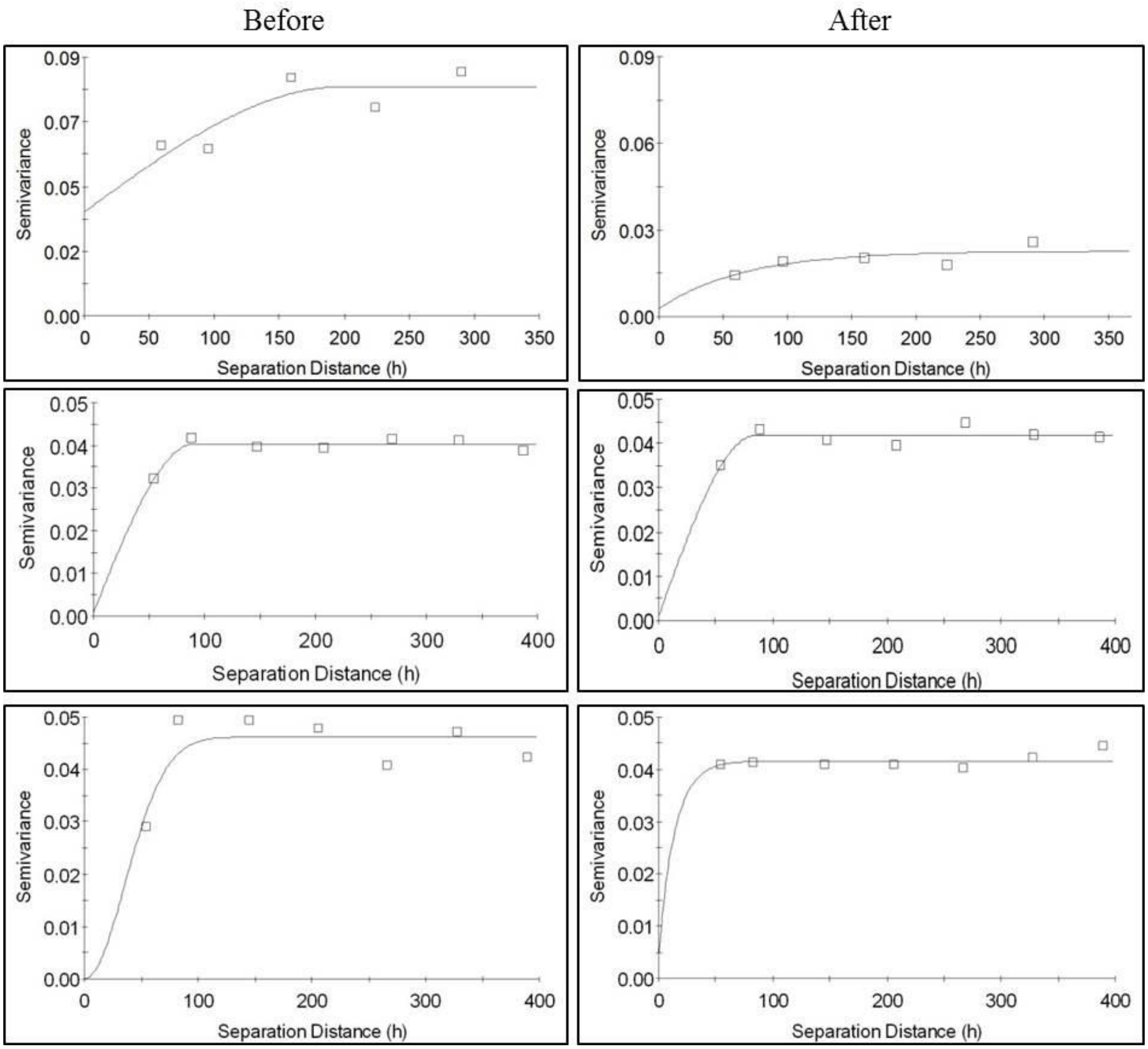

Fig. 4.4b. Variogram analysis of mean percentage of boll injury (arcsine transformed) induced by stink bugs, before and after the treatments in 3 conventionally treated fields: first row-Tifton; second row-Nashville-1; third row- Nashville-2. Variogram parameters are given in Table 4.4.

CHAPTER 5

CONCLUSIONS

The Food and Agriculture Organization (FAO) of the United Nations defines Integrated Pest Management (IPM) as “the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations while keeping pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment.” Several concepts of IPM, such as pest resistant cultivars, improved tillage operations, and crop rotations, have been successfully integrated into cotton pest management over the last several decades. Nearly universal adoption of Bt cotton technology and eradication of the boll weevil have reduced reliance on insecticides significantly. However, the observed increase in sucking pests, such as stink bugs and plant bugs, is an undesirable consequence of these shifts in production practices.

This research project demonstrated that planting early in May could possibly eliminate the need for insecticide treatment or greatly reduce the frequency of required applications for stink bugs. The author observed that the rate of increase in boll injury caused by stink bugs was significantly reduced in early-planted cotton. Similarly, early-planted cotton reached the Extension recommended threshold for boll injury less frequently than late-planted cotton. Conversely, cotton planted in June resulted in reduced yield, fiber quality, and economic returns when compared with cotton planted in May.

Spatial analyses of boll injury induced by stink bugs demonstrated that injury was spatially aggregated. Possible reasons for these aggregations include pronounced colonization of stink bugs along field edges, movement of stink bugs from adjacent habitats, presence of aggregation pheromones, and the innate nature of aggregation associated with multiple individuals hatching from egg masses. New knowledge about spatial aggregation could be integrated into decision making on insecticide application by only treating regions of the field where aggregations are present. Data also showed that neighboring sample points were often spatially associated, despite being located more than 50 m apart. These observations suggest that targeting stink bugs in a field should include treatment areas that are fairly large as opposed to only a few meters.

Comparisons of insecticide treatments applied to entire fields with those applied using the skip-spray approach demonstrated that both strategies significantly reduced injury by stink bugs. A single skip-spray treatment reduced stink bug injury below the Extension-recommended threshold in at least 50% of experimental fields. In skip-spray fields, the reduction in boll injury was observed in unsprayed strips as well. The skip-spray strategy was particularly effective when the field was in the fourth week of bloom or later. Conversely, fields that exceeded the Extension treatment threshold in the second or third week of bloom benefited from a whole-field application.

In conclusion, this research provided several practical ideas to augment current management practices and make pest management in cotton more efficient and cost effective. Extensive sampling of stink bugs and associated boll injury during this study revealed that there is a high degree of predictability in assessing stink bug injury in cotton fields. Regular (weekly) monitoring is a crucial step in early detection of pest incidence and mitigation of economically damaging boll injury.