5-1-2010

Optimization of an immunomarking technique for the study of tarnished plant bug movement between corn and cotton

Ankit Kumar

OPTIMIZATION OF AN IMMUNOMARKING TECHNIQUE FOR THE STUDY OF TARNISHED PLANT BUG MOVEMENT BETWEEN CORN AND COTTON

By

Ankit Kumar

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agricultural Life Sciences
in the Department of Entomology and Plant Pathology

Mississippi State, Mississippi

August 2010

OPTIMIZATION OF AN IMMUNOMARKING TECHNIQUE FOR THE STUDY OF TARNISHED PLANT BUG MOVEMENT BETWEEN CORN AND COTTON

By

Ankit Kumar

Approved:

Fred R. Musser Assistant Professor of Entomology Director of Thesis	John C. Schneider Professor/Research Entomologist Committee Member
Jeff Gore Assistant Professor of Entomology Committee Member	Clarence H. Collison Head of Entomology and Plant Pathology Department Graduate Coordinator
Melissa Mixon Interim Dean of College of Agriculture and Life Sciences	

Name: Ankit Kumar

Date of Degree: August 7, 2010

Institution: Mississippi State University

Major Field: Agricultural Life Sciences (Entomology)

Major Professor: Dr. Fred R. Musser

Title of Study: OPTIMIZATION OF AN IMMUNOMARKING TECHNIQUE FOR THE STUDY OF TARNISHED PLANT BUG MOVEMENT BETWEEN CORN AND COTTON

Pages in Study: 80

Candidate for Degree of Master for Science

A key economic pest of cotton in the mid-south is the tarnished plant bug (TPB), *Lygus lineolaris* (Hemiptera: Miridae). It is believed that early season crops like corn play a major role in building up TPB populations which then move to nearby cotton fields. The objective of this research was to determine the movement dynamics of TPB at the interface of these crops. Our 2009 data indicate TPB movement from corn into cotton occurred when corn was maturing from silk stage to milk. However, tasseling corn is more attractive compared to pre-squaring cotton to TPB.

A supporting study evaluated the retention time of protein markers under simulated midsouth summer conditions. We measured the impact of adding sorbitol to an egg protein solution on retention time of the protein on TPB acquisition. We found that sorbitol decreased acquisition time and raised the detection values of proteins in the absence of rain.

Key words: sorbitol, movement, *Lygus lineolaris*

DEDICATION

I would like to dedicate this research to Fred R. Musser who has been much more than just a major advisor to me.

ACKNOWLEDGEMENTS

First of all I acknowledge my Lord and Holy Spirit, Jesus Christ, who has always been with me when times seemed difficult. I thank my advisor, Dr. Fred Musser for his endless guidance, motivation, trust in me and who was always available to clarify my doubts. I thank my brother, Neelesh Sharma who lives back in India, for his encouragement to pursue graduate studies. A special thanks to Dr. John Schneider who was always there to clarify some of my notorious doubts. I also thank members of my graduate committee: Dr. Jeff Gore and Dr. Clarence H. Collison, for their input into the research and completion of this research.

Cotton Incorporated provided financial support for these studies. I also would like to thank all students, faculty, and staff members at Mississippi State University for making this a pleasant environment. I never felt away from home here at Mississippi. A special thanks to the people of Mississippi who accepted and welcomed me with their full heart.

Special thanks to Kathy Knighten for her technical support and help in this research. I thank Will Mackenzie, Jordan Clarke, Stephanie White, Justin Allard, Nicole Cox, Heather Pogue, and Teresa Knighten for helping me in this research study. I also thank Shweta Kailani, Sushant Khaire, Saurabh Dutta, Ritisha Hazarika and Ankush Saran for their patience when I frequently cancelled outdoor trips due to the nature of my research.

TABLE OF CONTENTS

DEDICATION ii

ACKNOWLEDGEMENTS iii

LIST OF TABLES vi

LIST OF FIGURES viii

CHAPTER

I. LITERATURE REVIEW 1

Genus *Lygus* 1
Tarnished Plant Bug, *Lygus lineolaris* (Palisot de Beauvois) 3
Tarnished Plant Bug of 8
Cotton 8
Corn 12
Marking Techniques 13
References Cited 16

II. IMPACT OF SORBITOL ON AN IMMUNOMARKING TECHNIQUE USING EGG ALBUMIN PROTEIN 25

Abstract 25
Introduction 26
Materials and Methods 28
Experiment 1 29
Experiment 2 31
Data Analysis 31
Results 33
Experiment 1 33
Experiment 2 35
Discussion 36
Acknowledgements 39
References Cited 40

III. INTER AND INTRA CROP MOVEMENT OF TARNISHED PLANT BUG ON CORN AND COTTON 52

Abstract 52
Introduction 53
Materials and Methods 54
Insect Sampling....55
Experimental Design for Mark-Release-Recapture (MRR) 55
Experimental Design for Mark Capture (MC)....57
Data Analysis....58
Results 61
Mark-Release-Recapture Experiment....61
Mark Capture Experiment....62
Natural Population Density....64
Discussion 65
Acknowledgements 68
References Cited....69

IV. SUMMARY 79

LIST OF TABLES

1.1 Percentage of known plant hosts of *Lygus* grouped by family (Goodell 1998) 23

1.2 Threshold for tarnished plant bug on cotton in Mississippi (Catchot 2010).....23

1.3 Annual production of cotton by country (1,000 metric tons) (FAS-USDA 2010) 24

1.4 Relationship between heat units and cotton growth stages (DD_{60}).Based on historical data from Tifton, GA (Ritchie et al. 2007). 24

2.1 Overall statistics of main effects and their interactions for Experiment 1.....42

2.2 Mean optical density reading (±SE, % marked) in the absence of simulated rain for egg albumin marker with or without sorbitol on tarnished plant bug exposed to cotton plants for the indicated number of hours and 3, 6, 9, and 12 days after application of the marker solution to the plants. Insects were considered positive for any reading > 0.0034. Each reading represents 32 insects. 43

2.3 Mean optical density reading (±SE, % marked) in the presence of simulated rain for egg albumin marker with or without sorbitol on tarnished plant bug exposed to cotton plants for the indicated number of hours and 3, 6, 9, and 12 days after application of the marker solution to the plants. Insects were considered positive for any reading > 0.0034. Each reading represents 32 insects. 44

2.4 Mean optical density reading (±SE, % marked) in the absence of simulated rain for egg albumin marker with or without sorbitol to the tarnished plant bug exposed to marked plants for 12 h and then to unmarked cotton plants for 3, 6, 9, and 12 days. Insects were considered positive for any reading > 0.0034. Each reading represents 32 insects. 45

3.1 Diffusion coefficient estimates, *D* (±SE m^2/d) (number of marked insects captured) for *L. lineolaris* adults in mark-release-recapture experiments at various phenological stages in corn and cotton in the Hills, Caledonia and Baldwyn, MS, 2008.....71

3.2 Diffusion coefficient estimates, *D* (±SE m^2/d) and number of marked insects recaptured per 3 days of sampling (N), for *L. lineolaris* adults in mark-release-recapture experiments at various phenological stages of corn and cotton in the Hills, Noxabee, MS, 2009.....72

3.3 Number of marked insects (±SE) captured per sampling days for *L. lineolaris* from mark-capture experiment in the Delta at Glendora, MS 2008; data pooled.. 73

3.4 Diffusion coefficient estimates, *D* (±SE m^2/d) and number of marked insects captured per sampling day (N), for *L. lineolaris* adults in mark-capture experiments at various phenological stages of corn and cotton in the Delta, Midnight, MS, 2009. 74

3.5 Mean (±SE) number of "wild" [unmarked (Hills) and marked + unmarked (Delta)] *L. lineolaris* collected per day in the Hills and Delta, MS in 2008. 75

3.6 Mean (±SE) number of "wild" [unmarked (Hills) and marked + unmarked (Delta)] *L. lineolaris* collected per day in the Hills and Delta, MS in 2009. 76

LIST OF FIGURES

2.1 A schematic arrangement of pots and rain gauges during simulated rainfall. The hose was held 1m away from the outer row and 2m above the base of the pots. The hose was moved slightly in a circular manner to obtain a uniform distribution of water. "R" represents the position of rain gauges. Rain gauge readings: 1R (1.0 cm), 4R (1.1 cm), 7R (1.0 cm), 11R (1.0 cm), 13R (1.0 cm), and 16R (0.9 cm).....46

2.2 Pots of cotton plants with netting to cage tarnished plant bugs in a greenhouse. 47

2.3 Mean optical density reading (±SD) for insects exposed three days after plants were marked. The line on Y axis = 0.0034 is the positive threshold for insects to be considered marked. 48

2.4 Mean optical density reading (±SD) for insects exposed six days after plants were marked. The line on Y axis = 0.0034 is the positive threshold for insects to be considered marked.....49

2.5 Mean optical density reading (±SD) for insects exposed nine days after plants were marked. The line on Y axis = 0.0034 is the positive threshold for insects to be considered marked. 50

2.6 Mean optical density reading (±SD) for insects exposed twelve days after plants were marked. The line on Y axis = 0.0034 is the positive threshold for insects to be considered marked. 51

3.1 A schematic diagram showing a insect release line and the 25-m sample units at various distances from the release line for each collection day. The same sampling distances were used for releases within corn, releases within cotton, and for the releases at corn/cotton interface.....77

3.2 A schematic diagram showing the marker sprayed rows and the 25 m sample units at various distances for each collection day from the middle of the sprayed rows in the same habitat. In the corn/cotton interiors, only 2 rows were sprayed with egg marker and similar to this design, insects were sampled 2.5 m, 5.5 m, 10.5 m, and 16 m away from the middle of sprayed rows in both directions. 78

CHAPTER I

LITERATURE REVIEW

Genus *Lygus*

The genus *Lygus* belongs in the family Miridae, the most species-rich family in the order Hemiptera. As many as 43 different species of *Lygus* have been reported from different areas of the world including North and South America, the United Kingdom, Europe, China, Africa, Russia, India, Japan, Australia, Fiji and New Zealand (Graham et al. 1984). About 34 of these species have been reported in the United States (Graham et al. 1984). Plant bugs in the family Miridae are important agricultural pests worldwide, especially *Lygus. Lygus pratensis* and *L. rugulipennis* are important pests in Europe (Easterbrook and Tooley 1999), while *L. hesperus* and *L. lineolaris* are important in the United States (Scott and Snodgrass 2000, Zink and Rosenheim 2008).

Lygus hesperus, western tarnished plant bug (WTPB), has about 100 host plant species within 24 plant families and is a serious pest of strawberries, cotton and alfalfa (Allen and Gaede 1963, Swezey et al. 2007, Sheller et al. 2008). *Lygus lineolaris* (Palisot de Beauvois), tarnished plant bug (TPB), a native species, is very well adapted for survival in a large number of habitats in North America, and attacks a wide variety of economically important herbaceous plants, including vegetables, agronomic crops, commercial flowering plants, fruit trees, and nursery stock (Dixon 1989). In fact, over

half of the cultivated plant species grown in the United States are listed as host plants for tarnished plant bug (Young 1986). It thrives in the southern states of Louisiana, Mississippi, Alabama, Georgia, Arkansas and some regions of Texas, and has about 385 reported host plant species in 55 families (Young 1986), including numerous important crops such as cotton (*Gossypium* spp.), alfalfa (*Medicago sativa*), apple (*Malus domestica*), strawberries (*Fragaria* spp.), cherry (*Prunus avium*), peach (*Prunus persica*), beans (*Phaseolus vulgaris*) and lettuce (*Lactuca sativa*) (Hagler and Blackmer 2007). Mississippi alone has over 169 host plant species in 36 plant families (Snodgrass et al. 1984). Major host plant families for both of these insect species are shown in Table 1.1 (Goodell 1998).

Tarnished plant bug is the most economically important plant bug in the eastern regions of the United States while western tarnished plant bug is the most important in the western regions. *Lygus* feeding results in economic damage on cotton grown throughout the United States (Williams 2009). Being a true bug, they feed on tender parts of plants and fruiting structures. They are also reported to be omnivorous feeders, eating other small insects (Pfannenstiel and Yeargan 2002, Rosenheim et al. 2004). Both tarnished plant bugs and western tarnished plant bugs are known to cause the abortion of developing flowering buds (squares) in cotton (Zink and Rosenheim 2005). However, *Lygus* also damage pre-squaring cotton and developing fruit (bolls) (Layton 2000, Hughes and Evans 2003). Tarnished plant bugs and western tarnished plant bugs feed on leaves and stems, but prefer to feed on fruiting structures of a wide variety of plants. On a world-wide basis, the described species of *Lygus* are mostly plant feeders which suck sap from young stems, leaves, fruits and flowers. Both tarnished plant bugs and western

tarnished plant bugs are highly mobile, active from early spring to early fall, and able to feed on a wide range of host plants. They are multivoltine, completing a generation in 20 to 24 days during warm weather, and they overwinter as adults in plant materials. These characteristics enable *Lygus* to build and maintain large populations, making them serious pests of crops, especially in the southern region of the United States.

Tarnished Plant Bug, *Lygus lineolaris* (Palisot de Beauvois)

Tarnished plant bugs overwinter as adults from late autumn or early winter until spring on wild hosts and in plant debris (Snodgrass 2003) . They move to broadleaf hosts like fleabanes (Genus: *Erigeron*), horseweed (Genus: *Conyza.*), common ragweed (Genus: *Ambrosia*), evening primrose (Genus: *Oenothera*), and chickweed (Genus: *Stellaria*) in the spring. Tarnished plant bug adults in the midsouth can be found on wild host plants during every month of the year (Snodgrass et al. 1984). The development threshold for tarnished plant bug is 10°C (Fleischer and Gaylor 1988) and 8°C to 12.2°C for western tarnished plant bug (Champlain and Butler 1967, Sevacherian et al. 1977, Pickel et al. 1990). Snodgrass (2003) reported that tarnished plant bug enters diapause in the second week of September in Mississippi, when day length is shorter than 13 hours. After this time, nymphs of tarnished plant bug will develop into diapausing adults. At the start of spring and with emergence of fresh vegetation, overwintered adult females start laying eggs. The time of year and environmental conditions determine the number of eggs an adult female lays (Bariola 1969, Snodgrass et al. 1984, Gerber 1995). It is believed that broadleaf plants are nutritionally more suitable compared to monocot plants (Snodgrass et al. 1984). However, cotton is less suitable for tarnished plant bugs than

other broadleaf hosts (Fleischer and Gaylor 1988). Bariola (1969) reported that the average time needed by tarnished plant bug to complete one generation on cotton at 27°C was nearly 33 days, with the approximate number of days required for each stage as follows: egg – 8 days; nymphs – 17 days; pre-ovipositional period – 8 days. Tarnished plant bug prefers squares and terminals compared to other cotton structures. Fleischer and Gaylor (1988) found that 32.1 percent of eggs were found in squares and 19.6 percent in terminals. The stable-stage distribution was unaffected by host and was about 0.50, 0.40, and 0.10 for eggs, nymphs, and adults, respectively, which suggests that tarnished plant bug tends to have an r-selected life strategy (Fleischer and Gaylor 1988). Gerber (1995) reported that under laboratory conditions, first generation female tarnished plant bugs reared on canola pods produced 303 ± 44 (N = 21; range= 6 - 704), 334 ± 33 (N = 18; range = 61 - 648), and 250 ± 27 eggs (N = 23; range = 26 - 497) when fed potato shoots in 1990, 1991, and 1992, respectively. Most eggs (> 87%) were laid during the first 5-7 days of the egg- laying period (Gerber 1995).

During the 1930's, calcium arsenate, paris green, and sulfur were used to control tarnished plant bugs and other cotton pests (Scott and Snodgrass 2000). In the 1940s, synthetic organic insecticides were used to control pests, including tarnished plant bugs (Scott and Snodgrass 2000). Even now most pest management practices for tarnished plant bugs still rely primarily on chemical control (Snodgrass 2003). Substantial work has been done on insecticidal efficacy and insecticide application methods for tarnished plant bug control (Scott and Snodgrass 2000). Almost all insecticide classes available are used against tarnished plant bugs (Scott and Snodgrass 2000). Major classes include organophosphates (OP), insect growth regulators (IGR), carbamates (C),

pyridinecarboxylic acids (PC), and neonicotinoids (CN). Within these classes there are multiple active ingredients registered for tarnished plant bug control. Currently recommended active ingredients in Mississippi for tarnished plant bug control in cotton are acephate (OP), malathion (OP), dicrotophos (OP), novaluron (IGR), oxamyl (C), flonicamid (PC), acetamiprid (CN), imidacloprid (CN), and thiamethoxam (CN) (Catchot 2010). One of the concerns of a chemically-dependent control strategy is the development of insecticide resistance. Recently, tarnished plant bugs were found to be resistant to pyrethroids in the Mississippi Delta region (Snodgrass and Scott 2000, Zhu and Snodgrass 2003). Alternate insecticides, such as imidacloprid, acephate and novaluron, have largely replaced the pyrethroids for tarnished plant bug control. Bt cotton and boll weevil eradication have reduced the frequency of insecticide applications in cotton. However, this benefit is threatened by increased insecticides being applied for tarnished plant bug (Williams and Hendrix 2008). To avoid this, non-chemical controls need to be incorporated into tarnished plant bug management. Several studies examined cultural and biological control methods on tarnished plant bug. In one study of a cultural method, early spring weeds were killed to keep the overwintering tarnished plant bug population low. It was effective for reducing the number of tarnished plant bugs in cotton; however, tarnished plant bug populations in cotton still exceeded thresholds, so while the number of insecticidal applications was reduced, they were still needed (Snodgrass et al. 2005, 2006). Killing spring hosts may also have non-target effects as it may reduce natural enemy densities. Other studies have evaluated trap crops in cotton (Sevacherian and Stern 1974) and strawberry (Easterbrook and Tooley 1999, Swezey et al. 2007) with some efficacies demonstrated. These studies showed effective use of

alfalfa as a trap crop to manage *Lygus* in cotton and strawberries. Efforts to establish biological control agents for tarnished plant bug have not been successful in cotton because of an absence of favorable non-crop habitats. One study indicated that the floral resources of some non-crop plants that serve as important reproductive hosts for *L. lineolaris* offer little or no benefit to *Anaphes iole* Girault, a hymenopteran egg parasitoid of *L. lineolaris* (Williams and Hendrix 2008). Other studies indicate that *Anaphes iole* releases, integrated with certain pesticide programs, can be used to control *L. hesperus* in strawberry field trials in California (Udayagiri et al. 2000). *Peristenus* spp. a nymphal parasitoid of *L. lineolaris* imported from Europe, has been successfully established in the United States and has been shown to suppress *L. lineolaris* very effectively in strawberry (Day et al. 1990, Tilmon and Hoffmann 2003). However, biocontrol efforts in Mississippi have not been able to reach the level of parasitization needed to effectively suppress tarnished plant bug densities in cotton (Snodgrass and Fayad 1991). Predators such as *Orius insidiosus, Geocoris punctipes* and *Chrysoperla* are commercially available, and are known to feed on tarnished plant bug eggs and nymphs. However, augmenting predator populations in cotton would be cost-prohibitive (Smith and Nordlund 2000). Fungal pathogens for tarnished plant bugs are another source of biological control agents that could provide biological control of tarnished plant bugs. *Beauveria bassiana* has been the most studied fungal pathogen and has been found to be the most successful fungal pathogen against tarnished plant bugs and western tarnished plant bugs. It has been used commercially in several crop systems and landscapes (Liu et al. 2003, Mazra'awi et al. 2006, McGuire et al. 2006, Sabbahi et al. 2007). However, using *B. bassiana* on a large scale in hot cotton fields may not be economically feasible. Host

plant resistance has also been explored for tarnished plant bug control. A genetically modified strawberry variety, *Fragaria virginiana,* resists tarnished plant bug (Dale et al. 2008). Kennedy et al. (1986) showed that super okra type cotton varieties produced the best response to exogenous chemical abscission of squares and the greatest chance of recovery from early season insect pressure. Cleft-shaped okra-leaf types offered advantages over normal-leaf isolines such as earlier maturity. Another experiment showed mixed responses where an okra leaf variety sometimes showed better yield, but at other times showed a lower yield compared to normal-leaf varieties (Heitholt and Meredith 1998). Although nectariless genes are not associated with higher yield in cotton, They are associated with reduced tarnished plant bug density (Meredith 1985, Scott et al. 1986, Thomson et al. 1987).

Tarnished plant bug flight patterns studied under laboratory conditions showed an average speed of 0.45 m/s (1.6 km/h) during sustained flights. Prereproductive stage bugs (<8 days old) flew much less than older individuals. Females made more long-duration flights than males (Stewart and Gaylor 1994). An interesting finding was that females with eggs flew more than females without eggs. This information supports studies showing that early-reproductive females were more likely to colonize new habitat patches than were males or prereproductive females (Stewart and Gaylor 1994, Blackmer et al. 2004). Expected colonization by reproductive females could alter the treatment threshold for tarnished plant bug. If one samples a field just before the mass immigration of tarnished plant bug to cotton, the pest density could change dramatically in a few days. Current thresholds for tarnished plant bugs are not adjusted for landscape considerations even though this may be an important factor in tarnished plant bug population dynamics.

The main period of insecticide application directed at tarnished plant bugs is from first week of squaring to late boll formation. The thresholds currently recommended during this period are listed in Table 1.2 (Catchot 2010).

Tarnished Plant Bug of

Cotton

Cotton (*Gossypium spp.*), constitutes a little over 35 percent of the total fiber used in the world (USDA-ERS 2009a). There are more than 40 different cotton species available around the world, but only four of them are actively cultivated and economically important. *G. hirsutum* L. and *G. barbadense* L. are the only two species grown in the United States. *G. hirsutum* L. is the main cotton of the United States and constitutes more than 97% of the cotton cultivated (USDA-ERS 2009b). Although China ranks first in terms of cotton production in the world, the United States is a significant contributor to the world's cotton supply. In 2009, cotton was grown on more than 3.11 million hectares in the United States with a total production of 2,700,000 MT which was about 12 percent of world cotton production (FAS-USDA 2010) (Table 1.3). The United States is the third largest cotton producer after China and India (Table 1.3), and due to low consumption in its domestic market, about 60 percent of the U.S. cotton is exported. The cotton industry has a significant impact on the overall economy of the United States. Cotton supports numerous business enterprises, such as seed production, agrochemicals, fertilizers, ginning, farm machinery, cattle feed and textiles. It is estimated that the revenue provided or initiated by cotton in the U.S. economy is about $120 billion (NCCA

2009). Cotton is grown in 17 U.S. states ranging from California to Virginia (NASS-USDA 2010). The largest production comes from Texas, Arkansas and Mississippi. In the five years since 2004/05, the United States has remained the world's largest cotton exporter. However, India continues to rapidly gain market share as the U.S. production has transitioned to more profitable alternatives (FAS-USDA 2010) . The United States planted cotton on 5,586,000 hectares in 2005/6 whereas in 2009/10, cotton was only planted on 3,112,000 hectares. This decrease in cotton acreage across the Unites States was largely due to several factors: stiff cotton price competition from India, higher commodity prices in the United States for corn and soybean, and within the Midsouth, continued development of insecticide resistance among hemipteran pests (Snodgrass and Scott 2000, Robinson 2009).

Agriculture continues to be a major economic force in Mississippi. At present, row crops together comprise the third largest commodity group in Mississippi behind poultry and forestry, with about $598 millions dollars of revenue produced each year (MSU-Cares 2010). The primary location for crop production is the highly fertile flood ("Delta") plain on the western side of the state near the Mississippi River. Among agronomic crops in Mississippi, soybean occupies the most acreage for most of the last four decades, whereas cotton remains the biggest cash crop for farmers. However, as recently as 2001, Mississippi farmers planted more acres of cotton than soybeans, and Mississippi produced more cotton than all states except Texas. But now as the cotton acreage is decreasing all over the United States, Mississippi is no exception. Mississippi farmers planted cotton on 480 thousand hectares in 2005, 267 thousand hectares in 2007, which was reduced to 123 thousand hectares in 2009 (FAS-USDA 2010).

Managing cotton requires an in-depth knowledge of its biology. Optimum air temperature for cotton growth is between 32° to 35°C. Cotton growth at temperatures below 15.5°C will be very slow, especially if soil moisture is low (Ritchie et al. 2007) . Cotton is usually considered drought tolerant because of its extensive root system (Wright and Sprenkel 2005). Unlike many other crops, the cotton plant continues vegetative growth after flowering begins. The development of fruiting structures ultimately reduces vegetative growth as the plant matures (Ritchie et al. 2007). Cotton in Mississippi is generally planted during May. It takes 4-14 days from planting to emergence depending on the temperature with an average time for emergence of 7 days. It takes approximately 38 days from planting to attain first square or flower bud. Planting to 1st bloom will take an average of 59 days. The first boll will open around 116 days from planting. Harvest follows 3-4 weeks after open boll. Hence it takes nearly 5 months from planting to harvest (Ritchie et al. 2007). Since cotton growth is greatly affected by temperature, cotton development can also be measured using heat units. Heat units, or DD_{60}, are an estimation of growth during a day, based on the average of the maximum and minimum daily temperatures in degrees Fahrenheit ($°F_{max}$ and $°F_{min}$, respectively). The number 60 is subtracted from this average, because 60ºF (15.5°C) is the lowest temperature at which cotton growth occurs (Ritchie et al. 2007). The formula for calculating heat units per day is: $DD_{60} = (°F_{max} + °F_{min}) /2 – 60$. Calculating the accumulated heat units of a crop over time can then be used to estimate the growth of the cotton during the season (Ritchie et al. 2007) (Table 1.4).

There are reports on *Lygus* in the cotton literature as early as the 1930's in Louisiana (Parencia 1978). However, this insect was not regarded as a potentially serious

pest of cotton until presentations were given at the 1953 Beltwide Cotton Production and Research Conferences (Snodgrass and Scott 2000). Subsequently, there have been numerous studies concerning this pest. Cotton is susceptible to tarnished plant bug damage in its early vegetative growth, during squaring, during blooming and during early boll formation. However, tarnished plant bug normally starts feeding on cotton from pinhead square to first bloom (Snodgrass 1998). Feeding on pinhead squares results in abortion of the squares. Feeding on larger squares may not result in abortion but leads to "dirty bloom", characterized by discoloration of anthers and anther damage. Feeding on small bolls may induce "cat-facing", distortion of the boll shape and reduction of the fiber content. Bolls more than 14 days old are typically not preferred feeding sites and are relatively immune to injury (Stewart 2004). With the constant and ever increasing modernization of farming systems, the broad acceptance of genetically modified plants, the application of more selective pesticides and pest eradication programs, pest management in cotton is different than a few years ago. Damage due to lepidopteran pests such as *Helicoverpa zea* and *Heliothis virescens* has been greatly reduced due to high adoption rates of cotton varieties expressing toxins derived from Bt (*Bacillus thuringiensis*) (Torres et al. 2006). Before the implementation of cotton boll weevil eradication programs across the cotton belt, the boll weevil (*Anthonomus grandis* Boheman) was a primary pest of cotton. Now boll weevil has been eradicated from Mississippi (Layton 2008). The adoption of Bt cotton and boll weevil eradication have reduced the number of insecticide applications, particularly during early growth stages, which has led to a pest shift. Plant bugs and stink bugs, which were secondary pests of cotton and were previously controlled incidentally by insecticide applications targeted at

boll weevil or heliothines, now have the opportunity to build large populations in the absence of insecticides (Layton et al. 1998). In addition to Bt cotton and boll weevil eradication, the development of tarnished plant bug populations resistant to pyrethroid insecticides has made tarnished plant bug one of the primary pests of cotton in Mississippi (Snodgrass and Scott 2000). In addition, some populations also have some low levels of resistance to a cyclodiene and several organophosphate insecticides (Hollingsworth et al. 1997, Snodgrass and Scott 2000). To develop an effective management strategy for this serious pest of cotton, it is necessary to know the biology and ecology of tarnished plant bug in relation to its hosts.

Corn

Corn is used in recently expanded ethanol production in the United States (24.7% of U.S production 2007-2008) as an alternative to fossil fuel (Dickey 2009). The United States produced 35 billion liters of ethanol in 2008, largely from corn. About 45% of total corn produced in the United States is used as a livestock food. Corn is also used for producing high fructose corn syrup (4%), with the remainder used for producing starch, cereal, sweeteners, alcohol, seed, and other products (Dickey 2009). Due to the ethanol-driven increase in demand for corn, corn has become an important crop in many states that traditionally have not grown much corn. In Mississippi 137 thousand hectares of corn were planted in 2006, yielding 6500 kg of corn per hectare for a total of 853 million kg. In contrast, 300 thousand hectares of corn were planted in Mississippi in 2008, yielding 9000 kg of corn per hectare, for a total of 2500 million kg corn (MSU-Cares 2010). During this same time period, cotton acreage decreased.

Corn is planted before cotton during March and April. It takes about 65 days to reach R1 (silking) stage when planted in optimum conditions (Ritchie et al. 1997). Depending upon the planting dates of corn and cotton, R1 corn and squaring cotton periods often overlap. Tarnished plant bugs can be observed in VT (tasseling) corn after the winter annual hosts are dying down (Abel and Snodgrass 2003). Tarnished plant bug is not known to cause any yield damage to corn, but some laboratory experiments have shown that tarnished plant bug can feed on corn silk and corn ears during blister and milk stages (Abel and Snodgrass 2003). In a field study, tarnished plant bug eggs were oviposited on the corn leaf sheath, leaf mid-rib, tassel, and ear silks (Abel 2004). While tarnished plant bug development in corn is possible, the role of corn in tarnished plant bug population dynamics is still not known. Although corn may be a source of tarnished plant bugs infesting cotton, the rate of movement between these crop fields is not understood or documented. A better understanding of movement of tarnished plant bug within and between these crops would improve our ability to predict the likely pressure of tarnished plant bugs at a particular point of time. This movement is likely to vary with the suitability of each crop for tarnished plant bug development.

Marking Techniques

There have been numerous studies concerning insect movement and distribution in space to understand the population dynamics of insects in crop habitats. Usually, a relatively small number of lab reared marked insects are released and recaptured. Based on movement by these insects, movement by the wild population is extrapolated. In general, trials marking insects are described as mark-release-recapture or mark-capture. In mark-release-recapture studies, the marks are applied to lab reared insects or field

collected insects, while the mark is applied directly in the wild in mark-capture studies. Each of these marking approaches has its advantages and disadvantages (Hagler and Jackson 2001). In both, some sort of trap is used to capture or recapture the insects, and the trapped insects are examined to determine if they are marked. Common marking methods include using fluorescent powders, trace elements, radioisotopes, genetic markers, insect mutilation, internal or external dyes and immunomarking techniques (Jones and Parrella 1986, Akey et al. 1991, Vincent and Chagnon 2000, Schellhorn et al. 2004, Carrière et al. 2006, Jones et al. 2006, Tillman 2006).

With the immunomarking technique, a protein is sprayed on insects or foliage in order to mark insects. Because of the low cost per insect labeled, this technique can allow the marking of more individuals than possible with the other techniques (Jones et al. 2006). The marked insects are identified using ELISA (Enzyme-Linked ImmunoSorbent Assay) targeted against the sprayed protein. The end product of the ELISA procedure, a change in color when the protein of interest is present, is detected using a spectrophotometer (Jones et al. 2006). Immunomarking techniques work better when the climatic conditions are moderate (Jones et al. 2006). However, in Mississippi, where summer temperatures are high, the protein marker may have a short life. To extend the duration of the protein mark in hot climates, a stabilizer can be added. Trehalose, sorbitol and some other polyols are known to raise the transition temperature (i.e, the temperature at which denaturation occurs) of proteins by 12 to 15ºC (Higashiyama 2002, Kaushik and Bhat 2003).

Chapter 2 describes the impact of sorbitol on probability of detection and longevity of egg albumin protein. Experiments were conducted under no rain and

simulated rain conditions. We also determined how long marked insects could walk over unmarked plants and still be clearly marked. We conducted these experiments with tarnished plant bug, *L. lineolaris* (Palisot de Beauvois) and cotton (*Gossypium hirsutum* L), but the principles are similar for many insect-plant systems. The primary goal of this research was to optimize an inexpensive protein marking technique (Jones et al. 2006) while working with crops in hot summer conditions with frequent rainfall.

In a field study described in chapter 3, the primary goal was to examine and understand tarnished plant bug movement in and between cotton and adjacent corn, and to monitor changes in movement as these crops matured. This goal was accomplished using the immunomarking method in mark-release-recapture and mark-recapture experiments.

References Cited

Abel, C. A. 2004. Corn and soybean crop influence. USDA, http://www.ars.usda.gov/Research/docs.htm?docid=7575 (viewed March 5, 2008).

Abel, C. A., and G. L. Snodgrass. 2003. The development of tarnished plant bug on various corn tissue. pp. 949-953. *In* Proceedings, Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Akey, D. H., J. L. Hayes, and S. J. Fieisher. 1991. Use of elemental markers in the study of arthropod movement and trophic interactions. Southwest. Entomol. 14(Suppl): 1-87.

Allen, W. W., and S. E. Gaede. 1963. The relationship of *Lygus* bugs and thrips to fruit deformity in strawberries. J. Econ. Entomol. 56: 823-825.

Bariola, L. A. 1969. The biology of the tarnished plant bug, *Lygus lineolaris* (Beauvois), and its nature of damage and control on cotton. PhD dissertation. Texas A&M University, College Station, TX.

Blackmer, J. L., S. E. Naranjo, and L. H. Williams. 2004. Tethered and untethered flight by *Lygus hesperus* and *Lygus lineolaris* (Heteroptera: Miridae). Environ. Entomol. 33: 1389-1400.

Carrière, Y., P. C. Ellsworth, P. Dutilleul, C. Ellers-Kirk, V. Barkley, and L. Antilla. 2006. A GIS-based approach for areawide pest management: the scales of *Lygus hesperus* movements to cotton from alfalfa, weeds, and cotton. Entomol. Exp. Appl. 118: 203-210.

Catchot, A. L. 2010. Insect control guide for agronomic crops. MSU Extension Serv. Pub 2471.

Champlain, R. A., and G. D. Butler. 1967. Temperature effects on development of the egg and nymphal stages of *Lygus hesperus* (Hemiptera: Miridae). Ann. Entomol. Soc. Am. 60: 519-521.

Dale, A., D. Galic, and R. H. Hallett. 2008. *Fragaria virginiana* resists tarnished plant bug. Entomol. Exp. Appl. 126: 203-210.

Day, W. H., R. C. Hedlund, L. B. Saunders, and D. Coutinot. 1990. Establishment of *Peristenus digoneutis* (Hymenoptera: Braconidae), a parasite of the tarnished plant bug (Hemiptera: Miridae) in the United States. Environ. Entomol. 19: 1528-1533.

Dickey, B. 2009. Uses of Corn. Nebraska Corn Board. http://www.nebraskacorn.org/usesofcorn/index.htm, (Viewed March 1, 2010)

Dixon, W. N. 1989. The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) in conifer nurseries (Heteroptera: Miridae). Fla. Dept. Agric. & Consumer Serv. Divison of Plant Industry, Entomol. Circu. No. 320.

Easterbrook, M. A., and J. A. Tooley. 1999. Assessment of trap plants to regulate numbers of the European tarnished plant bug, *Lygus rugulipennis,* on late-season strawberries. Entomol. Exp. Appl. 92: 119-125.

FAS-USDA. 2010. Cotton: World Markets and Trade Monthly Circular. http://www.fas.usda.gov/psdonline/psdreport.aspx?hidReportRetrievalName=BVS&hidReportRetrievalID=849&hidReportRetrievalTemplateID=8, (viewed January 23, 2010)

Fleischer, S. J., and M. J. Gaylor. 1988. *Lygus lineolaris* (Heteroptera: Miridae) population dynamics: nymphal development, life table, and leslie matrices on selected weeds and cotton. Environ. Entomol. 17: 246-253.

Gerber, G. H. 1995. Fecundity of *Lygus lineolaris* (Heteroptera: Miridae) Can. Entomol. 127: 263-264.

Goodell, P. B. 1998. Biology, ecology, and host plants of *Lygus lineolaris* and *Lygus hesperus*. pp. 949-951. *In* Proceedings, Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Graham, H. M., A. A. Negm, and L. R. Ertle. 1984. Worldwide literature of the *Lygus* complex (Hemiptera: Miridae) 1900-1980. U.S. Dept. Agric. Bibliogr. Lit. Agric. 30: 1-205.

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: Current technologies and future prospects. Annu. Rev. Entomol. 46: 511-543.

Hagler, J. R., and J. L. Blackmer. 2007. Potassium chloride deters *Lygus hesperus* feeding behavior. Entomol. Exp. Appl. 124: 337-345.

Heitholt, J. J., and J. W. R. Meredith. 1998. Yield, flowering, and leaf area index of okra-leaf and normal-leaf cotton isolines. Crop Sci. 38: 643-648.

Higashiyama, T. 2002. Novel functions and applications of trehalose. Pure Appl. Chem. 74: 1263-1269.

Hollingsworth, R. G., D. C. Steinkraus, and N. P. Tugwell. 1997. Responses of Arkansas populations of tarnished plant bugs (Heteroptera: Miridae) to insecticides, and tolerance differences between nymphs and adults. J. Econ. Entomol. 90: 21-26.

Hughes, J. M., and K. A. Evans. 2003. Lygid bug damage as a pod access mechanism for *Dasineura brassicae* (Dipt., Cecidomyiidae) oviposition. J. Appl. Ent. 127: 116-118.

Jones, V. P., and M. P. Parrella. 1986. The movement and dispersal of *Liriomyza trifolii* in a chrysanthemum green house. Ann. Appl. Biol. 109: 33-39.

Jones, V. P., J. R. Hagler, J. F. Brunner, C. C. Baker, and T. D. Wilburn. 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. Environ. Entomol. 35: 827-836.

Kaushik, J. K., and R. Bhat. 2003. Why is trehalose an exceptional protein stabilizer? J. Biol. Chem. 278: 26458-26465.

Kennedy, C. W., J. W. C. Smith, and J. E. Jones. 1986. Effect of early season square removal on three leaf types of cotton. Crop Sci. 26: 139-145.

Layton, M. B. 2000. Biology and damage of the tarnished plant bug, *Lygus lineolaris*, in cotton. Southwest. Entomol. 23: 7-20.

Layton, M. B. 2008. The boll weevil in Mississippi: gone, but not forgotten. MSU Extension Serv. Pub 2294.

Layton, M. B., S. D. Stewart, and M. R. Williams. 1998. Performance of Bt cotton in Mississippi, 1997. pp. 970-973. *In* Proceedings, Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Liu, H., M. Skinner, and B. L. Parker. 2003. Bioassay method for assessing the virulence of *Beauveria bassiana* against tarnished plant bug, *Lygus lineolaris* (Hem., Miridae) J. Appl. Entomol. 127: 299-304.

Mazra'awi, M. S. A., J. L. Shipp, A. B. Broadbent, and P. G. Kevan. 2006. Dissemination of *Beauveria bassiana* by honey bees (Hymenoptera: Apidae) for control of tarnished plant bug (Hemiptera: Miridae) on canola. Environ. Entomol. 35: 1569-1577.

McGuire, M. R., J. E. Leland, S. Dara, Y.-H. Park, and M. Ulloa. 2006. Effect of different isolates of *Beauveria bassiana* on field populations of *Lygus hesperus*. Biol. Control 38: 390-396.

Meredith, W. R. 1985. Performance of paired nectaried and nectariless F3 cotton hybrids. Crop Sci. 20: 757-760.

MSU-Cares. 2010. Crops: Cotton. http://msucares.com/crops/cotton/index.html, (viewed February 22, 2010)

NASS-USDA. 2010. Cotton: Acreage & Change from Previous Year by State. http://www.nass.usda.gov/Charts_and_Maps/Field_Crops/cotnacm.asp, (viewed April 19 ,2010)

NCCA. 2009. The world of cotton. http://www.cotton.org/econ/world/index.cfm, (viewed November 19, 2009)

Parencia, C. R. 1978. One hundred twenty years of research on cotton insects in the United States. USDA-ARS Agric. Handb. 515.

Pfannenstiel, R. S., and K. V. Yeargan. 2002. Identification and diel activity patterns of predators attacking *Helicoverpa zea* (Lepidoptera: Noctuidae) eggs in soybean and sweet corn. Environ. Entomol. 31: 231-241.

Pickel, C., N. C. Welch, and D. B. Walsh. 1990. Timing Lygus sprays using degree-days in Central Coast Strawberries. Santa Cruz County Agricultural Extension Publication. http://www.ipm.ucdavis.edu/PHENOLOGY/ma-lygus_bug.html, (viewed April 23, 2010)

Ritchie, G. L., C. W. Bednarz, P. H. Jost, and S. M. Brown. 2007. Cotton growth and development. http://pubs.caes.uga.edu/caespubs/pubs/PDF/B1252.pdf, (viewed March 5, 2008)

Ritchie, S. W., J. J. Hanway, and G. O. Benson. 1997. How a corn plant develops. Iowa State University of Science and Technology Cooperative Extension Service, Special Report 48.

Robinson, E. 2009. U.S. looks to win back some cotton acres. http://southwestfarmpress.com/cotton/cotton-acreage-0731/index.html, (viewed March 2, 2010)

Rosenheim, J. A., R. E. Goeriz, and E. F. Thacher. 2004. Omnivore or herbivore? Field observations of foraging by *Lygus hesperus* (Hemiptera: Miridae). Environ. Entomol. 33: 1362-1370.

Sabbahi, R., A. Merzouki, and C. Guertin. 2007. Efficacy of *Beauveria bassiana* against the strawberry pests, *Lygus lineolaris*, *Anthonomus signatus* and *Otiorhynchus ovatus*. J. Appl. Entomol. 132: 151-160.

Schellhorn, N. A., G. Siekmann, C. Paull, G. Furness, and G. Baker. 2004. The use of dyes to mark populations of beneficial insects in the field. Int. J. Pest Manag. 50: 153-159.

Scott, W. P., and G. L. Snodgrass. 2000. A review of chemical control of the tarnished plant bug in cotton. Southwest. Entomol. 23: 67-81.

Scott, W. P., J. W. Smith, and G. L. Snodgrass. 1986. Impact of early season use of selected insecticides on cotton arthropod populations and yield. J. Econ. Entomol. 79: 797-804.

Sevacherian, V., and V. M. Stern. 1974. Host plant preferences of *Lygus* bugs in alfalfa-interplanted cotton fields. Environ. Entomol. 3: 761-766.

Sevacherian, V., V. M. Stern, and A. J. Mueller. 1977. Heat accumulation for timing Lygus control measures in a safflower-cotton complex. J. Econ. Entomol. 70: 399-402.

Sheller, J. F., A. J. Rosenheim, and R. J. Hagler. 2008. Examining the dispersal capabilities of *Lygus hesperus* and its natural enemy *Geocoris pallens* in California's San Joaquin Valley. J. Insect Sci. 49: 1-27.

Smith, R. A., and D. A. Nordlund. 2000. Mass rearing technology for biological control agents of *Lygus spp.* Southwest. Entomol. 23: 121-127.

Snodgrass, G. L. 1998. Distribution of the tarnished plant bug (Heteroptera: Miridae) within cotton plants. Environ. Entomol. 27: 1089-1093.

Snodgrass, G. L. 2003. Role of reproductive diapause in the adaption of the tarnished plant bug (Heteroptera: Miridae) to its winter habitat in the Mississippi River Delta. Environ. Entomol. 32: 945-952.

Snodgrass, G. L., and Y. H. Fayad. 1991. Euphorine (Hymenoptera: Braconidae) parasitism of the tarnished plant bug (Heteroptera: Miridae) in areas of Washington County, Mississippi disturbed and undisturbed by agricultural production. J. Entomol. Sci. 26: 350-356.

Snodgrass, G. L., and W. P. Scott. 2000. Seasonal changes in pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations during a three-year period in the delta area of Arkansas, Louisiana, and Mississippi. J. Econ. Entomol. 93: 441-446.

Snodgrass, G. L., W. P. Scott, and J. W. Smith. 1984. Host plant and seasonal distribution of the tarnished plant bugs (Hemiptera: Miridae) in the delta of Arkansas, Louisiana, and Mississippi. Environ. Entomol. 13: 110-116.

Snodgrass, G. L., W. P. Scott, C. A. Abel, J. T. Robbins, J. Gore, and D. D. Hardee. 2005. Tarnished plant bug (Heteroptera: Miridae) populations near fields after early season herbicide treatment. Environ. Entomol. 34: 705-711.

Snodgrass, G. L., W. P. Scott, C. A. Abel, J. T. Robbins, J. Gore, and D. D. Hardee. 2006. Suppression of tarnished plant bugs (Heteroptera: Miridae) in cotton by control of early season wild host plants with herbicides. Environ. Entomol. 35: 1417-1422.

Stewart, S. D. 2004. Cotton insects, tarnished plant bug. University of Tennessee Agricultural Extension Service

http://www.utextension.utk.edu/publications/wfiles/W025.pdf, (viewed March 5 2008)

Stewart, S. D., and M. J. Gaylor. 1994. Effects of age, sex, and reproductive status on flight by the tarnished plant bug. Environ. Entomol. 23: 80-84.

Swezey, S. L., D. J. Nieto, and J. A. Bryer. 2007. Control of western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), in California organic strawberries using alfalfa trap crops and tractor-mounted vacuums. Environ. Entomol. 36: 1457-1467.

Thomson, N. J., P. E. Reid, and E. R. Williams. 1987. Effects of the okra leaf, nectariless, frego bract and glabrous conditions on yield and quality of cotton lines. Euphytica 36: 545-553.

Tillman, P. G. 2006. Sorghum as a trap crop for *Nezara viridul*a L. (Heteroptera: Pentatomidae) in cotton in the southern United States. Environ. Entomol. 35: 771-783.

Tilmon, K. J., and M. P. Hoffmann. 2003. Biological control of *Lygus lineolaris* by *Peristenus* spp. in strawberry. Biol. Control 26: 287-292.

Torres, J. B., J. R. Ruberson, and M. J. Adang. 2006. Expression of *Bacillus thuringiensis* Cry1Ac protein in cotton plants, acquisition by pests and predators: a tritrophic analysis. Agric. Forest Entomol. 8: 191-202.

Udayagiri, S., A. P. Norton, and S. C. Welter. 2000. Integrating pesticide effects with inundative biological control: interpretation of pesticide toxicity curves for *Anaphes iole* in strawberries. Entomol. Exp. Appl. 95: 87-95.

USDA-ERS. 2009b. Cotton briefing room. http://www.ers.usda.gov/Briefing/Cotton/, (viewed November 19, 2009)

USDA-ERS. 2009a. Cotton: Background. http://www.ers.usda.gov/Briefing/Cotton/background.htm, (viewed April 19, 2010)

Vincent, C., and R. Chagnon. 2000. Vacuuming tarnished plant bug on strawberry: a bench study of operational parameters versus insect behavior. Entomol. Exp. Appl. 97: 347-354.

Williams, L., and D. L. Hendrix. 2008. Comparing different floral resources on the longevity of a parasitic wasp. Agric. Forest Entomol. 10: 23-28.

Williams, M. R. 2009. Cotton insect losses. MSU Extension Serv. http://www.entomology.msstate.edu/resources/tips/cotton-losses/data/2009/2009loss.php, (viewed February 23, 2010)

Wright, D. L., and R. K. Sprenkel. 2005. Cotton growth and development. Publi. #SS-AGR-238, http://edis.ifas.ufl.edu/AG235, (viewed March 5, 2009)

Young, O. P. 1986. Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae). Ann. Entomol. Soc. Am. 79: 747-762.

Zhu, Y. C., and G. L. Snodgrass. 2003. Cytochrome P450 CYP6X1 cDNAs and mRNA expression levels in three strains of the tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae) having different susceptibilities to pyrethroid insecticide. Insect Mol. Biol. 12: 39-49.

Zink, A. G., and J. A. Rosenheim. 2005. Stage-dependent feeding behavior by western tarnished plant bugs influences flower bud abscission in cotton. Entomol. Exp. Appl. 117: 235-242.

.

Zink, A. G., and J. A. Rosenheim. 2008. Stage-specific predation on *Lygus hesperus* affects its population stage structure. Entomol. Exp. Appl. 126: 61-66.

Table 1.1 Percentage of known plant hosts of *Lygus* grouped by family (Goodell 1998)

Plant Family	*L. lineolaris*	*L. hesperus*
Asteraceae	26.17	23.42
Brassicaceae	7.70	6.31
Chenopodiaceae	3.31	6.31
Fabaceae	11.01	15.32
Gramineae	5.23	7.20
Plantaginaceae	1.10	7.21
Rosaceae	5.79	5.00
Others	39.69	29.00

Table 1.2 Threshold for tarnished plant bug on cotton in Mississippi (Catchot 2010)

Period	Economic Threshold			
	Sweep net	Drop cloth	Visual	Dirty squares
First 2 weeks of squaring	8/100 sweeps	1/1.83 row m	5/100 plants	NA
3rd week of squaring	15/100 sweeps	3/1.83 row m	10/100 plants	10%

NA – no threshold developed

Table 1.3 Annual production of cotton by country (1,000 metric tons) (FAS-USDA 2010).

	2005/06	**2006/07**	**2007/08**	**2008/09**	**2009/10**
China	6183	7729	8056	7991	6967
India	4148	4746	5225	4921	5117
United States	5201	4700	4182	2790	2700
Pakistan	2213	2155	1938	1960	2134
Brazil	1023	1524	1602	1193	1208
Uzbekistan	1208	1165	1165	1002	958
Australia	610	294	139	327	381
Other	4820	4238	3800	3213	2905
Total	25,407	26,552	26,107	23,395	22,370

Table 1.4 Relationship between heat units and cotton growth stages (DD_{60}).Based on historical data from Tifton, GA (Ritchie et al. 2007).

Growth stage	Heat Units (from planting)	Days (after planting)
Emergence	50	5
First Square	550	38
First Flower	950	59
Open Boll	2150	116
Harvest	2600	140

$DD_{60} = (°F_{max} + °F_{min}) /2 – 60$, all based on °F.

CHAPTER II

IMPACT OF SORBITOL ON AN IMMUNOMARKING TECHNIQUE USING EGG ALBUMIN PROTEIN

Abstract

Immunomarking methods are being widely used for determining insect movement patterns. This technique makes it possible to mark a large number of insects with inexpensive proteins such as chicken egg albumin. Enzyme–linked immunosorbent assay (ELISA) is used to detect marked insects. One of the critical issues when using external protein markers is their stability under various environment conditions. The acquisition and retention time of an external chicken egg albumin mark on tarnished plant bug (*Lygus lineolaris*) feeding on cotton under climate-controlled conditions was evaluated in this study. The temperature of the greenhouse was maintained in the range of typical summer conditions (21°C to 35°C) in the Midsouth United States. The effect of adding sorbitol, a polyol, to the egg albumin solution sprayed on plants was explored. Time to acquire the mark from foliage, the effect of rain on the ability of insects to acquire the protein from marked plants, and retention after marked insects walked and fed on unmarked plants were evaluated. The addition of sorbitol increased the rate at which the tarnished plant bug acquired the mark. Sorbitol also increased the intensity of the marks. Sorbitol had no significant effect on the probability of the protein detection

following rain. One cm of simulated rain was enough to wash the marker protein from cotton plants to the extent that no mark could be detected on insects exposed to them. Sorbitol increased the retention time of the marker when the marked insects were allowed to spend time on unmarked plants. In the absence of rain, egg albumin alone produced a mark that was detectable for about a week, and addition of sorbitol extended detection to nine days.

Key Words: *Lygus lineolaris*, sorbitol, egg albumin, mark

Introduction

Knowledge of insect pest dispersal can be important in the development of a program against the pest. One crop may act as a source of insects and another as a sink. A driving force for insect dispersal is the availability of food and oviposition sites. These tend to vary temporally and spatially due to plant development in the landscape mosaic. One method for measuring insect movement is marking, releasing, and recapturing them. Radioisotopes (Service 1993), fluorescent powders (Schroeder and Mitchell 1981, Jones and Parrella 1986, Schellhorn et al. 2004), internal or external dyes (Leeuwen 1940, Steiner et al. 1965, Hendricks and Graham 1970), mutilation (Murdoch 1963), rare elements (Akey et al. 1991), laser-marking (Griffiths et al. 2004), and immunomarking (Jones et al. 2006) techniques are among the many techniques that have been used to mark insects. Each of these marking methods has advantages and disadvantages (Southwood and Henderson 2000, Hagler and Jackson 2001).

The focus of this study is on the immunomarking technique, which is safe and does not impair the ability of an insect to behave normally (Jones et al. 2006). An

enzyme-linked immunosorbent assay (ELISA) mark detection method for protein markers was developed (Hagler et al. 1992, Hagler 1997, Hagler and Jackson 1998). This method used protein-specific antibodies to detect a specific vertebrate protein mark (rabbit or chicken IgG), which had been applied externally as a spray or internally by incorporating it into the insect's diet (Hagler 1997). The major limitation of this work was the high cost of purified protein marks: $30-50/g. The cost barrier limited its large scale application or marking a large number of insects. An alternative solution is to use of inexpensive protein such as chicken egg albumin, milk powder, or soy milk powder (Jones et al. 2006). These proteins make it affordable to externally mark a large number of insects in the laboratory or in nature. Before using these methods in diverse environments, a few areas that need to be evaluated are the denaturing rate of protein from exposure to high temperature conditions and the adherence of protein to the surface of the insect or plant during rain. These proteins were retained on most leaves for more than 4 weeks sampled from the lower two-thirds of cotton foliage in one study (Hagler and Jones 2010). However, low protein detection rates were observed in our preliminary field trials. This led to an exploration of the benefits of adding protein stabilizers to the marking solution.

Polyols, also known as sugar alcohols, are known to raise the transition temperature (i.e., the temperature at which denaturation occurs) of proteins. Trehalose, sorbitol, and mannitol are examples of polyols. Trehalose (2.0 m) was observed to raise the transition temperature of RNAase by 18 °C (Kaushik and Bhat 2003). Sorbitol has also been shown to stabilize proteins (Maury et al. 2004, Chang et al. 2005, Bakaltcheva

et al. 2007). In addition, sorbitol is one of the components in some Bt formulations to keep these formulations stable in field conditions (Brar et al. 2006).

We evaluated the effect of sorbitol on the probability of successfully marking *Lygus lineolaris* (Palisot de Beauvois) and on extending the longevity of the mark. Experiments were conducted with and without simulated rain. We also analyzed the amount of time that marked insects could spend on unmarked plants and retain a detectable mark. We conducted these experiments on cotton (*Gossypium hirsutum* L) but the results are expected to be similar for many insect-plant systems. The primary goal of this research was to develop an inexpensive but effective technique to mark insects under high temperatures and in the presence of rainfall.

Materials and Methods

All trials were conducted with a tarnished plant bug colony started in 2005 and maintained by the Department of Entomology and Plant Pathology, Mississippi State, MS. This colony was started from insects collected from wild hosts from various locations in Mississippi. Wild insects were periodically added to the colony to maintain its vigor. This colony was reared on artificial diet (Cohen 2000) and kept at 26°C, 16:8 LD. Cotton for all trials was grown in 3.78 liter pots with four plants per pot. The plants were grown on Promix-Bx media (Premier Horticulture Inc, Quakertown, PA, USA) and were fertilized once with Osmocote (Scotts Sierra Horticultural Products Comp, Marysville, OH, USA) at a rate of 2.5 gm/4 plants. Plants were watered every three days using tap water applied at the base of the pot. Squaring cotton plants (plants with flower buds) were sprayed with protein marker solutions using a garden sprayer until the

solution dripped from the leaves. Throughout this paper we will refer to the four plants in a pot as a "pot" unless otherwise stated. We sprayed two types of marker solutions. The egg albumin solution was one part liquid chicken egg albumin mixed with nine parts tap water (v:v) to achieve a 10% egg albumin solution. The egg albumin with sorbitol marker solution was the same 10% egg albumin solution plus 91.1 g sorbitol per 1000 ml egg solution to obtain a 10% egg albumin with 0.5M sorbitol solution.

Two studies were conducted to evaluate questions related to mark durability. In the first experiment, we tested the effects of exposure time, marker with or without sorbitol, and rain on mark acquisition by insects. In the second experiment, we tested the effect of marker with or without sorbitol on retention by marked insects feeding on unmarked plants.

Experiment 1

Thirty two pots were sprayed with egg albumin solution and 32 pots were sprayed with egg albumin + sorbitol solution. One and seven days after the solutions were sprayed, 16 pots from each treatment received one cm of simulated rainfall. We simulated rain using a garden hose fitted with a sprinkler nozzle. The nozzle was held facing up and moved as needed so that the water sprinkled evenly over the potted plants (Fig. 2.1). To assure an even distribution of water over the pots, six graduated cylinders were located as indicated to measure applied water. Three days after the plants were marked, 10 – 12 insects were caged on each pot using netting (Fig. 2.2). The nets were open from both ends and put on the plants from bottom of the pot (1 m: 0.4 m: length: diameter) and tied at the base of pot using rubber bands. The top of the net was closed

above the plants using rubber bands. Insects were caged on these marked plants for 1, 5, 12, or 22 h (exposure time). For each marking solution, exposure time, and rainfall treatment, we used 4 pots (replicates). We recovered 8 insects from each pot, which were directly and individually placed in a microcentrifuge tube and frozen at -80°C until mark evaluation. More insects were caged and treated as above at 6, 9, 12, and 15 days after marking the plants using the same sprayed pots for each exposure time. An overview of the indirect ELISA technique developed by Jones et al. (2006) to detect the protein mark on insects was as follows: Frozen insects were washed individually in TBS buffer for about 3 minutes, the insects were discarded, and the buffer was tested for the presence of the marker protein. Buffer samples were put in wells and incubated. After washes with phosphate buffered saline tween-20 (PBST), a blocker was added to the wells and incubated, and primary antibody was added and incubated. Then, the secondary antibody was added and incubated, a substrate was added, and finally acid was added to stop the reaction. Optical density (OD) of the contents of each well was determined with an absorbance microplate reader (BioTek-RLx800, Winooski, VT). The software used to collect data was Gen5 (BioTek, Winooski, VT).

The trial had a factorial design with repeated measures for the following factors and their interactions: exposure time (E, 4 levels), marker (M, 2 levels), rainfall (R, 2 levels), time elapsed in days since plants were sprayed (D, 5 levels), and associated subject and within subject error terms.

Experiment 2

Sixty four pots of plants were used in this trial. Sixteen pots were sprayed with egg albumin solution, 16 pots with egg albumin with sorbitol solution, and 32 pots were left unmarked. Five hours after the plants were marked, 12 – 15 insects were caged on each marked pot (Fig. 2.2). Twelve hours later, the insects were removed from the marked plants using an aspirator, and transferred to caged pots of unmarked plants. All insects from a given pot of marked plants were caged on the same pot of unmarked plants. The insects were removed from the pots of unmarked plants after 3, 6, 9, and 12 days, resulting in four replications for each marker and day combination. Eight insects were recovered for analysis from each pot. The insects were handled and analyzed in the same manner as in Experiment 1.

The trial had a completely randomized design with two factors: marker (M, 2 levels) and time in days on unmarked foliage (D, 4 levels).

Data Analysis

In both studies, an individual was considered marked if the ELISA optical density reading was at least three standard deviations above the mean for unmarked insects (Crowther 2001). Unmarked insects were ones collected from the wild. Ninety-six well plates (300 µl/well) were used for the indirect ELISA. Each plate had eight wells used for unmarked insects (negative control) and eight wells used for the antigen at 1 ppm (positive control). Before comparing the ELISA optical density values between the different treatments, the observed values were multiplied by the ratio of the average of the positive controls on the plate to the average across all plates (Jones et al. 2006).

In Experiment 1, analysis of variance with repeated measures based on a general linear model (PROC GLM, SAS Institute 2003) was used to test the effects of duration of exposure, marker solution, rainfall condition, period between spraying and exposure and their interactions on optical density readings. Following the full analysis, separate analyses for with and without simulated rainfall were performed. Percentages of insects marked are reported but were not statistically analyzed. REQWG at $P = 0.05$ was used for means separation (SAS Institute 2003). (Optical density + 0.001) was log transformed to equalize variance over the range of observed values. None of the insects exposed twelve and fifteen days after the plants were marked in the absence of simulated rainfall were found positive for mark. We did not have the data for fifteen days for the same experiment done in the presence of simulated rainfall. Hence, we eliminated fifteen day optical density readings from the data set to have a balanced design.

In Experiment 2, analysis of variance based on a general linear model (PROC GLM: SAS Institute 2003) was used to test the effects of marker solution, time spent on the unmarked plants, and their interaction on optical density readings. Percentages of insects marked are reported but not analyzed. REQWG at $P = 0.05$ was used for means separation (SAS Institute 2003). (Optical density + 0.001) was log transformed to equalize variance over the range of observed values.

Results

Experiment 1

The mean optical density readings differed significantly between all main effects and their interactions (Table 2.1). Simulated rain of one cm caused a highly significant reduction in the mean optical density readings.

In the absence of simulated rainfall, there was a significant interaction between marker and exposure time on optical density ($F = 20.00$; $df = 3, 24$; $P < 0.0001$) for insects exposed three days after the marker was applied to plants. Multiple comparison tests showed that for the egg albumin marker, the mean optical density after one hour exposure time was lower than after longer exposure times (5, 12, or 22 hours) (Table 2.2, Fig. 2.3). For insects infested on sorbitol marked plants, mean optical densities after one and five hr exposure times were lower than after 12 and 22 hr exposure times (Table 2.2, Fig 2.3). Mean optical density for one hr exposure time on egg albumin with sorbitol was higher than all exposure times for egg albumin alone, but the five hr exposure was not. All the insects were considered marked for both markers at all exposure times on day three.

For insects exposed six days after the marker was applied to plants, there was a significant interaction between marker and exposure time on optical density ($F = 4.84$; $df = 3, 24$; $P = 0.009$). Multiple comparisons tests showed that for egg albumin marker, the mean optical densities were not significantly different among the exposure times (Table 2.2, Fig. 2.4). Optical densities were different among exposure time for the egg albumin with sorbitol. However, similar to day three, the mean optical density for one hour

exposure time on egg albumin with sorbitol was higher than for all exposure times for egg albumin marker (Table 2.2, Fig 2.4). There was 40% and 6% drop in the mean optical density of the egg albumin and egg albumin with sorbitol markers, respectively, from day three to day six. All the insects were considered marked for both markers on day six.

For insects exposed nine days after the marker was applied to plants, there was a non-significant interaction between marker and exposure time on optical density ($F = 0.55$; $df = 3, 24$; $P = 0.6532$). The mean optical density did not differ significantly ($F = 0.03$; $df = 3, 24$; $P = 0.9927$) among the four exposure times. However, marker was a highly significant factor ($F = 19.33$; $df = 1, 24$; $P = 0.0002$) with egg albumin marks lower than egg albumin with sorbitol marks (Table 2.2, Fig. 2.5). There was a decrease in the optical density reading by 100 fold from day six to day nine. However, 91% (116/128) of insects were considered positively marked by the egg marker, and 99% (127/128) of insects were considered positively marked by the sorbitol marker.

For insects exposed twelve days after the marker was applied over plants, there was a non-significant interaction between marker and exposure times on optical density ($F=0.96$; $df = 3, 24$; $P = 0.4281$). The mean optical density did not differ significantly among the four exposure times ($F = 0.25$; $df = 3, 24$; $P = 0.8615$) or between markers ($F = 4.34$; $df = 1, 24$; $P = 0.0581$). The mean optical density reading dropped below the positive threshold for both markers, and none of the insects tested positive for either marker (Table 2.2, Fig. 2.6).

In the presence of simulated rain conditions, only the days between mark application and exposure had a significant affect on the mean optical density ($F = 31.2$; df

= 3, 24; P <0.001). All other main effects and interactions were non-significant (statistics not shown). Three days after the marker was applied to plants, 25% (32/128) and 35% (45/128) of insects were positively marked by the egg albumin marker and egg albumin with sorbitol marker, respectively (Table 2.3). Most of the positively marked individual insects were very lightly marked (optical density ≤ 0.0052) compared to optical densities > 1.00 for insects without rainfall. Six days after the mark was applied to plants, 7% (9/128) and 8% (11/128) of insects were positively marked by the egg albumin marker and egg albumin with sorbitol marker, respectively. A second one cm rain event was simulated on day seven and by day nine ≤ 6% of insects were marked by either marker. By day 12 there were no marked insects (Table 2.3, Fig. 2.6).

Experiment 2

In this experiment, conducted without simulated rain, there was a significant interaction between marker and time spent by marked insects on unmarked plants on mark intensity (F = 3.8; df = 3, 24; P = 0.0232). Mean optical densities were consistently higher for insects exposed to plants marked with egg albumin with sorbitol than with egg albumin marker (Table 2.4). As anticipated, mean optical densities decreased as time passed (Table 2.4). Nine days after marked insects were put on unmarked plants, the mark could be detected on 50% (16/32) of insects exposed to plants marked with egg albumin and 84% (27/32) exposed to egg albumin with sorbitol. Twelve days after insects were put on unmarked plants, marks could not be detected on any insects.

Discussion

Sorbitol is a component in wastewater sludge-based Bt formulations to stabilize them in field conditions (Brar et al. 2006). Polyethylene sorbitan monolaurate is a derivative of sorbitol which is used as a surfactant in dye granules of Rhodamine b (Schellhorn et al. 2004) to enhance this marking technique. There are a few limitations of this particular marker; Rhodamine b granules are not registered to be used on crops for human or animal consumption (Schellhorn et al. 2004). Moreover, the dye (Rhodamine b) was completely undetectable five days after application. In contrast, the immunomarking technique using egg albumin with sorbitol reported here is superior because it is completely non toxic to humans, animals, or plants, and it last longer. However, nothing is published regarding the use of sorbitol to stabilize egg albumin in the mark-recapture-technique. One of the basic assumptions of insect marking studies is that marked individuals do not lose their mark over the period of recapture. Our results demonstrated a decrease in protein mark detectability over time for tarnished plant bug and how detectability was reduced by rain and time spent on unmarked plants.

In the absence of simulated rain, the egg albumin mark on insects was reliably detected with or without the addition of sorbitol up to six days after exposure to protein marked plants. One hour exposure on egg albumin marked plants was sufficient for all insects to become marked although the optical density after one hr was lower than after longer exposure times on day three. In contrast, except for an anomalously high optical density for one hr exposure time for egg albumin with sorbitol, there was an increase in optical density with exposure time three and six days after application (Table 2.2). However, optical density for insects exposed to egg albumin with sorbitol marked plants

for one hr on day six was higher than the optical density for insects exposed to egg albumin marked plants for any other exposure times on any days and seven out of eight comparisons (3 and 6 days/ 4 exposure times) showed that optical density was higher for egg albumin with sorbitol than egg albumin alone. This indicates that sorbitol helped reduce required exposure time by insects to become marked by marked plants, which is important where the goal is to mark mobile insects by marking the habitat. Positive detection of insects was not a concern on days three and six because all insects tested were marked. However, sorbitol plays a role at the time when egg albumin alone is close to the positive threshold. On day nine, optical densities of insects exposed to plants marked with egg albumin without sorbitol were closer to the positive threshold, and the mark could only be detected on 91% of the insects (Table 2.2, Fig. 2.5). In contrast, 99% of sorbitol marked insects were still positive for the mark. Moreover, the detection rate of egg albumin for one hr exposure time was only 75% compared to 96% for egg albumin with sorbitol mark. Therefore, the addition of sorbitol to egg albumin allows mark-recapture experiments to continue longer than is possible using egg albumin alone. Sorbitol is inexpensive ($10 – 15/kg) so it can be added to the egg albumin solution to increase probability of mark detection for longer periods. Sorbitol may not be needed if the study period is less than a week.

Although sorbitol increased the probability of insects getting marked under simulated rain conditions, only 25% and 35% of insects were marked for egg albumin and egg albumin with sorbitol, respectively, three days after plants had been marked (Table 2.3). Since most of the marking methods to study insect movement assume that the mark is not being lost from the insect over the period of recapture, this level of

marking is inadequate for marking studies, so these protein-marking methods are only suitable during periods without rain.

Once an insect has acquired the mark and spends time on unmarked plants, the intensity of the mark declines. Although 100% of insects tested positive for both markers three and six days after the marked insects were put on unmarked plants, the mean optical reading decreased by about 2/3 from day three to day six for both markers (Table 2.4). Nine days after the insects were exposed to marked plants for 12 hr and were put on unmarked plants, 84 and 50% of insects were still marked using the egg albumin with sorbitol and egg albumin marker, respectively. In experiment 1, 100 and 95% of insects exposed 12 hr to plants that were marked nine days earlier became marked (Table 2.2). This suggests that the mark is less persistent on the insect than on the plant. Although we did not explore the effects of temperature, exposure to higher temperatures should increase the rate of protein denaturation (Kaushik and Bhat 2003). The experiments were done in a greenhouse during April, 2009, which was usually sunny. The greenhouse roof was glazed with glass which partially blocked ultraviolet light. Ultraviolet light may be another factor that reduces protein marker persistence. Although we did not study the mechanism through which sorbitol provides protection against protein denaturation, sugars, in general, provide protection to proteins by hydrogen bonding to the dried proteins by serving as a water substitute (Carpenter and Crowe 1989, Carpenter et al. 1993). This property might be useful since it keeps the protein moist and hence can increase the probability of protein persistence on plants and of an insect getting labeled by walking over marked plants. In these trials, 0.5 m sorbitol was added to the protein solution. A higher concentration of sorbitol may increase marker persistence even more.

However, since sorbitol has a high molecular weight (182.17 g/mol; solubility in water, 220g/100 ml at 20°C), the amount of sorbitol to be added to achieve higher molar concentrations results in sorbitol precipitation and a more viscous solution. This may clog spray equipment and affect the uniformity of the spray coverage in field, so may not be practical.

Every marking technique has advantages and disadvantages. The immunomarking technique using egg albumin with sorbitol is best suited for a study period of less than a week during summer conditions in the midsouth United States. It may be useful for longer periods in climates with lower temperatures. It is non-toxic, so it can be used in any habitat. However, this technique is not suitable in the presence of rainfall. In the absence of rain, the addition of sorbitol to egg albumin may help insects become marked more quickly and may enable detection of marked insects for a few more days than egg albumin alone, which may allow extension of the period of recapture.

Acknowledgements

The authors wish to thank Cotton Incorporated, Cary, NC, for providing funding for this research. I thank Kathy Knighten for her technical assistance in the lab; Heather Pogue, Stephanie White, Teresa Knighten, and Nicole Cox for their help in rearing; and Will Mackenzie, Justine Allard and Jordan Clark for their help in the field.

References Cited

Akey, D. H., J. L. Hayes, and S. J. Fieisher. 1991. Use of elemental markers in the study of arthropod movement and trophic interactions. Southwest. Entomol. 14(Suppl): 1-87.

Bakaltcheva, I., A. M. O'Sullivana, P. Hmela, and H. Ogbu. 2007. Freeze-dried whole plasma: Evaluating sucrose, trehalose, sorbitol, mannitol and glycine as stabilizers. Thrombosis Res. 120: 105-116.

Brar, S. K., M. Verma, R. D. Tyagi, J. R. Valero, and R. Y. Surampalli. 2006. Screening of different adjuvants for wastewater/wastewater sludge-based *Bacillus thuringiensis* formulations J. Econ. Entomol. 99: 1065-1079.

Carpenter, J. F., and J. H. Crowe. 1989. An infrared spectroscopy study of the interactions of carbohydrates with dried proteins. Biochemistry 28: 3916-3922.

Carpenter, J. F., S. J. Prestrelski, and T. Arakawa. 1993. Separation of freezing and drying-induced denaturation of lyophilized proteins using stress-specific stabilization. Arch. Biochem. Biophys. 303: 456-464.

Chang, L. L., D. Shepherd, J. Sun, X. C. Tang, and M. J. Pikal. 2005. Effect of sorbitol and residual moisture on the stability of lyophilized antibodies: Implications for the mechanism of protein stabilization in the solid state. J. Pharm. Sci. 94: 1445-1455.

Cohen, A. 2000. New oligidic production diet for *Lygus hesperus* Knight and *L. lineolaris* (Palisot de Beauvois). J. Entomol. Sci. 35: 301-310.

Crowther, J. R. 2001. The ELISA Guidebook. Humana Press, Totowa, NJ.

Griffiths, G. J. K., C. J. Alexander, A. Birt, J. M. Holland, P. J. Kennedy, J. N. Perry, R. Preston, and L. Winder. 2004. A method for rapidly mass laser-marking individually coded ground beetles (Coleoptera: Carabidae) in the field. Ecol. Entomol. 30: 391-396.

Hagler, J. R. 1997. Field retention of a novel mark-release-recapture method. Environ. Entomol. 26: 1079-1086.

Hagler, J. R., and C. G. Jackson. 1998. An immunomarking technique for labeling minute parasitoids. Environ. Entomol. 27: 1010-1016.

Hagler, J. R., and C. G. Jackson. 2001. Methods for marking insects: Current technologies and future prospects. Annu. Rev. Entomol. 46: 511-543.

Hagler, J. R., and V. P. Jones. 2010. A protein-based approach to mark arthopods for mark-capture type research. Entomol. Exp. Appl. 135: 177-192.

Hagler, J. R., A. C. Cohen, D. Dradley-Dunlop, and F. J. Enriquez. 1992. New approach to mark insects for feeding and dispersal studies. Environ. Entomol. 21: 20-25.

Hendricks, D. E., and H. M. Graham. 1970. Oil-soluble dye in larval diet for tagging moths, eggs, and spermatophores of tobaco budworms. J. Econ. Entomol. 63: 1019-1020.

Jones, V. P., and M. P. Parrella. 1986. The movement and dispersal of *Liriomyza trifolii* in a chrysanthemum green house. Ann. Appl. Biol. 109: 33-39.

Jones, V. P., J. R. Hagler, J. F. Brunner, C. C. Baker, and T. D. Wilburn. 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. Environ. Entomol. 35: 827-836.

Kaushik, J. K., and R. Bhat. 2003. Why is trehalose an exceptional protein stabilizer? J. Biol. Chem. 278: 26458-26465.

Leeuwen, E. R. V. 1940. The activity of adult codling moths as indicated by captures of marked moths. J. Econ. Entomol. 33: 162-168.

Maury, M., K. Murphy, S. Kumar, A. Mauerer, and G. Lee. 2004. Spray-drying of proteins: Effects of sorbitol and trehalose on aggregation and FT-IR amide I spectrum of an immunoglobulin G. Euro. J. Pharm. Biophar. 59: 251-261.

Murdoch, W. W. 1963. A method for marking Carabidae (Col.). Entomol. Monthly 99: 22-24.

SAS Institute. 2003. SAS/STAT user's guide. Version 9.1, SAS Institute, Cary, NC.

Schellhorn, N. A., G. Siekmann, C. Paull, G. Furness, and G. Baker. 2004. The use of dyes to mark populations of beneficial insects in the field. Int. J. Pest Manag. 50: 153-159.

Schroeder, W. J., and W. C. Mitchell. 1981. Marking tephritidae fruit fly adults in Hawaii for release-recovery studies. Proc. Hawaii. Entomol. Soc. 23: 437-440.

Service, M. W. 1993. Mosquito Ecology: Field Sampling Methods. Elsevier, New York, NY.

Southwood, T. R. E., and P. A. Henderson. 2000. Ecological Methods. 3rd ed. Blackwell, Malden, MA.

Steiner, L. F., W. C. Mitchell, E. J. Harris, T. T. Kozuma, and M. S. Fujimoto. 1965. Oriental fruit fly eradication by male annihilation. J. Econ. Entomol. 58: 961-964.

Table 2.1 Overall statistics of main effects and their interactions for Experiment 1.

Factors	*F*	df	*P*
Rain, R	36419.00	(1, 48)	<0.001
Marker, M	173.05	(1, 48)	<0.001
R*M	159.00	(1, 48)	<0.001
Exposure time, E	11.05	(3, 48)	<0.001
R*E	8.12	(3, 48)	<0.001
M*E	9.38	(3, 48)	<0.001
R*M*E	7.88	(3, 48)	<0.001
Days after mark application, D	12278.24	(3, 144)	<0.001
R*D	11876.45	(3, 144)	<0.001
M*D	42.94	(3, 144)	<0.001
R*M*D	41.55	(3, 144)	<0.001
E*D	6.40	(9, 144)	<0.001
R*E*D	6.28	(9, 144)	<0.001
M*E*D	4.37	(9, 144)	<0.001
R*M*E*D	3.62	(9, 144)	<0.001

Table 2.2 Mean optical density reading (±SE, % marked) in the absence of simulated rain for egg albumin marker with or without sorbitol on tarnished plant bug exposed to cotton plants for the indicated number of hours and 3, 6, 9, and 12 days after application of the marker solution to the plants. Insects were considered positive for any reading > 0.0034. Each reading represents 32 insects.

Days after Application	Spray Solution	Exposure Time, h1	Exposure Time, h5	Exposure Time, h12	Exposure Time, h22	Overall Mean
3	Albumin	0.69 (0.05, 100)e	1.34 (0.03, 100)d	1.25 (0.03, 100)d	1.30 (0.03, 100)d	1.15 (0.03, 100)
3	Albumin+ Sorbitol	1.64 (0.14, 100)c	1.36 (0.11, 100)cd	2.13 (0.08, 100)b	2.33 (0.15, 100)ab	1.87 (0.06, 100)
6	Albumin	0.61 (0.03, 100)e	0.68 (0.03, 100)e	0.74 (0.03, 100)e	0.72 (0.02, 100)e	0.68 (0.014, 100)
6	Albumin+ Sorbitol	1.67 (0.15, 100)c	1.01 (0.14, 100)d	1.92 (0.20, 100)bc	2.44 (0.21, 100)a	1.70 (0.09, 100)
9	Albumin	0.0062 (0.0008, 75)h	0.0063 (0.0003, 94)h	0.0064 (0.0002, 94)h	0.0062 (0.0002, 100)h	0.0063 (0.00002, 91)
9	Albumin+ Sorbitol	0.0081 (0.0001, 96)f	0.0083 (0.0003, 100)f	0.0074 (0.0002, 100)g	0.0084 (0.0003, 100)f	0.0080 (0.00001, 99)
12	Albumin	0.0030 (4.2E-5, 0)k	0.0029 (2.0E-5, 0)k	0.0031 (2.3E-5, 0)k	0.0030 (2.3E-5, 0)k	0.0030 (1.4E-5, 0)
12	Albumin+ Sorbitol	0.0030 (1.7E-5, 0)k	0.0030 (4.6E-5, 0)k	0.0030 (2.3E-5, 0)k	0.0030 (2.7E-5, 0)k	0.0030 (1.5E-5, 0)

Log transformation of optical density + 0.001 used for statistical comparisons. Means followed by the same letter are not significantly different according REGWQ to at $P \leq 0.05$. Means comparisons were made overall for Tables 2.2 and 2.3.

Table 2.3 Mean optical density reading (±SE, % marked) in the presence of simulated rain for egg albumin marker with or without sorbitol on tarnished plant bug exposed to cotton plants for the indicated number of hours and 3, 6, 9, and 12 days after application of the marker solution to the plants. Insects were considered positive for any reading > 0.0034. Each reading represents 32 insects.

Days after Application	Spray Solution	Exposure Time, h1	Exposure Time, h5	Exposure Time, h12	Exposure Time, h22	Overall Mean
3	Albumin	0.0033 (6.5E-5, 25)j	0.0032 (4.5E-5, 28)j	0.0032 (4.2E-5, 25)j	0.0033 (7.9E-5, 21)j	0.0033 (1.2E-5, 25)
3	Albumin+ Sorbitol	0.0041 (7.1E-4, 28)i	0.0031 (3.4E-5, 19)j	0.0036 (1.1E-4, 40)i	0.0035 (1.0E-4, 53)i	0.0036 (2.1E-5, 35)
6	Albumin	0.0030 (3.1E-5, 3)k	0.0031 (4.3E-5, 6)k	0.0031 (4.9E-5, 9)k	0.0031 (9.0E-4, 9)k	0.0031 (1.4E-5, 7)
6	Albumin+ Sorbitol	0.0030 (4.6E-5, 9)k	0.0030 (2.4E-5, 6)k	0.0031 (5.6E-5, 9)k	0.0031 (2.9E-5 ,9)k	0.0031 (1.0E-5, 8)
9	Albumin	0.0030 (2.1E-5, 0)k	0.0030 (2.8E-5, 3)k	0.0030 (1.7E-5, 3)k	0.0030 (1.8E-5, 6)k	0.0030 (0.9E-5, 3)
9	Albumin+ Sorbitol	0.0030 (3.0E-5 ,3)k	0.0030 (2.6E-5, 6)k	0.0030 (2.6E-5, 6)k	0.0030 (1.9E-5, 3)k	0.0030 (3.4E-6, 5)
12	Albumin	0.0030 (1.8E-5, 0)k	0.0030 (2.8E-5, 0)k	0.0030 (1.7E-5, 0)k	0.0030 (1.8E-5 ,0)k	0.0030 (2.6E-6, 0)
12	Albumin+ Sorbitol	0.0030 (1.2E-5, 0)k	0.0030 (2.0E-5, 0)k	0.0030 (1.6E-5, 0)k	0.0030 (1.9E-5, 0)k	0.0030 (5.3E-6, 0)

Log transformation of optical density + 0.001 used for statistical comparisons. Means followed by the same letter are not significantly different according REGWQ to at $P \leq 0.05$. Means comparisons were made overall for Tables 2.2 and 2.3.

Table 2.4 Mean optical density reading (±SE, % marked) in the absence of simulated rain for egg albumin marker with or without sorbitol to the tarnished plant bug exposed to marked plants for 12 h and then to unmarked cotton plants for 3, 6, 9, and 12 days. Insects were considered positive for any reading > 0.0034. Each reading represents 32 insects.

Period After Exposure, d	Albumin Marker	Albumin + Sorbitol Marker
3	1.20 (0.03, 100)b	1.56 (0.05, 100)a
6	0.41 (0.02, 100)d	0.57 (0.03, 100)c
9	0.0035 (0.0002, 50)f	0.0047 (0.0002, 84)e
12	0.0022 (0.0003, 0)g	0.0022 (0.0002, 0)g

Means followed by the same letter are not significantly different according to REGWQ at $P \leq 0.05$.

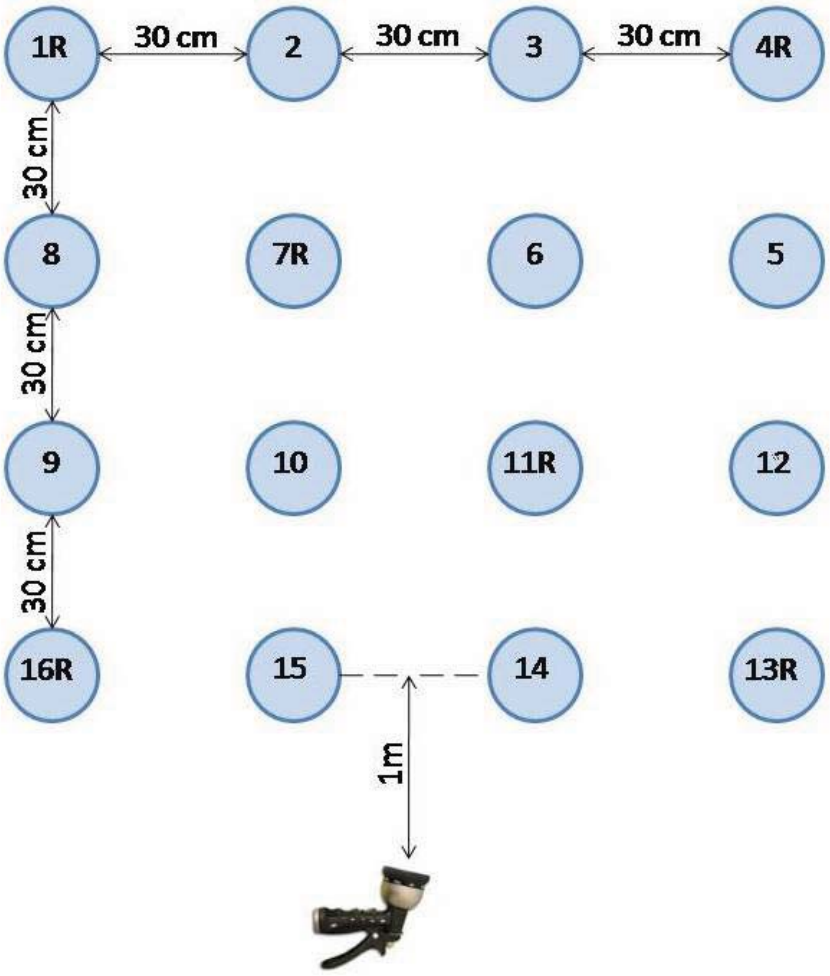

Figure 2.1 A schematic arrangement of pots and rain gauges during simulated rainfall. The hose was held 1m away from the outer row and 2m above the base of the pots. The hose was moved slightly in a circular manner to obtain a uniform distribution of water. “R” represents the position of rain gauges. Rain gauge readings: 1R (1.0 cm), 4R (1.1 cm), 7R (1.0 cm), 11R (1.0 cm), 13R (1.0 cm), and 16R (0.9 cm).

Figure 2.2 Pots of cotton plants with netting to cage tarnished plant bugs in a greenhouse.

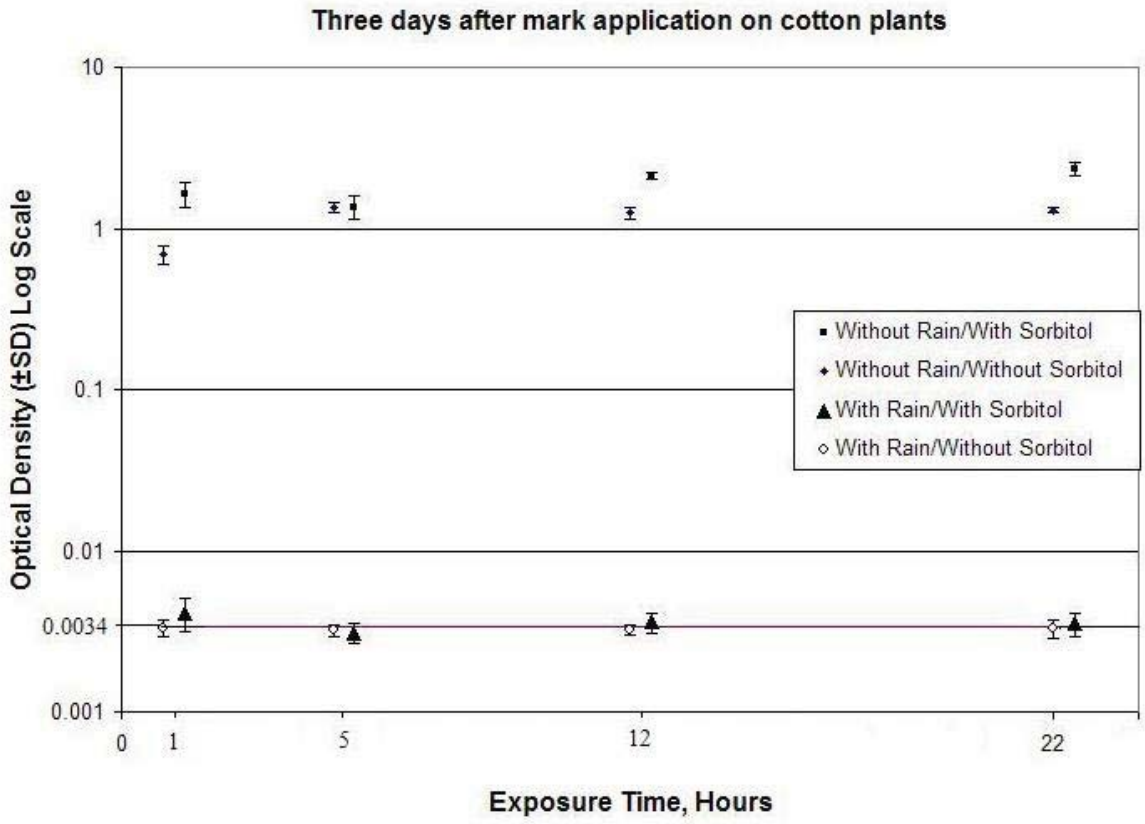

Figure 2.3 Mean optical density reading (±SD) for insects exposed three days after plants were marked. The line on Y axis = 0.0034 is the positive threshold for insects to be considered marked.

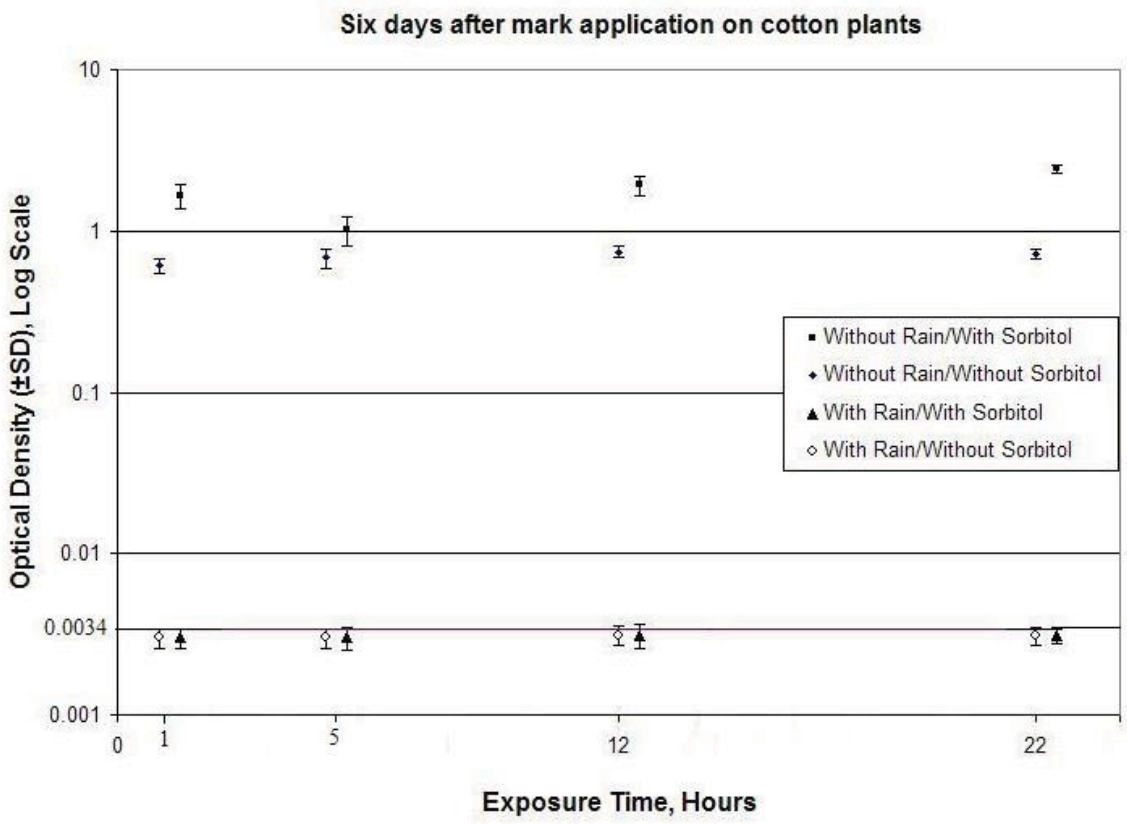

Figure 2.4 Mean optical density reading (±SD) for insects exposed six days after plants were marked. The line on Y axis = 0.0034 is the positive threshold for insects to be considered marked.

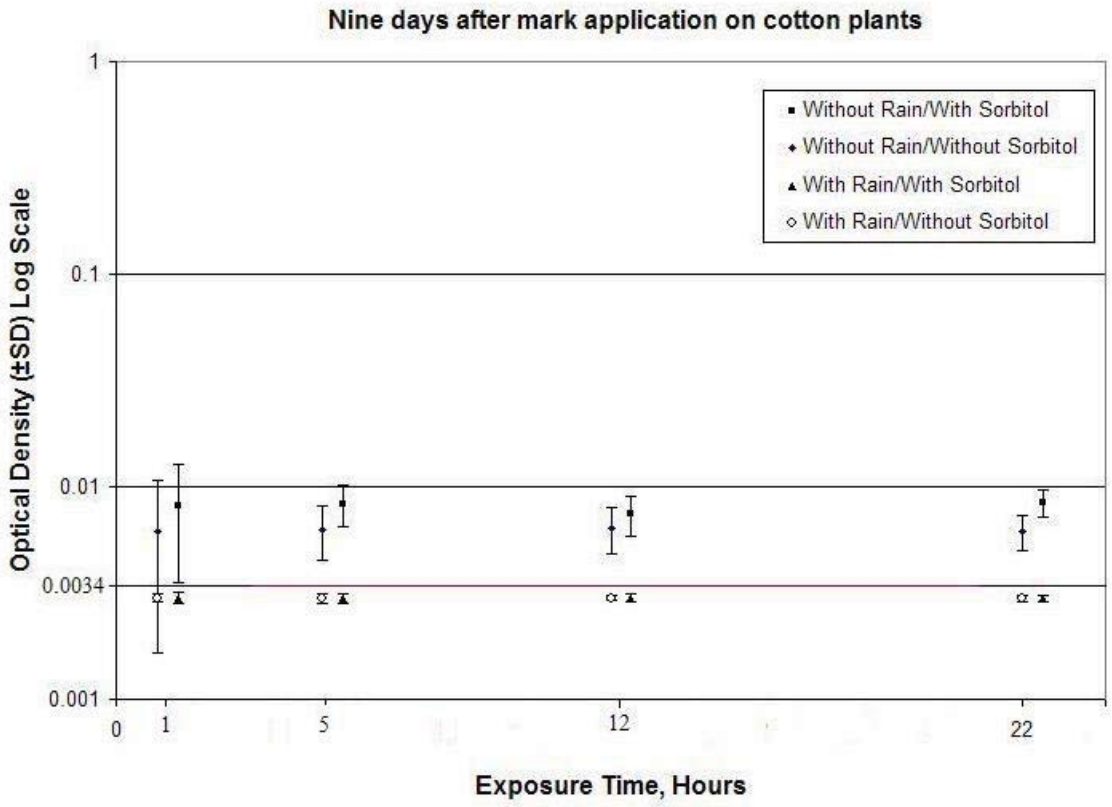

Figure 2.5 Mean optical density reading (±SD) for insects exposed nine days after plants were marked. The line on Y axis = 0.0034 is the positive threshold for insects to be considered marked.

.

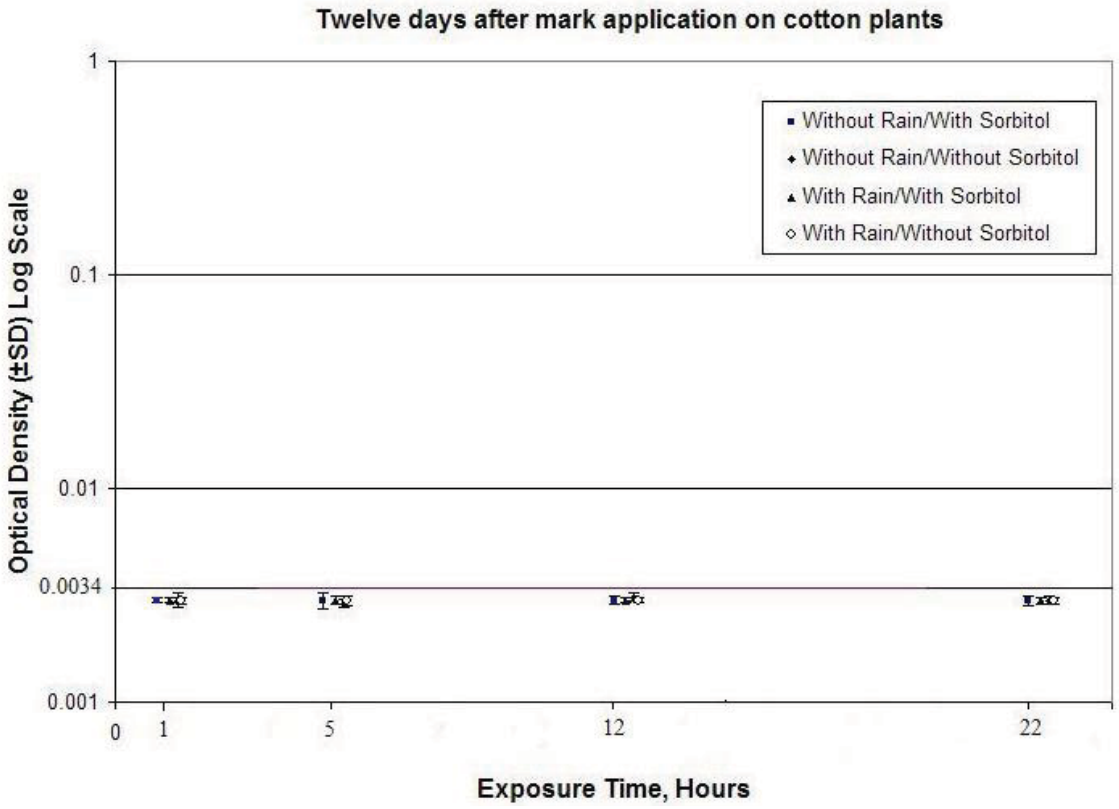

Figure 2.6 Mean optical density reading (±SD) for insects exposed twelve days after plants were marked. The line on Y axis = 0.0034 is the positive threshold for insects to be considered marked.

CHAPTER III

INTER AND INTRA CROP MOVEMENT OF TARNISHED PLANT BUG ON CORN AND COTTON

Abstract

Lygus lineolaris (Palisot de Beauvois), the tarnished plant bug, is a key economic pest of cotton (*Gossypium hirsutum* L.) in the mid-south USA. It is believed that early season crops like corn (*Zea mays* L.) play a major role in the increase of *L. lineolaris* populations, which then move to nearby cotton fields. *L. lineolaris* densities within cotton fields are often much higher near corn fields, but this phenomenon has not been well quantified. A better understanding of this movement could play a key role in managing *L. lineolaris*. The mark-capture and mark-release-recapture studies reported here show that corn at tasseling stage is a more suitable habitat to *L. lineolaris* than pre-squaring cotton. Dispersal of *L. lineolaris* from corn to cotton starts during the period when corn is at silking stage and cotton is at squaring stage. By the time corn reaches milk stage and cotton is at first bloom, most adult *L. lineolaris* have moved out of corn to infest cotton. Corn is a host of *L. lineolaris* immediately before cotton is a suitable host, so the arrangement of these crops in the landscape will impact the location and severity of pest pressure in cotton.

Key Words: *Lygus lineolaris*, corn, cotton, mark, diffusion coefficients

Introduction

Lygus lineolaris (Palisot de Beauvois), the tarnished plant bug, is an important pest of cotton in the mid-south United States, especially in the Mississippi River delta of Arkansas, Louisiana, and Mississippi (Snodgrass et al. 1984). Most pest management practices for *L. lineolaris* rely heavily on chemical control. One of the concerns of a chemically-dependent control strategy is the development of insecticide resistance. Recently *L. lineolaris* was found to be resistant to pyrethroids in the Mississippi Delta region (Snodgrass and Scott 2000, Zhu and Snodgrass 2003). *Lygus lineolaris* overwinters as adults from late autumn or early winter until spring in broadleaf species or other ground cover including plant debris. Adults may become active periodically on warm days during the winter (Johnson et al. 2005). They move to broadleaf species like crimson clover, fleabane, horseweed, ragweed, evening primrose, and chickweed in the spring (Snodgrass et al. 1984). On these plants, *L. lineolaris* live one to two generations (Snodgrass et al. 2006). The time period during which corn tassels generally coincides with the time that spring hosts start to senesce, and some *L. lineolaris* move to corn. Although *L. lineolaris* is not considered a pest of corn, laboratory experiments have shown that *L. lineolaris* can feed and reproduce on corn silk and corn ears during blister and milk stages (Abel and Snodgrass 2003). In a field study, *L. lineolaris* eggs were oviposited on the corn leaf sheath, leaf mid-rib, tassel, and ear silks; and development to the adult stage was successful (Abel and Snodgrass 2003, Abel 2004). While *L. lineolaris* development in corn is possible, the importance of corn to *L. lineolaris* population dynamics is not known. Because corn is generally suitable for *L. lineolaris* development earlier than cotton, corn may be the source of *L. lineolaris* immigrating into some cotton

fields. The rate and timing of movement of *L. lineolaris* between these crops has not been well quantified. A better understanding of movement of *L. lineolaris* within and between corn and cotton would improve our ability to manage *L. lineolaris.* For example, depending on the relative maturities of corn and cotton, corn may be a source of *L. lineolaris* and cotton a sink at some times but this relationship may change at other times.

The primary goal of this research project was to understand the dispersal rates of *L. lineolaris* within and between adjacent corn and cotton fields, and how these rates change as the crops mature. This goal was accomplished through two marking studies using immunomarking techniques (Jones et al. 2006).

Materials and Methods

A total of six corn-cotton field locations were studied in 2008 and 2009. This study was conducted on commercial farms where corn and cotton were planted adjacent to one another. We conducted two similar experiments each year using two different marking approaches: mark-release-recapture (MRR) and mark-capture (MC). In each corn-cotton farmscape, corn and cotton were planted side by side with rows running parallel. The row width was 0.96 m in both crops. For MRR, trials were conducted with *L. lineolaris* adults collected from field populations of wild host plants, primarily fleabanes (*Erigeron* spp.) and pigweed (*Amaranthus* spp.). Collected insects were placed in cages with host plant material initially with access to artificial diet (Cohen 2000) as well. Only artificial diet was maintained and field collected insects were reared up to one generation on artificial diet. When needed, this F1 generation adult population was supplemented with field-collected *L. lineolaris* and *L. lineolaris* from a colony started in 2005 and

maintained by the Department of Entomology and Plant Pathology, Mississippi State, MS. This colony was reared on artificial diet (Cohen 2000) at 25°C and 16:8 LD. Wild *L. lineolaris* were added to the colony periodically to maintain colony vigor.

Insect Sampling

Lygus lineolaris was sampled in cotton using a sweep net 38 cm in diameter and 84 cm in length as recommended by Musser et al. (2007). Preliminary observations in tasseling corn indicated that *L. lineolaris* was found primarily on tassels. Therefore, sampling in tasseling corn was accomplished using a sweep net 46 cm in diameter and 124 cm in length (7221NA, BioQuip) by quickly covering the plants with the sweepnet from the top down as far as possible. The sweepnet bag was clamped around the plant as low as possible and shaken thoroughly to collect *L. lineolaris* in the net. *Lygus lineolaris* were recovered from the net using an aspirator. Since sampling in tasseling corn disturbed adjacent plants, two plants were sampled together using one net, then the next two plants were skipped, resulting in half of each row being sampled. To compensate for this incomplete sampling, the counts were multiplied by two. For corn at silking and milk stages, preliminary observations showed that *L. lineolaris* was found mostly on corn silks, so a visual search of corn ears was made and the insects were collected with an aspirator. For the silking and milk stages, all plants in the row were sampled.

Experimental Design for Mark-Release-Recapture (MRR)

Two similar experiments were conducted during 2008 and 2009. During 2008, two locations were chosen in commercial fields near Caledonia and Baldwyn, MS in the

'Hills' region where natural *L. lineolaris* densities are typically low. At both locations, corn was at silking stage and cotton was squaring. Nine hundred marked *L. lineolaris* were divided into groups of 30 and placed in 250 ml plastic cups with green bean pieces. *Lygus lineolaris* were marked a day before releasing them with 10% egg albumin+0.5M sorbitol solution using a garden sprayer set to deliver a fine spray into each 250 ml individual cup. Cups with *L. lineolaris* and green bean pieces were kept at room condition overnight. Care was taken to avoid excessive spraying, which could hinder their ability to fly normally. On the next day between 9 and 11 am, marked insects were released opening cups at ground level at 2.5 m intervals between two rows in the corn interior, cotton interior, and at the interface of the corn and cotton fields (Fig. 3.1). Field interiors were at least 200 m from the field edge. One, three, and six days after the release, *L. lineolaris* were collected at 0.5, 2.5, 5.5, 10.5, and 16 m on both sides away from the release lines. A separate, but adjacent, 25 m of row was sampled on each collection day in each habitat. Collected insects were immediately dumped into kill jars to minimize the risk of unmarked insects becoming contaminated by marked ones. Collection of *L. lineolaris* began at the furthest distance from the release line and moved toward the release line. Collected *L. lineolaris* were brought to the lab and placed individually into microcentrifuge tubes. They were agitated in 1 ml TBS buffer solution for 3 minutes and then removed. The buffer was stored at -70°C in the microcentrifuge tubes labeled with the day, habitat, and distance. They were analyzed later for the marker using indirect ELISA (Jones et al. 2006). The experiment was repeated when corn was at milk stage and cotton was at first week of bloom, and again when corn was at dent stage and cotton was in the third week of bloom.

In 2009, a protocol similar to that described for 2008 was followed except for two changes. First, 3300 insects were released along a line of 275 m. This made nine 25 m release segments flanked on each side by a 25 m release segment adjacent to which tarnished plant bugs were not sampled. Three hundred adults were released in each 25 m segment as in 2008. Rather than two locations with one replicate at each location as in 2008, there was one location (Noxubee, MS) with three replications. The second change was the crop maturities at release. In 2009, the first release was made when corn was tasseling and cotton pre-squaring, the second when corn was silking and cotton squaring, and the third when corn was at milk and cotton at the first week of bloom. The rest of the protocol was similar to 2008.

Experimental Design for Mark Capture (MC)

Two similar experiments were conducted in the Delta regions of MS. during 2008 and 2009 where *L. lineolaris* densities tend to be high. Field selection was similar to that described above for MRR. The two study sites were located near Glendora, MS in 2008. Seventy five meters of each of two adjacent rows in corn and cotton field interiors were sprayed with a 10% egg albumin + 0.5M sorbitol solution using a CO_2 sprayer. Foliage was saturated completely with the spray solution. Field interiors were at least 200 m from any field edge. The design and insect collection protocol was similar to the MRR experiment (Fig. 3.1b). At the interface between cotton and corn, 75 m of each of two adjacent rows of cotton neighboring the corn field were sprayed with 10% egg + 0.5M sorbitol solution. Similarly, each of two adjacent rows of corn neighboring the cotton field were sprayed with 20% soy milk + 0.5M sorbitol solution (Fig. 3.2). The rest of the

protocol was the same as described for MRR. As with MRR in 2008, MC experiments during 2008 were repeated at two additional time periods: corn at milk stage and cotton in the first week of bloom, and corn at dent stage and cotton in the third week of bloom.

In 2009, a protocol similar to that described for 2008 was followed except for two changes. First, instead of spraying 75 m as done in 2008, 400 m of each habitat (corn interior, cotton interior, and corn-cotton interface) were sprayed, giving nine 25-m spray segments flanked on each side by 87.5 m of sprayed row adjacent to which *L. lineolaris* were not sampled. There was one location (Midnight, MS) in 2009 with three replicates. The second change was as done for MRR in 2009 the time periods of the MC experiments were shifted earlier so the first observations were made when corn was tasseling and cotton was not yet squaring, the second when corn was silking and cotton was squaring, and the third when corn was at milk stage and cotton was in the first week of bloom. The rest of the protocol was similar to 2008.

Data Analysis

In all the studies, an individual was considered marked if the ELISA optical density reading was three standard deviations above the mean of unmarked, control insects (Crowther 2001). Control insects (unmarked) were collected from other parts of a cotton field before the start of the experiments. We used 96 well (300 µml capacity per well) plates for the indirect ELISA. Each plate had 8 wells used for unmarked insects (negative control) and 8 wells used for the antigen at 1 ppm (positive control).

Data from the two sites in 2008 for MRR were combined, but each release period was analyzed separately. In both years, a diffusion model was used to estimate the rates

of movement of *L. lineolaris* for each crop and time period. Although individual insects do not diffuse passively, the dispersal of many insect populations can be described with the equations developed to describe diffusion (Rudd and Gandour 1985). Insect dispersal models are best modeled by diffusion when the environment is homogeneous relative to the scale of movement (Turchin 1998). The interface of the crops was not homogeneous, so the data were analyzed for movement into each crop separately. Diffusion rates were estimated by fitting the following model to the data:

$$Y(r) = \{N_0/(4\pi Dt)\}\exp(-kt)*\exp(-r^2/(4Dt) \quad \text{Eq. 1.}$$

where, $Y(r)$ is the number of marked insects at a distance r (m) from the release line at time t (day). D is the diffusion rate (m^2/d), N_0 (= 300) is the number of marked insects released in each 25-m segment, k is the loss rate (mortality and emigration) of released insects (Rudd and Gandour 1985).

A different diffusion model was used for MC study which assumes marked organisms were released continuously at r = 0 such that a constant proportion of insects were marked each day and the population density remains constant at the line of release. Although marked individuals were not released continuously in this study, it is reasonable to assume that the density of marked individuals at the marked rows was approximately constant. All insects captured from the marked rows were marked, so this assumption appears to be valid. In addition, it was assumed that the probability of individuals becoming marked was constant over the study period. All the *L. lineolaris* exposed to cotton plants sprayed up to 6 days earlier with marker solution were

detectably marked (Chap 2), so this assumption appears to be valid. Consequently, diffusion rates were estimated by fitting the following model to the data:

$$Y(\mathrm{r}) = N_0(1\text{-erf}[r/2\sqrt{(Dt)}]) \qquad \text{Eq. 2.}$$

where Y(r) is the number of marked insects at a distance r (m) from the middle of the two sprayed rows at time t (d) and erf is the mathematical error function. D is the diffusion rate (m^2/d) and N_0 is the number of insects becoming marked per unit time (Crank 1989, Turchin 1998).

The diffusion rate (D) in each habitat of each experiment was estimated using the Gauss-Newton iterative method as implemented in PROC NLIN (SAS Institute, 2003). The Habitat in the MRR experiment was the crop in which the insects were captured. For the MC experiment habitat was the combination of crop in which the insect was marked plus the crop where the insect was captured. The default convergence criterion of SSE (c = 10^{-8}) was used. We analyzed the estimated diffusion rates for each habitat and maturity using PROC GLM (SAS Institute, 2003). REQWG at $P = 0.05$ was used for means separation (SAS Institute 2003). Comparisons were made for crop locations and crop maturities using CONTRAST (SAS Institute, 2003). All the interaction levels of factors were analyzed. When higher level interactions were not significant, they were removed from the model and the revised model was used for final analysis. In addition to marked insects, the unmarked populations of *L. lineolaris* were sampled. The natural population densities of *L. lineolaris* in cotton and corn at the various maturity stages were analyzed using PROC GLM (SAS Institute, 2003).

Results

Mark-Release-Recapture Experiment

In 2008 in the Hills region (Caledonia and Baldwyn, MS; data pooled), estimates of diffusion coefficients (*D*) for *Lygus lineolaris* for combinations of crop (cotton, corn), release location (interface, interior), and crop phenological stages (silking/squaring, milk/1st wk bloom, dent/3rd wk bloom) could not be compared statistically due to a lack of replication. Point estimates and associated standard errors of the diffusion estimates (SE) and the number of marked insects recaptured are reported (Table 3.1). There were no recaptures of marked insects following release in the corn interior, while twice as many marked insects were recaptured in cotton at the interface than in the cotton interior. When corn was at the milk stage and cotton in the first week of bloom and also when corn was at dent stage and cotton in the third week of bloom, 100% of the marked insects released at the interface and subsequently recaptured were recaptured in cotton.

In 2009 in the Hills region (Noxubee,MS), the crop*site*phenological stage interaction was significant ($F=5.23$; $df = 1, 20$, $p = 0.033$) for estimates of *D,* so a multiple comparison procedure was applied for this experiment (Table 3.2). *D* values for *L. lineolaris* in tasseling corn were similar to those in pre-squaring cotton, but higher in silking corn than in squaring cotton. *D* values in pre-squaring cotton were generally higher than those estimated for squaring and 1st week bloom cotton, which were similar to one another (Table 3.2). Given that the interaction habitat*phenological stage was significant for the number of marked individuals recaptured ($F = 65.17$; $df = 6, 22$; $p < 0.0001$), a multiple comparison procedure was applied on a table-wide basis (Table 3.2).

When corn was tasseling and cotton pre-squaring, more marked insects released at the interface and subsequently recaptured were recaptured in corn than in cotton. At later maturities, more marked insects released at the interface were recaptured in cotton. No marked insects were found in corn during milk stage.

Mark-Capture Experiment

In 2008, in the Delta (two locations at Glendora, MS; data pooled), estimates of *D* could not be made because at one location there was a barren/weedy area of 50 m width between the corn and cotton fields and at the other there was a roadway of 15 m width between two fields. In each case, the sprayed pairs of rows for the crops at the interface were separated by the intervening non-crop areas. The numbers of marked insects captured during sampling are presented in Table 3.3. Given that the habitat*phenological stage interaction was significant ($F = 9.98$; 10, 36; $P < 0.0001$), a multiple comparison procedure was applied for the whole experiment (Table 3.3). When corn was silking and cotton squaring, the number of insects marked in cotton and captured in corn was significantly lower than the number of insects marked in corn and captured in cotton. When corn was in the milk stage and cotton in the first week of bloom, no insects were captured in corn.

In 2009 in the Delta (Midnight, MS), estimates of *D* for the combinations of habitat, and phenological stages were compared statistically. The habitat*phenological stage interaction was significant ($F = 6.61$; df = 7, 30; $P < 0.001$), so a multiple comparison procedure was applied on the overall experiment (Table 3.4).

When corn was tasseling and cotton pre-squaring, the diffusion coefficients were significantly lower for *L. lineolaris* marked and captured in the same crop compared to the individuals marked in one crop and captured in another crop at the interface ($t = -3.91$; $df = 12$; $P = 0.0021$) (Table 3.4), indicating higher movement rates for the individuals which have been exposed to more than one habitat. The diffusion coefficients were also significantly lower for the *L. lineolaris* captured in corn compared to cotton ($t = 5.59$; $df = 12$; $P < 0.0001$) (Table 3.4). The diffusion estimate for *L. lineolaris* marked in corn and captured in cotton was higher than the one for those marked in cotton and captured in corn (Table 3.4).

When corn was silking and cotton was squaring, the observed diffusion rates were significantly lower for *L. lineolaris* marked and captured in the same crop compared to the individuals marked in one crop and captured in another crop at the interface ($t = -4.07$; $df = 12$; $P = 0.0016$). The diffusion coefficients were similar for the *L. lineolaris* captured in corn compared to those captured in cotton ($t = -0.75$; $df = 12$; $P < 0.4663$) (Table 3.4). There were also no differences in diffusion coefficients for *L. lineolaris* captured in corn at the interface compared to the corn interior ($t = -1.93$; $df = 12$; $P = 0.0778$); however, diffusion estimates were significantly higher for *L. lineolaris* captured in cotton at the interface compared to cotton in the interior ($t = 2.9$; $df = 12$; $P = 0.0112$). When corn was in milk stage and cotton was in the first week of bloom, where calculated, diffusion coefficients in each habitat were similar to corresponding diffusion coefficients during the silking/squaring period (Table 3.4).

The numbers of marked insects captured during sampling are also presented in Table 3.4. The habitat*phenological stage interaction was significant ($F = 84.07$; $df = 10$,

36; P < 0.0001), so a multiple comparison procedure was used (Table 3.4). When corn was tasseling and cotton was pre-squaring, the number of insects marked in cotton and captured in corn was significantly higher than the number of insects marked in corn and captured in cotton (Table 3.4). This relationship was reversed when corn was silking and cotton squaring, as the number of insects marked in cotton and captured in corn was significantly lower than the number of insects marked in corn and captured in cotton. When corn was at milk stage and cotton was at first week of bloom, no insects were captured in corn, but 66 insects marked in corn were captured in cotton.

Natural Population Density

In 2008, the natural population densities of *L. lineolaris* for several combinations of site (Hills, Delta), habitat (corn interior, corn interface, cotton interface, cotton interior) and phenological stage (silking/squaring, milk/1st wk bloom, dent/3rd wk bloom) were compared. The habitat*site interaction was significant (F = 13.17; df = 3; 26; P < 0.0001) and the habitat*crop phenological stage interaction was also significant (F = 5.90; df = 4, 26; P = 0.0023). However, the three way interaction of site*habitat*crop phenological stage was not significant (F = 1.01; df = 4, 20; P = 0.6114). Consequently, a multiple comparison procedure was applied on a table-wide basis (Table 3.5). When corn was silking and cotton was squaring, natural *L. lineolaris* densities in the corn interior and corn interface in the Delta were higher than corn interior and corn interface densities in the Hills, respectively (Table 3.5). *Lygus lineolaris* densities in the cotton interior and cotton interface in the Delta were similar to cotton interior and cotton interface densities in the Hills, respectively (Table 3.5). However, when corn was at milk and cotton at first week of bloom, natural *L. lineolaris*

densities in the cotton interior and cotton interface in the Delta were lower compared to the cotton interior and cotton interface in the Hills, respectively (Table 3.5).

In 2009, the habitat*site*crop phenological stage interaction was significant ($F = 17.21$; $df = 6, 48$; $P < 0.0001$), so a multiple comparison procedure was applied on a table-wide basis (Table 3.6). The Delta site had a higher *L. lineolaris* density for each habitat compared to the Hills (Table 3.6). In both the Hills and the Delta, tasseling corn at the interface had higher densities of *L. lineolaris* than tasseling corn in the interior. When corn was silking and cotton squaring, the natural *L. lineolaris* densities in the corn interface was similar to densities in the corn interior in both the Hills and Delta (Table 3.6).

Discussion

High levels of *L. lineolaris* damage have been frequently observed in cotton bordering corn. In this study, natural *L. lineolaris* densities were often higher in cotton near corn then in the interior of the same field (Tables 3.5 and 3.6). More released *L. lineolaris* moved into squaring cotton than silking corn in 2008 and 2009 (Tables 3.1 and 3.2). Similarly for mark-capture studies in 2008 and 2009, a high number of marked *L. lineolaris* were found in squaring cotton compared to silking corn.

In 2009, we marked-released-recaptured *L. lineolaris* when corn was at tassel stage and cotton pre-squaring, which was not done in 2008. Since diffusion estimates were lower in tasseling corn than later stages and a greater proportion of marked insects were collected in tasseling corn than at later stages, it seems that corn is more attractive to *L. lineolaris* during tasseling even though both tasseling and silking corn are suitable to ovipositing *L. lineolaris* (Abel and Snodgrass 2003). Similarly, diffusion coefficients were generally lower

in squaring and blooming cotton compared to pre-squaring cotton, and more insects were generally captured in reproductive stage cotton than pre-squaring cotton, suggesting that cotton is more attractive to *L. lineolaris* during reproductive growth than during vegetative growth. We observed no *L. lineolaris* in milk stage corn, indicating that movement out of corn occurs before milk stage. Although silking corn is an excellent food source for developing *L. lineolaris* (Abel and Snodgrass 2003), flowering cotton is a more attractive host than silking corn, consistent with other observations that *L. lineolaris* prefers flowering plants (Pack and Tugwell 1976, Snodgrass 1998). Moreover, *L. lineolaris* released could not be found in the corn interior when corn was in the milk stage, so adults apparently moved a long distance at this time to find a more desirable feeding site.

Although no data were collected on nymphs, we observed more nymphs near the cotton interface than in the cotton interiors on several occasions. One explanation for this is that *L. lineolaris* adults move out of corn after tasseling stage, lay eggs on cotton near the interface, and then disperse further into the cotton field. Since nymphs have a strong preference for fruiting structures within the cotton plants and nymphs cannot fly (Snodgrass 1998), high densities of nymphs cause damage to cotton near the corn - cotton interface. Although the diffusion estimates for the Hills and Delta locations could not be compared statistically because of the difference in the way insects were handled, the diffusion estimates were usually higher for MRR in the Hills than for MC in the Delta. This may be because the insects were handled in the lab and required to move to feed in the MRR experiment, but not in the MC experiment. Regardless, the trends in diffusion coefficients between habitats with changes in phenological developments in crops were similar for both experimental methods.

A low cost protein marking technique has enabled us to estimate the movement of *L. lineolaris* in cotton-corn interfaces. A similar study has been reported for *L. hesperus* in alfalfa-cotton farmscape in Texas (Parajulee et al. 2007). Although that study did not estimate diffusion coefficients, the research shows similar results of *Lygus* movement from one crop to another. *Lygus hesperus* showed movement dynamics in both directions at the interface of an alfalfa field next to a cotton field. A study done in Italy reported that *Lygus rugulipennis* Poppius migrates onto peach trees when winter cereals are harvested and usual host plants are lacking. Adult *Lygus rugulipennis* Poppius also migrated into fields from other host plants such as alfalfa and clover, causing serious injuries to fruits (Tavella and Pansa 2007). These studies indicate the adaptability of *Lygus* to a wide range of hosts and their ability to move from one habitat to another, depending on habitat attractiveness. Hence, movement of *L. lineolaris* will be affected by the farmscape, and crop phenology within the farmscape is crucial to understanding and predicting *L. lineolaris* movement so that pest management practices can be utilized most efficiently.

High insect densities due to crop to crop migration have been observed for other hemipteran pests. In Georgia, reduced lint quality due to southern green stink bug damage has been noted in the cotton-peanut farmscape (Tillman 2006, Tillman et al. 2009). A management tool suggested for this farmscape was the use of sorghum as a trap crop since it has been shown to be highly effective for southern green stink bug control in cotton (Tillman 2006). A similar approach has been suggested for control of *L. hesperus* in strawberries in California using alfalfa as the trap crop (Swezey et al. 2007). A trap crop approach could also be beneficial for *L. lineolaris* management in the Midsouth states, where insecticides remain the primary means of control. Even without a trap crop, growers and consultants can

minimize crop to crop movement by arranging their crops so that the amounts of interfaces are minimized. This can be achieved by planting bigger fields or clustering similar crops together.

Milk stage corn kernels are a good source of food for *L. lineolaris* (Abel and Snodgrass 2003). However, in the field, corn kernels are not exposed and available for *L. lineolaris* feeding, so milk stage corn is an unsuitable host for *L. lineolaris*. Corn is only a suitable host for *L. lineolaris* from tasseling to silking. Typically during this period in Mississippi, cotton begins to square and becomes a suitable host for *L. lineolaris.* If corn is planted earlier, or cotton is planted later, then this corn to cotton migration will be disrupted, which could reduce *L. lineolaris* densities. However, crop planting times are constrained by environmental conditions and agronomic needs. Planting cotton late may reduce *L. lineolaris,* but it may increase the problems from late season insects and cause problems with harvesting. Further research is needed to evaluate the impacts of management strategies that could minimize *L. lineolaris* movement within the farmscape.

Acknowledgements

The authors wish to thank Cotton Incorporated, Cary, NC for providing funding for this research. We thank Kathy Knighten for her technical assistance in the lab. We also thank Heather Pogue, Stephanie White, Teresa Knighten and Nicole Cox for their help in rearing the insect colony. We also thank Will Mackenzie, Justine Allard and Jordan Clark for their help in the field.

References Cited

Abel, C. A. 2004. Corn and soybean crop influence. USDA, http://www.ars.usda.gov/Research/docs.htm?docid=7575 (viewed March 5, 2008).

Abel, C. A., and G. L. Snodgrass. 2003. The development of tarnished plant bug on various corn tissue. pp. 949-953. *In* Proceedings, Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Cohen, A. 2000. New oligidic production diet for *Lygus hesperus* Knight and *L. lineolaris* (Palisot de Beauvois). J. Entomol. Sci. 35: 301-310.

Crank, J. 1989. The Mathematics of Diffusion. Oxford University Press, New York.

Crowther, J. R. 2001. The ELISA Guidebook. Humana Press, Totowa, NJ.

Johnson, D., T. Cottrell, and D. Horton. Plant bugs and stink bugs. Southestern Peach Grower's Handbook http://www.ent.uga.edu/Peach/peachhbk/insects/plantbugs.pdf, (viewed April 3, 2010).

Jones, V. P., J. R. Hagler, J. F. Brunner, C. C. Baker, and T. D. Wilburn. 2006. An inexpensive immunomarking technique for studying movement patterns of naturally occurring insect populations. Environ. Entomol. 35: 827-836.

Musser, F., S. Stewart, R. Bagwell, G. Lorenz, A. Catchot, E. Burris, D. Cook, J. Robbins, J. Greene, G. Studebaker, and J. Gore. 2007. Comparison of direct and Indirect sampling methods for tarnished plant bug (Hemiptera: Miridae) in flowering cotton. J. Econ. Entomol. 100: 1916-1923.

Pack, T. M., and P. Tugwell. 1976. Clouded and tarnished plant bugs on cotton: a comparison on injury symptoms and damage on fruit parts. Ark. Agric. Exp. Stn. Rep. Ser. 226: 1-17.

Parajulee, N. M., B. R. Shrestha, and C. S. Carroll. 2007. Intercrop movement of *Lygus hesperus* in the Texas High Plains: Potential for landscape level management. J. Insect Sci. http://www.insectscience.org/8.49/ref/abstract44.html, (viewed February 25, 2010)

Rudd, W. G., and R. W. Gandour. 1985. Diffusion model for insect dispersal. J. Econ. Entomol. 78: 295-301.

SAS Institute. 2003. SAS/STAT user's guide. Version 9.1, SAS Institute, Cary, NC.

Snodgrass, G. L. 1998. Distribution of the tarnished plant bug (Heteroptera: Miridae) within cotton plants. Environ. Entomol. 27: 1089-1093.

Snodgrass, G. L., and W. P. Scott. 2000. Seasonal changes in pyrethroid resistance in tarnished plant bug (Heteroptera: Miridae) populations during a three-year period in the delta area of Arkansas, Louisiana, and Mississippi. J. Econ. Entomol. 93: 441-446.

Snodgrass, G. L., W. P. Scott, and J. W. Smith. 1984. Host plant and seasonal distribution of the tarnished plant bugs (Hemiptera: Miridae) in the delta of Arkansas, Louisiana, and Mississippi. Environ. Entomol. 13: 110-116.

Snodgrass, G. L., W. P. Scott, C. A. Abel, J. T. Robbins, J. Gore, and D. D. Hardee. 2006. Suppression of tarnished plant bugs (Heteroptera: Miridae) in cotton by control of early season wild host plants with herbicides. Environ. Entomol. 35: 1417-1422.

Swezey, S. L., D. J. Nieto, and J. A. Bryer. 2007. Control of western tarnished plant bug, *Lygus hesperus* Knight (Hemiptera: Miridae), in California organic strawberries using alfalfa trap crops and tractor-mounted vacuums. Environ. Entomol. 36: 1457-1467.

Tavella, L., and M. Pansa. 2007. Population dynamics of *Lygus rugulipennis* in agroecosystems of NW Italy. Journal of Insect Science. http://www.insectscience.org/8.49/ref/abstract53.html, (Viewed February 25, 20010)

Tillman, P. G. 2006. Sorghum as a trap crop for *Nezara viridul*a L. (Heteroptera: Pentatomidae) in cotton in the southern United States. Environ. Entomol. 35: 771-783.

Tillman, P. G., T. D. Northfield, R. F. Mizell, and T. C. Riddle. 2009. Spatiotemporal patterns and dispersal of stink bugs (Heteroptera: Pentatomidae) in peanut-cotton farmscapes. Environ. Entomol. 38: 1038-1052.

Turchin, P. 1998. Quantitative Analysis of Movement. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

Zhu, Y. C., and G. L. Snodgrass. 2003. Cytochrome P450 CYP6X1 cDNAs and mRNA expression levels in three strains of the tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae) having different susceptibilities to pyrethroid insecticide. Insect Mol. Biol. 12: 39-49.

Table 3.1 Diffusion coefficient estimates, *D* (±SE m^2/d) (number of marked insects captured) for *L. lineolaris* adults in mark-release-recapture experiments at various phenological stages in corn and cotton in the Hills, Caledonia and Baldwyn, MS, 2008.

Habitat	Silking/Squaring	Milk/1st wk Bloom	Dent/3rd wk Bloom
Corn Interior	[1] (0)	[1] (0)	[2]
Interface into Corn	22.2 ± 14.4 (7)	[1] (0)	[1] (0)
Interface into Cotton	13.4 ± 6.5 (14)	22.5 ± 8.1 (33)	23.6 ± 7.9 (12) [3]
Cotton Interior	9.0 ± 2.8 (28)	18.5 ± 4.8 (26)	82.5 ±156.0 (4) [3]

[1] Not able to estimate diffusion coefficients.
[2] No marked *L. lineolaris* released.
[3] The estimates based only on the insects recaptured 1 and 3 days after release due to heavy rainfall before day six that likely removed the marker from the insects.

Table 3.2 Diffusion coefficient estimates, *D* (±SE m^2/d) and number of marked insects recaptured per 3 days of sampling (N), for *L. lineolaris* adults in mark-release-recapture experiments at various phenological stages of corn and cotton in the Hills, Noxabee, MS, 2009.

Habitat	D for Crop Stages in Corn/Cotton			N for Crop Stages in Corn/Cotton		
	Tasseling/Pre-square	Silking/ Squaring	Milk/1st Bloom	Tasseling/Pre-square	Silking/ Squaring	Milk/1st Bloom
Corn Interior	7.2 ± 1.5 c	16.7 ± 2.4 ab	[1]	67.3 ± 2.4 c	30.7 ± 3.0 e	0.0 ± 0.0 f
Interface into Corn	10.1 ± 2.7 bc	17.7 ± 3.4 a	[1]	89.0 ± 4.8 b[2]	49.0 ± 3.5 d[2]	0.0 ± 0.0 f[2]
Interface into Cotton	14.9 ± 3.5 b	2.4 ± 0.3 d	4.5 ± 0.7 cd	57.6 ± 6.1 d[2]	123.0 ± 5.4 a[2]	100.0 ± 3.6 a[2]
Cotton Interior	6.6 ± 1.0 c	2.9 ± 0.3 d	3.1 ± 0.4 d	30.3 ± 3.4 e	63.3 ± 5.5 bcd	63.3 ± 5.4 bcd

Multiple comparisons for *D* estimates are separate from number of marked insects recaptured (N).
Means (± SE) followed by the same letter are not significantly different according to REGWQ at $P \leq 0.05$.
[1] Not able to estimate diffusion coefficients
[2] Ten 25-m rows of corn and cotton interiors were sampled, and five 25-m rows of corn and cotton interface were sampled. The interface samples were multiplied by a factor of 2 to adjust for the sampling difference.

Table 3.3 Number of marked insects (±SE) captured per sampling days for *L. lineolaris* from mark-capture experiment in the Delta at Glendora, MS 2008; data pooled.

Habitat		Crop Stages in Corn/Cotton		
Mark Location	Sample Location	Silking/Squaring	Milk/ 1st wk Bloom	Dent/3rd wk Bloom
Corn Interior[1]		1.7 ± 0.3 cd	0.0 ± 0.0 e	[2]
Corn	Corn[3]	2.0 ± 1.5 cd	0.0 ± 0.0 e	0.0 ± 0.0 e
Cotton	Corn[4]	0.5 ± 0.5 d	0.0 ± 0.0 e	0.0 ± 0.0 e
Corn	Cotton[4]	16.53 ± 1.4 a	10.7 ± 3.0 b	0.0 ± 0.0 e
Cotton	Cotton[3]	24.0 ± 2.3 a	9.3 ± 2.2 bc	10.7 ± 1.3 b
Cotton Interior[1]		10.33 ± 1.8 b	5.67 ± 1.7 c	2.67 ± 0.7 c

Means (±SE) followed by the same letter are not significantly different according to REGWQ at $P \leq 0.05$.

[1]Eight 25- m rows sampled outside marked habitat.

[2]Habitat not sampled.

[3]Four 25- m rows sampled outside marked habitat, so sample values multiplied by 2 to put on an equal basis with field interior values.

[4]Five 25- m rows sampled outside marked habitat, so sample values multiplied by 1.6 to put on an equal basis with field interior values.

Table 3.4 Diffusion coefficient estimates, *D* (±SE m^2/d) and number of marked insects captured per sampling day (N), for *L. lineolaris* adults in mark-capture experiments at various phenological stages of corn and cotton in the Delta, Midnight, MS, 2009.

Habitat		D for Crop Stages in Corn/Cotton			N for Crop Stages in Corn/Cotton		
Mark Location	Sample Location	Tasseling/Pre-Square	Silking/ Squaring	Milk/1st Bloom	Tasseling/Pre-Square	Silking/ Squaring	Milk/1st Bloom
Corn Interior		3.7 ± 1.3 c	2.4 ± 0.7 c	[1]	10.0 ± 1.0[2] b	4.7 ± 0.3[2] d	0.0 ± 0.0[2] f
Corn	Corn	5.8 ± 2.1 bc	1.8 ± 1.0 c	[1]	12.5 ± 1.1[3] b	2.4 ± 0.3[3] e	0.0 ± 0.0[3] f
Cotton	Corn	6.9 ± 3.2 bc	9.8 ± 4.1 b	[1]	5.7 ± 0.4[4] c	5.68 ± 0.6[4] c	0.0 ± 0.0[4] f
Corn	Cotton	24.0 ± 6.4 a	7.4 ± 2.5 bc	7.7 ± 3.1[5] bc	1.4 ± 0.2[4] e	8.5 ± 0.5[4] b	17.6 ± 1.0[4] a
Cotton	Cotton	9.6 ± 4.2 b	3.7 ± 1.0 c	2.1 ± 0.8[5] c	4.9 ± 0.4[3] d	8 ± 0.7[3] c	11 ± 1.0[3] b
Cotton Interior		9.8 ± 2.4 b	0.3 ± 0.2 d	0.2 ± 0.04[5] d	4.9 ± 0.3[2] d	4.6 ± 0.3[2] d	3.3 ± 0.4[2] d

Multiple comparisons for D estimates are separate from number of marked insects recaptured (N).
Means (± SE) followed by the same letter are not significantly different according to REGWQ at $P \leq 0.05$.
[1]Not able to estimate diffusion coefficients
[2]Eight 25- m rows sampled outside marked habitat.
[3]Four 25- m rows sampled outside marked habitat, so sample values multiplied by 2 to put on an equal basis with field interior values.
[4]Five 25- m rows sampled outside marked habitat, so sample values multiplied by 1.6 to put on an equal basis with field interior values.
[5]D estimates are based only on the insects captured 1 and 3 days after the habitats were marked due to rainfall before day six sampling.

Table 3.5 Mean (±SE) number of "wild" [unmarked (Hills) and marked + unmarked (Delta)] *L. lineolaris* collected per day in the Hills and Delta, MS in 2008.

Habitat	Crop Stages in Corn/Cotton		
	Silking/ Squaring	Milk/1st wk Bloom	Dent/3rd wk Bloom
Corn Interior (Hills)[2]	06.0 ± 1.2 c	0.0 ± 0.0 e	[1]
Corn Interface (Hills)[3]	17.0 ± 2.1 c	0.0 ± 0.0 e	0.0 ± 0.0 e
Cotton Interface(Hills)[3]	28.4 ± 5.9 bc	52.0 ± 5.8 a	36.0 ± 2.2 b
Cotton Interior (Hills) [2]	32.8 ± 3.2 b	36.0 ± 2.0 b	20.0 ± 5.0 c
Corn Interior (Delta) [2]	10.7 ± 1.3 d	0.0 ± 0.0 e	[1]
Corn Interface (Delta)[3]	34.5 ± 4.2 b	0.0 ± 0.0 e	0.0 ± 0.0 e
Cotton Interface (Delta)[3]	26.0 ± 5.3 bc	31.7 ± 9.3 b	16.7 ± 2.8 c
Cotton Interior (Delta)[2]	22.0 ± 6.8 bc	15.2 ± 3.2 c	15.3 ± 2.1 c

Means (± SE) followed by the same letter are not significantly different according to REGWQ at $P \leq 0.05$.
[1]Habitat not sampled
[2]Ten 25- m rows sampled
[3]Five 25- m rows sampled so sample values multiplied by 2 to put an equal basis with field interior data.

Table 3.6 Mean (±SE) number of "wild" [unmarked (Hills) and marked + unmarked (Delta)] *L. lineolaris* collected per day in the Hills and Delta, MS in 2009.

Habitat	Crop Stages in Corn/Cotton		
	Tasseling/Pre-Square	Silking/Square	Milk/1st Bloom
Corn Interior (Hills)[1]	14.4 ± 1.5 f	7.8 ± 1.2 g	0.0 ± 0.0 h
Corn Interface (Hills)[2]	35.5 ± 2.9 cd	12.4 ± 1.6 fg	0.0 ± 0.0 h
Cotton Interface(Hills)[2]	21.3 ± 2.6 ef	25.3 ± 2.2 e	33.7 ± 1.3 d
Cotton Interior (Hills)[1]	14.1 ± 2.2 f	14.0 ± 1.6 f	19.5 ± 0.7 e
Corn Interior (Delta)[1]	38.1 ± 2.1 c	30.8 ± 2.2 cd	0.0 ± 0.0 h
Corn Interface (Delta)[2]	84.9 ± 4.7 a	44.7 ± 3.5 bc	0.0 ± 0.0 h
Cotton Interface (Delta)[2]	44.7 ± 2.7 bc	51.8 ± 3.6 b	54.3 ± 2.7 b
Cotton Interior (Delta)[1]	35.7 ± 1.2 c	40.0 ± 1.5 c	36.2 ± 3.1 c

Means (± SE) followed by the same letter are not significantly different according to REGWQ at $P \leq 0.05$.

[1]Ten 25- m rows sampled

[2]Five 25- m rows sampled so sample data multiplied by 2 to put an equal basis with field interior data

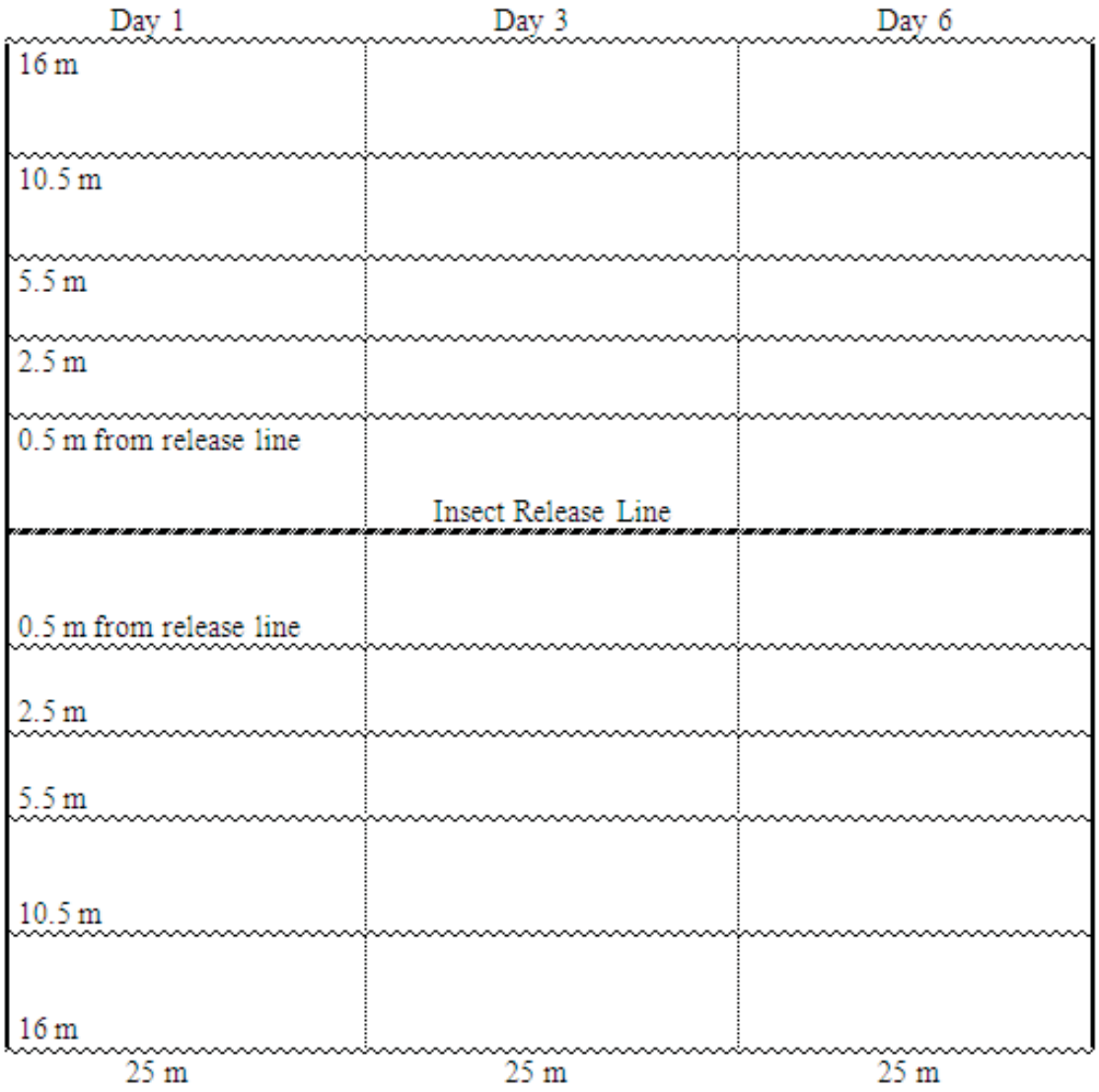

Figure 3.1 A schematic diagram showing a insect release line and the 25-m sample units at various distances from the release line for each collection day. The same sampling distances were used for releases within corn, releases within cotton, and for the releases at corn/cotton interface.

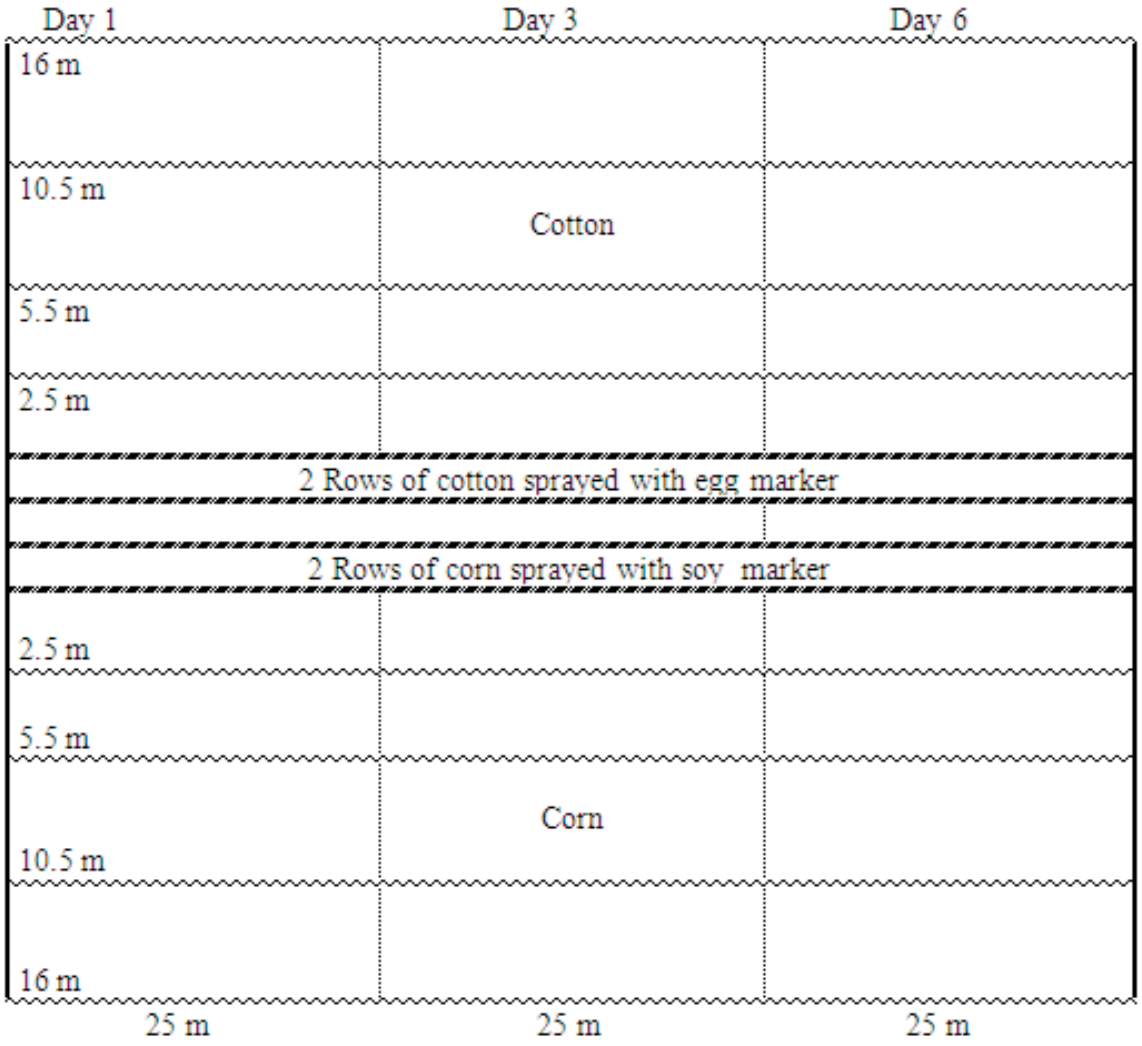

Figure 3.2 A schematic diagram showing the marker sprayed rows and the 25 m sample units at various distances for each collection day from the middle of the sprayed rows in the same habitat. In the corn/cotton interiors, only 2 rows were sprayed with egg marker and similar to this design, insects were sampled 2.5 m, 5.5 m, 10.5 m, and 16 m away from the middle of sprayed rows in both directions.

CHAPTER IV

SUMMARY

The immunomarking technique as used in these studies is best suited for a study period of less than a week during summer conditions in midsouth United States. It may be useful for longer periods in climates with less heat or ultraviolet radiation. It is non-toxic so it can be used in any habitat. However, this technique is not suitable under frequent rainfall conditions. The addition of sorbitol to egg albumin provides little protection to egg albumin against rain, but it helps insects become marked more quickly and enables detection of marked insects for a few more days than egg albumin alone in the absence of rain.

A comprehensive pest management program involving cultural, biological, and chemical controls is needed to manage tarnished plant bug in cotton. Corn in an important host of tarnished plant bug before cotton is attractive to tarnished plant bugs. Movement from corn to cotton primarily occurs between green silk and milk stages. This frequently coincides with cotton beginning to produce squares, so as corn get less attractive to tarnished plant bug, cotton becomes more attractive. The result is increased tarnished plant densities in cotton near corn interfaces. Management options could include a trap crop or simply adjusting crop arrangements in the landscape to minimize

corn-cotton interfaces. If corn is planted next to cotton, then scout cotton, based not only on the growth stage of cotton but also on the growth stage of adjacent corn. Tasseling corn could be sampled for tarnished plant bug to estimate the likelihood of a *L. lineolaris* migration later into cotton.