

5-1-2018

A Transgenic Approach to Managing Tarnished Plant Bug (Hemiptera: Miridae) in Cotton

John Cameron Corbin

Follow this and additional works at: <https://scholarsjunction.msstate.edu/td>

Recommended Citation

Corbin, John Cameron, "A Transgenic Approach to Managing Tarnished Plant Bug (Hemiptera: Miridae) in Cotton" (2018). *Theses and Dissertations*. 333.
<https://scholarsjunction.msstate.edu/td/333>

This Graduate Thesis - Open Access is brought to you for free and open access by the Theses and Dissertations at Scholars Junction. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of Scholars Junction. For more information, please contact scholcomm@msstate.libanswers.com.

A transgenic approach to managing tarnished plant bug (*Hemiptera: Miridae*) in cotton

By

John Cameron Corbin

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Entomology
in the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology

Mississippi State, Mississippi

August 2018

Copyright by
John Cameron Corbin
2018

A transgenic approach to controlling tarnished plant bug (*Hemiptera: Miridae*) in cotton

By

John Cameron Corbin

Approved:

Angus L. Catchot
(Major Professor)

Jeffrey Gore
(Co-Major Professor)

Don Cook
(Committee Member)

Darrin Dodds
(Committee Member)

Chris Daves
(Committee Member)

Kenneth Willeford
(Graduate Coordinator)

George Hopper
Dean
College of Agriculture and Life Sciences

Name: John Cameron Corbin

Date of Degree: August 10, 2018

Institution: Mississippi State University

Major Field: Entomology

Major Professor: Angus L. Catchot and Jeffrey Gore

Title of Study: A transgenic approach to controlling tarnished plant bug (Hemiptera: Miridae) in cotton

Pages in Study: 67

Candidate for Degree of Master of Science

Field experiments were conducted in Stoneville, MS and Sidon, MS in 2016 and 2017 to evaluate a new transgenic cotton variety (MON 88702) (Cry51Aa2.834_16) and its effects on tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), populations, frequency of insecticide applications, and yield. Experiments were designed to compare the interactions between the *Bt* traited plots and non-traited plots and the insecticide spray treatments, which consisted of different application thresholds as well as treatments sprayed only during the early season and only during the late season. MON 88702 provided a significant reduction in later instar tarnished plant bug nymphs, dirty squares, and insecticide applications, especially during the late season. MON 88702 also provided a significant increase in yield compared to non-traited plots. By implementing this transgenic approach, the number of insecticide applications necessary to effectively manage tarnished plant bug will be reduced compared to non-transgenic isolines, while also protecting yield.

DEDICATION

I would like to dedicate this research first and foremost to my Lord and Savior Jesus Christ. Without His grace and guidance, none of this would be possible. Next I would like to dedicate this research to my mother, Kim Corbin, my grandmothers, Annette Caffey and Helen Corbin, and my sister, Katherine Shook. I would not be where I am today without their love, patience, support, and encouragement. Not only have they served as an inspiration in pursuing a degree in agriculture due to farming being my family's livelihood, but the lessons I have learned from these people are truly invaluable and have molded me into the person I am today. Lastly I would like to dedicate this research to my brothers, Kellen and Kyle Corbin, my late grandfather, Jerry Caffey, and my father, Steve Corbin. The impact these men have had on my life in developing my love for the great outdoors and agriculture has been instrumental in developing me into the man I am today.

ACKNOWLEDGEMENTS

I would like to offer my deepest thanks and gratitude to all those that have given so much of their time to aid in this research. First, my most sincere appreciation to my co-major advisors, Dr. Angus Catchot and Dr. Jeff Gore. I am very appreciative, and always will be, for their support and guidance throughout my graduate school experience. The experience gained and friendships made during these two years will have a significant impact on my years ahead. I would also like to thank my other committee members, Dr. Don Cook, Dr. Darrin Dodds, and Chris Daves for the aid and direction they have provided me throughout this degree. I am deeply grateful for their willingness to work with me and help me achieve my goals.

My fellow graduate students Bill McRight, Tyler Towles, Whitney Crow, Chelsie Darnell, Ben Thrash, Adam Whalen, John North, Ty Smith, Brittany Lipsey, Keiton Croom, Joel Moor, Read Kelly, and Nick Bateman were a great support system teaching me the ins and outs of small plot research and helping me with any problems that arose throughout the course of this research. I am extremely grateful for all the hours spent working with them developing friendships. Also a special thanks to Dung Bao, not only for his help in the field, but also for his friendship. I would like to thank Monsanto Company for their financial support of this research. Lastly, I would like to thank all the summer workers for their help and hard work.

TABLE OF CONTENTS

DEDICATION	ii
ACKNOWLEDGEMENTS	iii
LIST OF TABLES	vi
LIST OF FIGURES	vii
CHAPTER	
I. INTRODUCTION	1
Cotton	1
Background and Development	1
Insect Pests of Cotton	3
Tarnished Plant Bug	5
Biology and Ecology	5
Feeding and Damage to Cotton	6
Thresholds and Sampling	7
Management Practices	8
<i>Bacillus thuringiensis</i> in Row Crops	10
Biology of <i>Bacillus thuringiensis</i>	10
History of <i>Bacillus thuringiensis</i>	11
Introduction of <i>Bacillus thuringiensis</i> to Row Crops	12
Justification for Research	13
References	15
II. DETERMINING THE APPROPRIATE THRESHOLD FOR TARNISHED PLANT BUG ON MON 88702 COTTON	19
Abstract	19
Introduction	20
Materials and Methods	23
Results	26
All Site Years except Sidon 2017	26
Pre-Bloom Samples	26
Bloom Samples	27
Insecticide Applications	28
Yield	28
Sidon 2017	28
Pre-Bloom Samples	28
Bloom Samples	29
Insecticide Applications	29
Yield	29

Discussion.....	30
References	40
III. TO DETERMINE THE VALUE OF EARLY SEASON PROTECTION COMPARED TO MID-LATE SEASON PROTECTION ON COTTON MODIFIED TO BE RESISTANT TO LYGUS (MON 88702)	42
Abstract.....	42
Introduction	42
Materials and Methods	46
Results	50
All Sites except Sidon 2017	50
Pre-Bloom Samples	50
Square Retention	51
Bloom Samples.....	51
Dirty Squares	52
Insecticide Applications	53
Yield	53
Sidon 2017.....	53
Pre-Bloom Samples	53
Square Retention	53
Bloom Samples.....	54
Dirty Squares	54
Insecticide Applications	55
Yield	55
Discussion.....	55
References	65

LIST OF TABLES

1.1	Estimates of cotton growth to select growth stages based on heat unit accumulation adapted from Jenkins et al. (1990).	3
-----	--	---

LIST OF FIGURES

2.1	Interaction between trait and threshold treatment on mean number of small tarnished plant bug nymphs per 15 sweeps in pre-bloom cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	33
2.2	Interaction between trait and threshold treatment on the mean number of large nymphs per two drops in blooming cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	34
2.3	Mean number of insecticide applications needed to manage tarnished plant bug in traited and non-traited cotton using a 1X threshold and 2X threshold averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	35
2.4	Impact of threshold spray treatment on cotton yields averaged across traited and non-traited lines in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	36
2.5	Impact of trait on cotton yields averaged across threshold treatments in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	37
2.6	Interaction between trait and threshold treatment on the mean number of large nymphs per two drops in blooming cotton averaged across all sample dates in Sidon, MS in 2017.....	38
2.7	Interaction between trait and threshold treatment on mean cotton yields in Sidon, MS in 2017.....	39
3.1	Interaction between trait and threshold treatment on the mean number of medium nymphs per 15 sweeps in pre-bloom cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	58
3.2	Interaction between trait and threshold treatment on the mean number of medium nymphs per 2 drops in blooming cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	59

3.3	Interaction between trait and threshold treatment on the mean number of dirty squares per 25 fruiting sites in blooming cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	60
3.4	Mean number of insecticide applications needed to manage tarnished plant bug in traited and non-traited cotton for the early season and late season treatments averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	61
3.5	Interaction between trait and spray treatment on mean cotton yields in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.....	62
3.6	Interaction between trait and threshold treatment on the mean number of large nymphs per 2 drops in blooming cotton averaged across all sample dates in Sidon, MS in 2017.....	63
3.7	Interaction between trait and spray treatment on mean cotton yields in Sidon, MS in 2017.	64

CHAPTER I INTRODUCTION

Cotton

Background and Development

Although there are four species of cotton cultivated across the world, upland cotton, *Gossypium hirsutum* (L.), is the most prominently grown species in the Mid-South, making up over 90% of the cultivated area. It was first introduced to the United States in Florida in 1556 (Smith and Cothren 1999). Since then, cotton has grown into a major agronomic crop across the mid-southern United States. In 2016, 4,077,005 hectares of cotton were planted in the United States and averaged 908 kilograms of cotton per hectare (NASS 2017).

Cotton development progresses through several growth phases, though these phases have been split into five main growth stages for practical purposes. These five main growth stages include germination and emergence, seedling establishment, leaf area and canopy development, flowering and boll development, and maturation (Oosterhuis 1990). The progression of these successive stages is rarely distinct, however. The use of a degree day model [$((\text{Maximum temperature} + \text{Minimum temperature})/2) - 15.55^{\circ}\text{C}$ or 60°F] can aid in predicting cotton growth stages based on heat unit accumulation (Table 1.1) (Jenkins et al. 1990). Germination and radical appearance occurs three days after planting. Seedling emergence occurs approximately six days after planting and the

cotyledons unfold one day after emergence. At 14 days after planting the first true leaf unfolds and photosynthesis begins. The first flower bud, or square, appears approximately 35 days after planting. At 55-65 days after planting the first white flower will emerge, which begins pollination and sexual fertilization. Full bloom, or peak flower, is typically reached about 93 days after planting. Boll and fiber development are at the highest level at this point in the growing season. The first boll generally opens around 110 days after planting, which signals the plant is approaching physiological maturity.

Cotton is exclusively grown in warmer climates such as the Southeastern and Southwestern United States due to the heat unit requirements. Cotton has an indeterminate growth habit, meaning that vegetative growth continues after reproductive growth begins, unlike most other cultivated crops grown in the Mid-South (Smith et al. 1999). Due to this indeterminate growth habit, cotton has a much longer flowering period than most other crops and may flower for > 8 weeks. Once cotton begins to flower, growth can be measured by counting nodes above white flower (NAWF). Nodes above white flower is determined by counting the number of main stem nodes above the highest first position white flower (Bourland et al. 1992). A first position flower can be defined as the uppermost fruiting branch that has a white flower at the first position from the main stem (Bourland et al. 1992).

In the Mid-South, cotton is typically planted from the beginning of April until the end of May. Cotton in the Mid-South is grown in a variety of row spacings. Among the most common of these are 91 cm (36"), 96.5 cm (38"), and 102 cm (40") rows. Typically, cotton is planted between 1.25 cm (0.5") and 3.8 cm (1.5") deep (Collins and

Whitaker 2013). Optimal plant populations per hectare range between 98,842 to 123,553 seeds (40,000-50,000 seed per acre). Cotton yield is most commonly measured in bales per hectare or kilograms of lint per hectare. A single bale of harvested seedcotton weighs 217.72 kg. In 2016, the United States' cotton crop yielded an average of 958 kg/ha of lint (NASS 2017).

Table 1.1 Estimates of cotton growth to select growth stages based on heat unit accumulation adapted from Jenkins et al. (1990).

Growth Phase	Heat Units Required
Planting to Emergence	50-60
Each Successive Node up Main Stem	45-60
Emergence to First Square	425-475
Square to White Flower	300-350
Planting to White Flower	775-850
White Flower to Open Boll	850
Planting to Harvest	2600

Insect Pests of Cotton

During the course of the growing season there are numerous arthropod pests that will infest and damage cotton. However, only a small percentage of these pests have the capability to cause economic damage on a yearly basis when if not managed. A number of these economically important pests are commonly found in the Mid-South (Leigh et al. 1996). Thrips in the genus *Franklinella* are important pests from emergence until the

fourth true leaf. Thrips damage is characterized by silvering of lower leaf surfaces as well as deformed, or mouse-eared, leaves (Leigh et al. 1996). Damage from thrips usually causes a delay in cotton maturity, as well as, possible yield loss. Traditionally, the bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), were the primary pests from first-square to cutout. These pests feed directly on cotton fruiting structures. Early instars tend to feed on terminals and small squares while later instars will begin to feed on more mature squares. This feeding on cotton squares cause the squares to “flare” and abscise from the plant (Leigh et al. 1996).

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), was once considered a secondary pest in cotton. However, the eradication of the boll weevil, *Anthonomus grandis grandis* (Boheman), combined with the decrease in foliar insecticide applications for lepidopteran pests due to *Bt* technology has led to the rise of the tarnished plant bug as the most economically damaging insect pest of Mid-South cotton. Tarnished plant bug can feed on cotton at any stage, but is most damaging from first-square to early bloom. This pest can feed on terminals, squares, flowers, and bolls (Leigh et al. 1996). Stink bugs, Pentatomidae, are another late-season pest of cotton. Stink bugs cause damage by inserting their stylet into bolls and feeding on developing seed. This penetration results in warts on the inside of the boll, which causes stained lint and/or shrunken seeds. Stink bug feeding on smaller bolls can cause boll abortion (Leigh et al. 1996).

Tarnished Plant Bug

Biology and Ecology

Taxonomically, tarnished plant bug is a true bug belonging to the family Miridae in the order Hemiptera (Triplehorn and Johnson 2005). Insects in this family are recognized by the presence of a cuneus, as well as, the presence of only one or two closed cells at the base of the wing membrane (Triplehorn and Johnson 2005). Tarnished plant bugs have a Y-shaped mark on the scutellum (Triplehorn and Johnson 2005). Adult tarnished plant bugs are 3.97 to 4.76 mm long, reddish brown in color, and have long slender and typically reddish brown antennae (Leigh et al. 1996). Early instar tarnished plant bugs are often mistaken for aphids due to their pale green color, but can be distinguished by a more rapid pace of movement, as well as, reddish tips on their antennae (Leigh et al. 1996). Later instar tarnished plant bugs can be distinguished by five characteristic black spots on their backs, with two of these spots being on the first segment of the thorax directly behind the head, two more spots on the next segment, and the last spot appearing in the center of the abdomen (Leigh et al. 1996). Adult female tarnished plant bugs lay eggs inside cotton terminals or flower buds (Fleischer and Gaylor 1988). Studies have shown that females are capable of laying up to 175 eggs in their lifetime, at a rate of around 10 eggs per day at 27°C (Ugine 2012). Ridgway and Gyrisco (1960) showed it takes an average of 7.62 days for a nymph to emerge from the egg. From egg to adult there are five nymphal instars present in the life cycle of the tarnished plant bug. Between each nymphal stage there are 4.77 days, 3.08 days, 3.28 days, 3.33 days, and 5.22 days on average, respectively (Ridgway and Gyrisco 1960). Ugine (2012) showed at 30°C, the generation time for a population was 21 days and the

doubling time was 3.7 days. In the Mid-South, a tarnished plant bug typically takes 30 to 40 days to fully complete development and may have several generations per year.

The tarnished plant bug is a polyphagous insect pest with an extremely wide host range. It has been observed to use over 385 host plants and possibly has the widest host range of any insect (Young 1986). These host plants range from agronomic crops such as corn, soybean, and cotton to weeds, fruits, and vegetables. Generally, the first 1-2 generations will occur on these alternate wild early season hosts. As alternate hosts begin to senesce in late spring tarnished plant bug moves into agronomic crops such as corn, soybean, and cotton (Layton 1995). The senescence of corn typically corresponds with the beginning of cotton bloom, resulting in a movement of large populations of tarnished plant bug out of corn into cotton. Tarnished plant bug comprises 94% of the collected bugs in flowering cotton within the Mid-South (Musser et al. 2007). Layton (1995) showed that the intensity and extent of populations moving into cotton typically varies between years, but has a correlation with the number of alternate hosts present, as well as, the availability of reproductive structures.

Feeding and Damage to Cotton

As previously mentioned, tarnished plant bug is the most important insect pest of cotton in the Mid-South. This importance stems from the fact that this pest can cause damage at any growth stage, from emergence to the last maturing bolls, although most economic damage occurs from first-square to early bloom (Black 1973, Layton 2000). Tarnished plant bug prefers to feed on reproductive structures of cotton, such as flower buds, flowers, and bolls. Tarnished plant bugs feed by injecting digestive salivary enzymes into plant tissue which allows ingestion of nutrients (Layton 1995). This

feeding results in swollen nodes, shortened internodes, deformed leaves, aborted terminals, excessive branching along the main stem, as well as delayed fruiting (Hanny et al. 1977). Scales and Furr (1968) noted that in pre-squaring cotton tarnished plant bug causes a loss of apical dominance by feeding on terminal tissues. This results in the formation of multiple secondary terminals, often referred to as “crazy cotton”. Tarnished plant bug preferentially feeds on squares less than 3.18 mm diameter in contrast to squares greater in size or bolls (Tugwell et al. 1976). Feeding on small squares leads to their abscission, causing a direct loss to yield (Layton 1995). Gutierrez et al. (1977) showed a single tarnished plant bug is capable of causing 0.6 to 2.1 squares to abscise per day due to feeding. Tarnished plant bug will continue to feed on cotton during the flowering period and is capable of causing significant yield loss during this growth stage. Feeding during the flowering period will produce symptoms such as staining in the bloom, brown or black anthers in the flower, and the presence of black necrotic spots on the outside of bolls. Pack and Tugwell (1976) observed a correlation between dirty anthers and boll damage. At 30% anther damage from tarnished plant bug feeding there is little to no effect on yield. When anther damage is more severe than 30%, the damaged anthers can result in an increase in malformed bolls and higher percent of boll shed. After anthesis, bolls are susceptible to damage by tarnished plant bug feeding for up to eight days (Greene et al. 1999). Once the boll has accumulated 250-300 heat units after anthesis, the lint yield is safe from tarnished plant bug damage (Horn et al. 1999).

Thresholds and Sampling

Due to the presence of tarnished plant bug in cotton throughout the entire growing season and the physiology of cotton there are several sampling techniques to determine

plant bug densities. A 38 cm diameter sweep net is used during the squaring period, with the purpose being to sweep back and forth across a row to dislodge insects from the plant and “sweeping” them into the net. In Mississippi, the current recommended sweep net threshold during the squaring period is eight tarnished plant bugs per 100 sweeps (Catchot et al. 2013). When cotton has begun to flower the use of a 0.76 m black drop cloth is implemented. The drop cloth is placed between adjacent rows and all cotton plants within the width of the drop cloth are shaken forcefully over the cloth. The number of insects that have fallen on the drop cloth are then counted. The current recommended drop cloth threshold in Mississippi is three tarnished plant bugs per 1.83m or three tarnished plant bugs per one drop cloth sample (Catchot et al. 2013). A plant-based sampling technique to determine tarnished plant bug damage during the squaring period is square retention counts. The application threshold for square retention is to treat when less than 80% of first position squares remain on the plant prior to first bloom. Additionally there is a plant-based sampling technique to determine tarnished plant bug damage during the flowering period is dirty square counts. The application threshold for “dirty square” counts is 10% dirty squares, looking at medium-sized squares with exposed buds that have been discolored by plant bug feeding. There is also a visual sampling technique and the threshold for this method is ten tarnished plant bugs per 100 plants (Catchot et al. 2013).

Management Practices

Tarnished plant bug is almost exclusively managed with foliar insecticide applications. The occurrence of insecticide resistance to several chemical classes of insecticides such as organophosphates, carbamates, and pyrethroids over the past decade

has intensified the necessity of other control options for this pest. Another insecticide option that has risen in popularity due to resistance is neonicotinoids, primarily imidicloprid (Admire Pro, Bayer Crop Science, Raleigh, NC) and thiamethoxam (Centric, Syngenta Crop Protection, Greensboro, NC). Sulfoxaflor (Transform, Dow AgroSciences, Indianapolis, IN) has recently become another effective, albeit more expensive, management option for tarnished plant bug. The insect growth regulator novaluron (Diamond® 0.83EC, ADAMA USA, Raleigh, NC) is a class of insecticide that can be effective at managing tarnished plant bugs, especially if administered at peak adult migration into cotton, which usually occurs around the third week of squaring. This third week of squaring application is effective because it is generally the same point in the growing season that corn begins to senesce, leading to large populations of tarnished plant bug migrating from corn into cotton. The 2017 Insect Control Guide for Agronomic Crops from Mississippi State University has outlined an insecticide rotation strategy for tarnished plant bugs for the purpose of avoiding further resistance selection (Catchot et al. 2017).

In conjunction with foliar insecticides, planting date and certain plant characteristics have been shown to assist in managing tarnished plant bug. Foliar insecticide applications can be significantly reduced by implementing an early planting date, as well as, planting an early maturing variety (Adams et al. 2012). Controlling host plants such as *Lamium amplexicaule* (L.) and *Capsella bursa-pastoris* (L.) in the spring with a selective herbicide can also reduce control costs for tarnished plant bug later in the growing season (Gore et al. 2010). Cotton field edges, particularly those that border corn, have been shown to hold a higher density of tarnished plant bug populations. To

combat this “edge effect”, Gore et al. (2010) promotes that cotton should be planted in large continuous blocks and planting next to other crops that tarnished plant bug prefer, such as corn, should be minimized. Cotton varieties with high leaf trichome densities have been shown to reduce tarnished plant bug damage (Wood et al. 2017). Biological control is another major factor in the management of tarnished plant bug populations in cotton. A small wasp, *Anaphes iole* (Girault), parasitizes tarnished plant bug eggs and lays its own eggs inside, consequently killing the tarnished plant bug eggs. A myriad of other beneficial insects prey on tarnished plant bug populations such as green lacewings, *Chrysoperia rufilabris* (Brumeister), minute pirate bugs, *Orius insidiosus* (Say), and big eyed bugs, *Geocoris* spp.

***Bacillus thuringiensis* in Row Crops**

Biology of *Bacillus thuringiensis*

Bacillus thuringiensis, or *Bt*, is a soil bacterium which forms spores during the stationary phase of its growth cycle (Sanahuja et al. 2011). *Bt* produces insecticidal proteins during the sporulation phase as parasporal proteins (Bravo et al. 2007). The crystals are comprised of mostly Crystal (Cry) and Cytolytic (Cyt) toxins. There are many *Bt* strains, each of which produce different toxins and can affect different taxonomic groups of insects (Sanahuja et al. 2011). These toxins have several beneficial characteristics such as being extremely specific to their target insect, harmless to humans, vertebrates and plants, and are completely biodegradable, making them an excellent option for the management of insect pests in agriculture (Bravo et al. 2005). Early experiments verified that *Bt* toxins require activation in the insect’s gut, which requires an alkaline environment and the presence of specific proteases, which transforms the pro-

toxin into its active form (Sanahuja et al. 2011). Once the pro-toxin is activated, each toxin adheres to receptors in the brush border membrane, causing pores to form. This opening of pores disrupts the progression of solutes across the gut epithelium, resulting in the influx of water (Sanahuja et al. 2011).

History of *Bacillus thuringiensis*

In 1901 the bacterium *Bacillus thuringiensis*, or *Bt*, was isolated by Japanese biologist Shigetane Ishiwatari while researching wilt disease in silk worms (Sanahuja et al. 2011). A decade later, Ernst Berliner also isolated *Bt*, this time in a diseased *Ephestia kuehniella* (Zeller). In 1927, O. Mattes recognized the insecticidal potential of *Bt*, isolated the *Bt* strain discovered by Berliner and conducted field trials against the European corn borer, *Ostrinia nubilalis* (Hübner). Mattes work led to the development and production of the first commercial *Bt* insecticide, Sporeine, used for the first time in 1938 in France (Sanahuja et al. 2011). In 1961 Sporeine was introduced to the United States as a registered pesticide. At this point several other *Bt* products had made an appearance on the market including Thuricide. New products were based on the *kurstaki* strain HD1 and were effective against lepidopteran pests (Sanahuja et al. 2011). Early *Bt* strains struggled to gain market-share in the U.S. market due to their poor performance compared to current chemical pesticide options. Two strategies were implemented to improve the performance of these *Bt* products, process development to increase the efficiency of the *Bt* products and strain improvement to increase the intrinsic toxicity of the bacteria (Sanahuja et al. 2011). *Bt* products saw a sixfold increase in efficacy in the field due to these efforts (Burgess and Jones 1998). The 1960s saw a revolution of *Bt*

strains that were far more potent than their predecessors and the search for improved strains continues to this day (Sanahuja et al. 2011).

Introduction of *Bacillus thuringiensis* to Row Crops

Early experiments with foliar *Bt* pesticides encountered several issues, including a short window of effectiveness, as well as, poor weather rendering the product useless within hours (Sanahuja et al. 2011). This resulted in the need for multiple applications throughout the growing season, which increased the amount of product necessary to be effective as well as the excessive fuel being used to make spray applications. These issues were potentially solved in the mid-1980s when scientists discovered the ability to introduce *Bt cry* genes directly into tobacco and tomato plants so the proteins would express directly in plant tissues (Sanahuja et al. 2011). In 1986 field trials were conducted with *Bt* transgenic tobacco in the United States and France in an effort to protect the plants from leaf damage caused by corn earworm, *Helicoverpa zea*. The success of these field trials, as well as others being conducted around the same time, resulted in trials with cotton, maize, and rice shortly thereafter (Sanahuja et al. 2011). The US Environmental Protection Agency approved the first registration of *Bt* potato, corn and cotton in 1995. The positive effects of *Bt* crops had become abundantly clear by 1998 across the agricultural and environmental spectrum. In 2002, Monsanto's Bollgard II cotton (event 15985), which expressed two *Bt* toxins, Cry1Ac and Cry2Ab, was approved. In 2003, the first stacked variety was developed by crossing two previously released *Bt* varieties. This product was Monsanto's YieldGard Plus (event MON 810 + MON 863) which expressed Cry1Ab1 and Cry3Bb1 (Sanahuja et al. 2011).

Justification for Research

Control costs for tarnished plant bugs has become an exceedingly troubling issue for cotton growers in the Mid-South since the mid-to late 1990s. This increase in control costs is partly due to the development of resistance to many once effective insecticide classes such as organophosphates, carbamates, and pyrethroids. The eradication of the boll weevil, as well as the introduction of transgenic cotton varieties in 1996 targeting lepidopteran pests of the heliothine complex have dramatically reduced the number of insecticide applications that had previously provided excellent control of tarnished plant bugs (Musser et al. 2009). Cleveland and Furr (1979) noted tarnished plant bug resistance to methyl parathion in the Mississippi River Delta. Later, dimethoate resistance was studied in the Mississippi Delta by Snodgrass and Scott (1988). An increase in resistance to pyrethroids, organophosphates, and cyclodiene insecticides in the Mississippi Delta were observed in 1996 (Snodgrass 1996). The first documentation of acephate resistance was in 2005 in one county in Mississippi. By 2006, acephate resistance was discovered to be widespread across the entire region (Snodgrass and Gore 2007).

This increase in control costs for tarnished plant bug coupled with growing resistance to a wide range of insecticides has left a concerning void in current integrated pest management strategies for this pest. An experimental transgenic cotton variety from Monsanto, MON 88702 (Cry51Aa2.834_16), is currently being tested to help fill this gap in current strategies. MON 88702 targets hemipteran and thysanopteran insect pests, particularly the tarnished plant bug and thrips, *Frankliniella* spp. (Bachman et al. 2017). Little research has been conducted on this new technology, so the following objectives

have been proposed. The objectives of this research were to determine the appropriate threshold for tarnished plant bug on cotton modified to be resistant to *Lygus* (MON 88702) and to determine the value of early season (pre-flowering) protection compared to mid-late season (flowering) protection on cotton modified to be resistant to *Lygus* (MON 88702)

References

- Adams, B. 2012. The biology and management of tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois), in cotton, *Gossypium hirsutum* (L.), in the Mississippi Delta. M.S. Thesis, Mississippi State University, Mississippi State, MS.
- Bachman, P.M., A. Ahmad, J.E. Ahrens, W. Akbar, J.A. Baum, S. Brown, T.L. Clark, J.M. Fridley, A. Gowda, J.T. Greenplate, P.D. Jensen, G.M. Mueller, M.L. Odegaard, J. Tan, J.P. Uffman, and S.L. Levine. 2017. Characterization of the activity spectrum of Mon 88702 and the plant-incorporated protectant Cry51aa2. 834_16. PloS one 12.1: e0169409.
- Black, E.R. 1973. Economic threshold studies of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), in cotton. 108 pp. Ph.D. Dissertation, Department of Entomology, Mississippi State University. Mississippi State, MS.
- Bourland, F.M., N.P. Tugwell, and D.M. Oosterhuis. 1992. Concept for monitoring the growth and development of cotton plants using the mainstem node counts. J. Prod. Agric. 5: 532-538.
- Bravo, A., M. Soberon, and S.S. Gill. 2005. *Bacillus thuringiensis* mechanisms and use. Comprehensive Molecular Insect Science 6: 175-205.
- Bravo, A., M. Soberon, and S.S. Gill. 2007. Mode of action of *Bacillus thuringiensis* Cry and Cyt toxins and their potential for insect control. Toxicon 49: 423-435.
- Burges, H.D. and K.A. Jones 1998. Trends in formulation of microorganisms and future research requirements. Formulation of microbial biopesticides, beneficial microorganisms, nematodes and seed treatment. Dordrecht (DE): Kluwer Academic Publication. Hlm: 311-332.
- Catchot, A., D.R. Cook, J. Gore, F. Musser, D. Dodds, C. Allen, R. Jackson, E. Larson, B. Layton, R. Luttrell, and B. Von Kanel. 2013. Insect control guide for agronomic crops, Mississippi State University Extension Service. Mississippi State, MS.
- Catchot, A., C. Allen, J. Bibb, D. Cook, W. Crow, J. Dean, D. Fleming, J. Gore, B. Layton, N. Little, J. MacGown, F. Musser, S. Winter, D. Dodds, T. Irby, E. Larson, and S. Meyers. 2017. Insect control guide for agronomic crops, Mississippi State University Extension Service. Mississippi State, MS.
- Cleveland, T. and R. Furr 1979. Toxicity of methyl parathion applied topically to tarnished plant bugs. J. Georgia Entomol. Soc. 15: 304-307.
- Collins, G.D., J. Whitaker, S. Culpepper, G. Harris, B. Kemerait, C. Perry, P. Roberts, D. Shurley, and A. Smith. 2013. Georgia cotton production guide. Publication No. CSS-13-01. University of Georgia Cooperative Extension Service, Tifton, GA, 2013.

- Fleischer, S.J. and M.J. Gaylor 1988. *Lygus lineolaris* (Hemiptera: Miridae) population dynamics: nymphal development, life tables, and leslie matrices on selected weeds and cotton. *Environ. Entomol.* 17: 246-253.
- Gore, J., G. Andrews, D. Cook, A. Catchot, F. Musser, J. Smith, G. Snodgrass, R. Jackson, C. Allen, D. Dodds, G. Lorenz, S. Akin, G. Studebaker, R. Leonard, S. Stewart and K. Tindall. 2010. Strategies for managing tarnished plant bugs in cotton. *MAFES Bulletin* 1179.
- Greene, J.K., S.G. Turnipseed, M.J. Sullivan and G.A. Herzog. 1999. Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. *J. Econ. Entomol.* 92: 941-944.
- Gutierrez, A.P., T.F. Leigh, Y. Wang, and R.D. Cave. 1977. An analysis of cotton production of cotton production in California: *Lygus hesperus* (Heteroptera: Miridae) injury- an evaluation. *Can. Entomol.* 109: 1375-1386.
- Hanny, B.W., T.C. Cleveland and J.W.R. Meredith 1977. Effects of tarnished plant bug, *Lygus lineolaris*, infestation on presquaring cotton, *Gossypium hirsutum*. *Environ. Entomol.* 6: 460-462.
- Horn, T.O., F.A. Harris, J.T. Robbins and R.E. Furr, Jr. 1999. Influence of boll age on susceptibility to tarnished plant bug injury. *In Proc. Beltwide Cotton Conference*, Orlando, FL. 1044-1045.
- Jenkins, J.N., J.C. McCarty and W.L. Parrott. 1990. Effectiveness of fruiting sites in cotton: yield. *Crop Sci.* 30: 365-369.
- Layton, M. 1995. Tarnished plant bug: biology, thresholds, sampling, and status of resistance, pp. 131-134. *In Proc. Beltwide Cotton Res. Conf., National Cotton Council*, Memphis, TN.
- Layton, M.B. 2000. Biology and damage of the tarnished plant bug, *Lygus lineolaris*, in cotton. *Southwest. Entomol. Suppl.* 23: 7-20.
- Leigh, T.F., S.H. Roach, and T.F. Watson. 1996. Biology and ecology of important insect and mite pests of cotton, pp. 17-85. *In E. G. King, J.R. Phillips, and R.J. Coleman [eds.], Cotton insects and mites: characterization and management.* The Cotton Foundation, Memphis, TN.
- 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.

Musser, F.R., A.L. Catchot, S.D. Stewart, R.D. Bagwell, G.M. Lorenz, K.V. Tindall, G.E. Studebaker, B.R. Leonard, D.S. Akin, D.R. Cook and C.A. Daves. 2009. Tarnished plant bug (Hemiptera: Miridae) thresholds and sampling comparisons for flowering cotton in the midsouthern United States. *J. Econ. Entomol.* 102: 1827-1836.

NASS. 2017. Cotton production data.

https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=MISSISSIPPI

Oosterhuis, D.M. 1990. Growth and development of a cotton plant, *In*: W.N. Miley and D.M. Oosterhuis (eds) Nitrogen Nutrition in Cotton: Practical Issues. Proc. Southern Branch.

Pack, T.M., and N.P. Tugwell, 1976. Clouded and tarnished plant bugs on cotton: a comparison of injury symptoms and damage of fruit parts, 17 pp. AR Agric. Exp. Stn. Report University of Arkansas. Fayetteville, AR.226

Ridgway, R.L., and G.C. Gyrisco, 1960. Effect of temperature on the rate of development of *Lygus lineolaris* (Hemiptera: Miridae). *Ann. Entomol. Soc. Am.* 53: 691-694.

Sanahuja, G., R. Banakar, R.M. Twyman, T. Capell, and P. Christou. 2011. *Bacillus thuringiensis*: a century of research, development and commercial applications. *Plant biotechnology journal* 9, no. 3: 283-300.

Scales, A.L., and R.E. Furr, 1968. Relationship between the tarnished plant bug and deformed cotton plants. *J. Econ. Entomol.* 61: 114-118.

Smith, C.W., and J.T. Cothren, 1999. Cotton: origin, history, technology, and production. Vol. 4. John Wiley & Sons.

Snodgrass, G.L. 1996. Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. *J. Econ. Entomol.* 89: 783-790.

Snodgrass, G., and J. Gore, 2007. Status of insecticide resistance for the tarnished plant bug, pp. 56-59. *In* Proc. Beltwide Cotton Res. Conference. Nation Cotton Council, Memphis, TN.

Snodgrass, G. and W. Scott 1988. Tolerance of the tarnished plant bug to dimethoate and acephate in different areas of the Mississippi delta. pp. 294-295. *In* Proc. Beltwide Cotton Res. Conf., National Cotton Council, Memphis, TN.

Triplehorn, C.A. and N.F. Johnson 2005. Borror and DeLong's introduction to the study of insects. Thomson Brooks/Cole, Belmont, CA.

Tugwell, P., S.C. Young, B.A. Dumas and J.R. Phillips 1976. Plant bugs in cotton: importance of infestation time, types of cotton injury, and significance of wild hosts near cotton pp. AR Agric. Exp. Stn. Report University of Arkansas. Fayetteville, AR

Ugine, J.A. 2012. Developmental times and age specific life tables for *Lygus lineolaris* (Heteroptera: Miridae), reared at multiple constant temperatures. Environ. Entomol. 41: 1-10.

Wood, W., J. Gore, A. Catchot, D. Cook, D. Dodds, and L.J. Krutz. 2017. Effect of leaf pubescence on tarnished plant bug (Hemiptera: Miridae) ability to cause damage and yield loss in cotton. J. of Cott. Sci. 21, no. 2: 122-127.

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

CHAPTER II
DETERMINING THE APPROPRIATE THRESHOLD FOR TARNISHED PLANT
BUG ON MON 88702 COTTON

Abstract

An experiment was conducted in Sidon, MS and Stoneville, MS during 2016 and 2017 to compare the impact of insecticide application thresholds for tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), on *Bt* traited cotton (MON 88702, Cry51Aa2.834_16) and non-traited cotton (DP 393). Insecticide applications were made when tarnished plant bug populations reached or exceeded current Mississippi thresholds. An additional insecticide treatment was applied when double the current Mississippi threshold was met. These treatments were compared to an untreated control and a weekly control. Planting MON 88702 resulted in a significant reduction in later instar tarnished plant bug nymphs, while having little effect on smaller nymphs and adults. MON 88702 cotton resulted in significantly fewer insecticide applications for both treatment regimes. Averaged across traited and non-traited plots, plots treated at the current Mississippi threshold yielded significantly greater than plots sprayed at 2X the current Mississippi threshold and the untreated control. Averaged across all threshold treatments, MON 88702 yielded significantly greater than DP 393. Results from this experiment will be important for incorporating this new trait into the current IPM program for tarnished plant bug in cotton.

Introduction

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is the most economically damaging insect pest of cotton in the Mid-South region of the U.S. including the states of Arkansas, Mississippi, Louisiana, Tennessee, and Missouri (Musser et al. 2007, Gore et al. 2012). Tarnished plant bug resistance to numerous insecticide classes has become prevalent across the Mid-South (Snodgrass 1996, Snodgrass et al. 2009). In the Mississippi Delta, tarnished plant bug has been found to be 54-fold more tolerant to permethrin and 35-fold more tolerant to bifenthrin when compared to other populations (Snodgrass 1994). Resistance to pyrethroid insecticides was documented in field populations of tarnished plant bug in 1996 (Snodgrass 1996). Tarnished plant bugs collected in the Mississippi Delta have been found to be more resistant to dimethoate than tarnished plant bugs collected in other areas of Mississippi (Snodgrass and Scott 1988). Tarnished plant bug resistance to organophosphate insecticides was documented in 2009 (Snodgrass et al. 2009). Williams (2014) reported that 5-7 insecticide applications were necessary throughout the season to prevent economic losses due to this resistance, with each application averaging a cost of \$26.63 per hectare. Increasing costs for tarnished plant bug management with foliar insecticides, in addition to other input costs such as technology fees associated with the purchase of transgenic seed varieties, seed treatments, increased fuel and fertilizer costs, and increased herbicide use due to resistant weed species, as well as control costs for other insect pests in cotton (Riley et al. 2010), have led to a decrease in cotton acres across the Mid-South.

Tarnished plant bug can cause damage to cotton at any growth stage, with most of the economic damage occurring from first-square until early bloom (Scales and Furr 1968). Early season by feeding on terminals by tarnished plant bugs may result in “crazy cotton”. This damage can be identified by the presence of multiple secondary terminals due to the loss of apical dominance. Tarnished plant bug populations exceeding current action thresholds in early season cotton can result in a reduction of plant height and boll weight, as well as swollen nodes, deformed leaves, and delayed maturity (Scales and Furr 1968, Hanny et al. 1977). Damage from tarnished plant bug remains localized to the area of feeding, and does not appear systemically in other parts of the cotton plant (Layton 1995). Cotton squares less than 3.18 mm in diameter are preferred feeding sites for tarnished plant bug as opposed to bolls and larger squares (Tugwell et al. 1976). Feeding on small cotton squares results in abscission of those squares (Layton 1995) which can result in an altered fruiting pattern if early square loss is excessive. A single tarnished plant bug can cause the abscission of 0.6 to 2.1 squares per day (Gutierrez et al. 1977). Tarnished plant bug will also feed on larger squares, which may also abscise, but generally remain on the plant and produce a bloom depending on the severity of the feeding damage. This damage will be apparent on cotton blooms as the anthers will be dark brown in color. Flowers with discolored anthers are often referred to as “dirty blooms”. There has been shown to be a correlation between “dirty blooms” and boll damage. When $\leq 30\%$ of anthers are damaged there is little to no yield loss, compared to an increase in malformed bolls and percent of boll shed when anther damage is $> 30\%$ (Pack and Tugwell 1976). Boll loss and malformed bolls is likely the result of poor pollination due to damaged anthers caused by tarnished plant bug feeding. Tarnished

plant bug also causes direct boll damage by feeding directly on bolls, causing sunken lesions on the outside of the boll that eventually turns black and necrotic (Pack and Tugwell 1976). Tarnished plant bug feeding damage on larger, more developed bolls is not as common, but can result in individual seed damage which results in discolored lint and a reduction in overall boll weight (Pack and Tugwell 1976).

Historically, the tarnished plant bug was considered a secondary pest of cotton in the Mid-South; however, the reduced need for insecticide applications due to the eradication of the boll weevil, *Anthonomus grandis grandis* (Boheman), and control of lepidopteran pests with *Bt* technology has resulted in tarnished plant bug being the most economically important insect pest cotton growers in the Mid-South encounter. Until recently, *Bt* cotton has been confined to the control of lepidopteran pests in cotton. Monsanto Company is currently developing a transgenic event for cotton (MON 88702) that expresses the Cry51Aa2.834_16 protein from *Bacillus thuringiensis* that targets hemipteran and thysanopteran insect pests, particularly tarnished plant bug and thrips, *Frankliniella* spp. (Bachman et al. 2017). This new *Bt* event is being developed with hopes of reducing insecticide control costs for tarnished plant bug. MON 88702 should also extend the useful life of other insecticides by reducing the exposure of tarnished plant bug to current insecticides, resulting in a decreased rate of resistance. MON 88702 is not expected to be 100% effective on tarnished plant bug. Research is needed to determine at what population density this event provides effective control of tarnished plant bug without sustaining economic loss. Therefore, research was conducted to determine the appropriate threshold for tarnished plant bug on cotton expressing MON 88702.

Materials and Methods

Experiments were conducted at the Delta Research and Extension Center in Stoneville, MS and on Sidon Plantation in Sidon, MS in 2016 and 2017 to determine the appropriate threshold for tarnished plant bug on cotton containing the MON 88702 gene. Trials were arranged in a randomized complete block design with a two by four factorial arrangement of treatments. Factor A was traited cotton variety [MON 88702] that expresses the Cry51Aa2.834_16 protein from *Bacillus thuringiensis* versus a conventional cotton variety (Delta Pine 393). Factor B consisted of threshold treatments which included an untreated control, weekly control, treatments applied using the current Mississippi threshold, and treatments applied using 2X the current Mississippi threshold. The current Mississippi square retention threshold is to treat when less than 80% of first position squares remain on the plant prior to first bloom (Catchot et al. 2018). The current Mississippi sweep net threshold is eight tarnished plant bugs per 100 sweeps during the first two weeks of squaring and fifteen tarnished plant bugs per 100 sweeps from the third week of squaring through bloom (Catchot et al. 2018). The current Mississippi drop cloth threshold is one tarnished plant bug per 1.5 row meters during the first two weeks of squaring and three tarnished plant bugs per 1.5 row meters from the third week of squaring through bloom (Catchot et al. 2018). The current Mississippi threshold for dirty squares is when 10% of the squares are dirty, looking at medium sized squares with exposed buds that have been discolored by plant bug feeding (Catchot et al. 2018).

Planting dates during 2016 were 17 May in Sidon, MS and 23 May in Stoneville, MS. Both trials were planted on 17 May during 2017. Plots consisted of 97 cm rows in Sidon, MS and 102 cm rows in Stoneville, MS with 12.2 meter length in both locations.

Cotton was planted at 120,000 seed per hectare into raised conventional tilled beds. Seed were treated with a commercial premix of thiodicarb, imidiclopid, trifloxystrobin, triadimenol, and metalaxyl (Aeris Trilex Advanced, Bayer CropScience, Raleigh, NC). A preemergence application of locally recommended herbicides was utilized within the trial area for control of summer annual weeds. Planting depth was approximately 2.5 cm below the soil surface. Furrow irrigation was applied to the trial area as needed. Other insect pests were managed as needed with insecticides that do not have activity against tarnished plant bug.

Data collection methods for tarnished plant bug varied throughout the growing season depending on cotton growth stage. During the first three weeks of squaring, square retention was recorded weekly in each plot. First position fruiting sites from the upper three nodes on 25 plants per plot were examined to determine square (flower bud) retention. Missing cotton squares were considered abscised. The presence of an abscission scar at the first position on each fruiting branch was used as an indicator for a missing square. Squares with evidence of tarnished plant bug damage such as blasted squares and squares with open (flared) bracts were also considered abscised. Also during the first three weeks of squaring, samples were taken with a 38 cm diameter sweep net twice per week. 15 sweeps per plot were taken, alternating sampling on row one and row four. During the flowering stages, all plots were sampled once per week with a 1.52 m black drop cloth. Two drop cloth samples were collected per plot. Samples were taken by positioning the drop cloth between the second and third rows near the center of the plot and vigorously shaking all of the cotton plants from each row onto the cloth. All tarnished plant bug numbers were separated into four categories: adults, small nymphs

(first and second instars), medium nymphs (third and fourth instars), and large nymphs (fifth instars). In conjunction with drop cloth samples, dirty square samples were also taken weekly during the flowering period. A total of 25 randomly selected squares per plot were visually examined for evidence of tarnished plant bug feeding.

The untreated control plots were not sprayed for tarnished plant bug at any point during the growing season, regardless of tarnished plant bug population densities. The weekly control plots were sprayed once per week, regardless of tarnished plant bug population densities. The current Mississippi threshold plots and double current Mississippi threshold plots were sprayed the same day as sampling when the threshold was exceeded. Thresholds were based on an average of all four replications. Insecticides utilized for control of tarnished plant bug included sulfoxaflor (Transform WG, DOW AgroSciences, Indianapolis, IN), thiamethoxam (Centric 40 WG, Syngenta Crop Protection, Greensboro, NC), acephate (Orthene 90S, Valent Corporation, Walnut Creek, CA), and acephate plus bifenthrin (Brigade® 2EC, FMC Corporation, Princeton, NJ). Tarnished plant bug sampling was terminated when cotton reached five nodes above white flower (NAWF) plus 350-400 heat units (DD60s). To calculate nodes above white flower, main stem nodes were counted above the uppermost first position white flower (Bourland et al. 1992). Tarnished plant bug cannot damage cotton bolls once they have accumulated at least 300 heat units (Russell et al. 1999). Consequently, it can be assumed that the latest harvestable bolls are safe from tarnished plant damage when plants average five nodes above white flower plus 300 heat units. At the end of the growing season, rows two and three of every plot were harvested mechanically with a cotton picker modified for small plot harvest and seedcotton weights were recorded. Lint

yield was calculated using a lint percentage of 39%. All data were analyzed using analysis of variance (PROC GLIMMIX, SAS version 9.3, Cary, NC). Trait and insecticide regime were considered fixed effects, while site-year was designated as a random effect. Due to low tarnished plant bug populations throughout the 2017 growing season in Sidon, MS, a separate analysis was conducted on this location. Tarnished plant bug densities, number of dirty squares, square retention, number of insecticide applications looking at only current thresholds and 2X thresholds, and yield were considered response variables. For all analyses, degrees of freedom were calculated using the Kenwood-Rogers method. Means were calculated using the LSMEANS statement and separated based on Fisher's protected least significant difference ($\alpha=0.05$).

Results

All Site Years except Sidon 2017

Pre-Bloom Samples

For small nymphs, an interaction between trait and insecticide regime was observed ($F=3.67$; $df=3, 14$; $P=0.04$). For the 2X threshold treatment, there was a significant difference between the traited and non-traited cotton but not for the other spray treatments (Fig. 2.1). No significant differences were observed for medium nymphs ($F=1.36$; $df=3$; $P=0.30$). For large nymphs ($F=3.65$, $df=3, 16$; $P=0.04$) and adults ($F=4.02$, $df=3, 14$; $P=0.03$), there was a significant effect of spray treatment. In general, the weekly spray treatment had fewer tarnished plant bugs than the untreated control (data not shown). There was no effect on square retention due to trait ($F=3.52$; $df=1$; $P=0.08$) or spray treatment ($F=1.84$; $df=3$; $P=0.18$) nor their interaction ($F=0.72$; $df=3$; $P=0.55$).

Bloom Samples

Small and medium nymphs were affected by trait ($F=4.88$; $df=1, 16$; $P=0.04$) and spray treatment ($F=5.59$; $df=3, 16$; $P<0.01$), but not by the interaction of trait and spray treatment ($F=0.58$; $df=3$; $P=0.63$). Non-treated cotton had more small (3.96 ± 0.55) and medium (1.73 ± 0.18) nymphs than the cotton with MON 88702 (2.35 ± 0.25 and 0.93 ± 0.10 , respectively). Additionally, the weekly application and current threshold treatments resulted in fewer small and medium nymphs than the untreated control and the 2X threshold. For large nymphs, there was a significant trait by spray treatment interaction ($F=3.79$; $df=3, 14$; $P=0.04$). Fewer nymphs were observed on cotton with MON 88702 than conventional cotton for the current threshold treatment and untreated control. No differences were observed between the cotton with MON 88702 and conventional cotton for the double current standard threshold treatment or weekly sprays (Fig. 2.2). For adults, there was significant effect of trait ($F=17.9$; $df=1, 14$; $P<0.01$) and spray treatment ($F=13.76$; $df=3, 14$; $P<0.01$), but the interaction was not significant ($F=2.64$; $df=3$; $P=0.09$). The conventional cotton (1.13 ± 0.10) had more adults than the cotton with MON 88702 (0.63 ± 0.063).

There was a significant effect of spray treatment for number of dirty squares ($F=16.48$; $df=3, 14$; $P<0.01$); however, trait ($F=4.14$; $df=1$; $P=0.06$) and trait and spray treatment interaction was not significant ($F=3.05$; $df=3$; $P=0.06$). Plots treated weekly (0.76 ± 0.10) had significantly fewer dirty squares than the non-treated plots (2.31 ± 0.22) or plots treated at the 2X threshold (1.54 ± 0.18).

Insecticide Applications

There was a significant trait by spray treatment interaction for number of insecticide applications ($F=9.45$; $df=1, 42$; $P<0.01$). For both the current and 2X threshold control spray treatments, there was a significant difference between the cotton with MON 88702 and conventional cotton. Cotton with MON 88702 required significantly fewer insecticide applications than the conventional cotton (Fig. 2.3). The current threshold control spray treatment required more insecticide applications (4.17 ± 0.28) than the 2X threshold control spray treatment (2.33 ± 0.29).

Yield

There was a significant effect of trait ($F=16.9$; $df=1, 86$; $P<0.01$) and spray treatment ($F=40.47$; $df=3, 86$; $P<0.01$) in yield, but the interaction was not significant ($F=2.14$; $df=3$; $P=0.10$). The weekly control and current threshold treatments resulted in greater cotton yields than the 2X threshold treatment and the untreated control (Fig. 2.4). The 2X threshold treatment resulted in greater cotton yields than the untreated control. Additionally, the cotton with MON 88702 had greater yields than the conventional cotton (Fig. 2.5).

Sidon 2017

Pre-Bloom Samples

Trait affected adult tarnished plant bug populations ($F=4.45$; $df=1, 120$; $P=0.04$). Conventional cotton (1.03 ± 0.18) had more adults than the cotton with MON 88702 (0.59 ± 0.10). There were no differences in nymph populations due to the presence or absence of *Bt* traits. Presence of a *Bt* trait affected square retention ($F=11.28$; $df=1, 88$;

P<0.01). Square retention was greater in cotton with MON 88702 (84.08±1.39) than in conventional cotton (75.46±2.19).

Bloom Samples

Spray treatment affected small (F=9.92; df=3, 152; P<0.01) and medium (F=7.9; df=3, 152; P<0.01) nymphs. For large nymphs, there was a significant trait by spray treatment interaction (F=3.02; df=3, 152; P=0.03). For the 2X threshold treatment and the untreated control treatment, there was a significant difference between the cotton with MON 88702 and conventional cotton, but not for the other spray treatments (Fig. 2.6). Adult tarnished plant bug populations were affected by spray treatment (F=5.1; df=3, 152; P<0.01) but not by trait (F=1.07; df=1; P=0.30). The number of dirty squares was affected by spray treatment (F=4.86; df=3, 120; P<0.01), but not by trait (F=0.09; df=1; P=0.76). Untreated control treatments (1.03±0.21) had significantly more dirty squares than weekly control spray treatments (0.31±0.09).

Insecticide Applications

The number of required insecticide applications was affected by trait (F=14; df=1, 14; P<0.01) and spray treatment (F=14; df=1, 14; P<0.01). The current threshold control spray treatment required more insecticide applications (1.5±0.19) than the 2X threshold control spray treatment (0.5±0.19). More insecticide applications were required in conventional cotton than in cotton with MON 88702.

Yield

Yield was affected by a trait by spray treatment interaction (F=4.86; df=3, 24; P=0.01). For the weekly control spray treatment, conventional cotton produced greater

yields than cotton with MON 88702 (Fig. 2.7). No significant yield differences were observed due to spray treatment ($P>0.05$).

Discussion

Based on these data, it is evident that cotton containing the MON 88702 trait will need to be managed differently than other crops where traits provide near total control of a given pest. MON 88702 will be most valuable when used in conjunction with other IPM practices. Graham (2015) listed some of the “best management strategies” for control of tarnished plant bug which included practices such as planting hairy leaf varieties, planting early with early maturing varieties (Adams et al. 2013), and lowering the rates of nitrogen fertilizer. These practices are critical due to their importance in lowering the number of insecticide applications for tarnished plant bug in cotton. Increased input costs for weed control, seed treatments, fuel and fertilizer inputs, purchase of transgenic seed, and control of other insect pests have been observed (Riley et al. 2010). Cost to grow cotton is generally greater than other major crops due to the increased frequency of in-season insect control costs. Almost \$366 per hectare was spent on foliar insecticide applications in cotton in the Mississippi Delta in 2011 (Williams 2012). Tarnished plant bug control alone averaged \$240 per hectare, making it by far the most economically important insect pest in cotton in the Mid-South (Williams 2012). In conjunction with increased control costs, insecticide resistance has played a major factor in a decline in cotton acres in the midsouthern United States. Insecticide applications for tarnished plant bugs have selected for resistance (Snodgrass et al. 2009). Tarnished plant bug was first documented to have developed resistance to methyl parathion in 1978 (Cleveland and Furr 1979). Tarnished plant bug resistance to pyrethroids was

documented in 1994 (Snodgrass 1994). Resistance to organophosphates has also been documented (Snodgrass et al. 2009). In 2006, there were over 485,600 hectares of cotton planted in Mississippi, whereas 255,150 hectares were planted in 2017 (NASS 2017).

These data suggest that implementation of MON 88702 will save cotton producers 1-2 insecticide applications per growing season. The current Mississippi standard threshold is the most economically viable option for triggering tarnished plant bug applications in regard to yield when growing cotton containing the MON 88702 trait. These data agree with Bachman et al. (2017), in that MON 88702 had a significant impact on large tarnished plant bug nymphs. These data also agree with Bachman et al. (2017) in that the Cry51Aa2.834_16 protein had no impact on small tarnished plant bug nymphs or adult tarnished plant bugs. The use of cotton with MON 88702 along with IPM strategies may potentially reduce the overall number of insecticide applications necessary to control tarnished plant bug and in turn reduce the financial inputs needed to grow cotton in this region.

When determining an appropriate tarnished plant bug threshold for cotton with MON 88702, it is important to weigh all factors such as population densities, insecticide applications, and lint yield. While the double current standard threshold treatment triggered fewer insecticide applications, the current standard threshold treatment resulted in greater yields. Insecticide costs for tarnished plant bug control in cotton in the Mississippi Delta averaged \$32.27 per hectare per application, averaging seven applications (Williams 2012). This equates to approximately \$240 per hectare of total cost for controlling tarnished plant bug throughout the growing season. Computing this with the 1.33 difference in insecticide applications, on average the double current

standard threshold treatment would potentially save growers \$42.93 per hectare on insecticide applications compared to the current standard threshold treatment, not taking into consideration the differences in yield. For cotton selling at \$0.32 per kg and the current standard threshold treatment having significantly higher yield by 243 kg per hectare, cotton growers would bring in \$77.76 more per hectare more if triggering applications at the current standard threshold rather than the double current standard threshold, not considering insecticide application costs. The potential of a higher yield by triggering applications at the current standard threshold is more profitable than the potential of saving insecticide application costs by triggering applications at double the current standard threshold by \$34.83 per hectare. Therefore, in regards to tarnished plant bug control, MON 88702 will be most profitable to cotton growers when used in conjunction with established IPM strategies and insecticide applications for tarnished plant bug are triggered at current Mississippi thresholds.

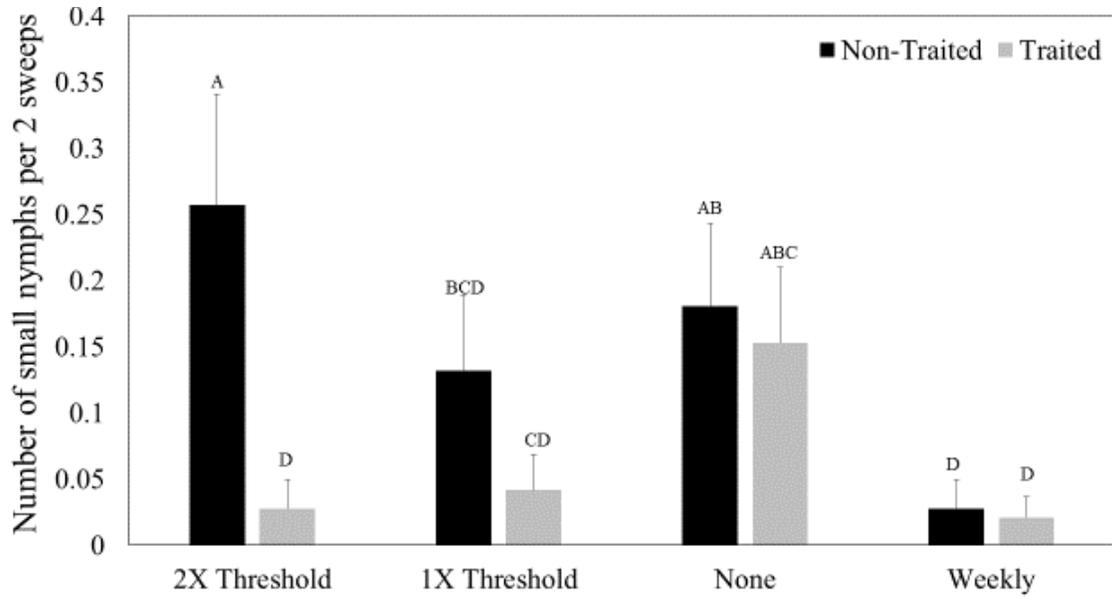


Figure 2.1 Interaction between trait and threshold treatment on mean number of small tarnished plant bug nymphs per 15 sweeps in pre-bloom cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

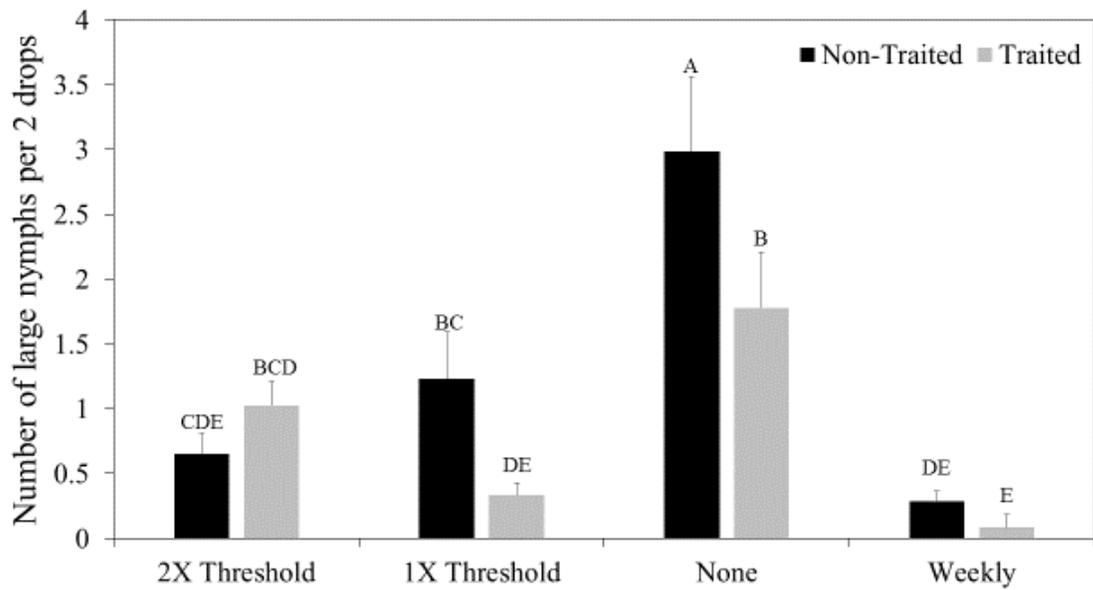


Figure 2.2 Interaction between trait and threshold treatment on the mean number of large nymphs per two drops in blooming cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

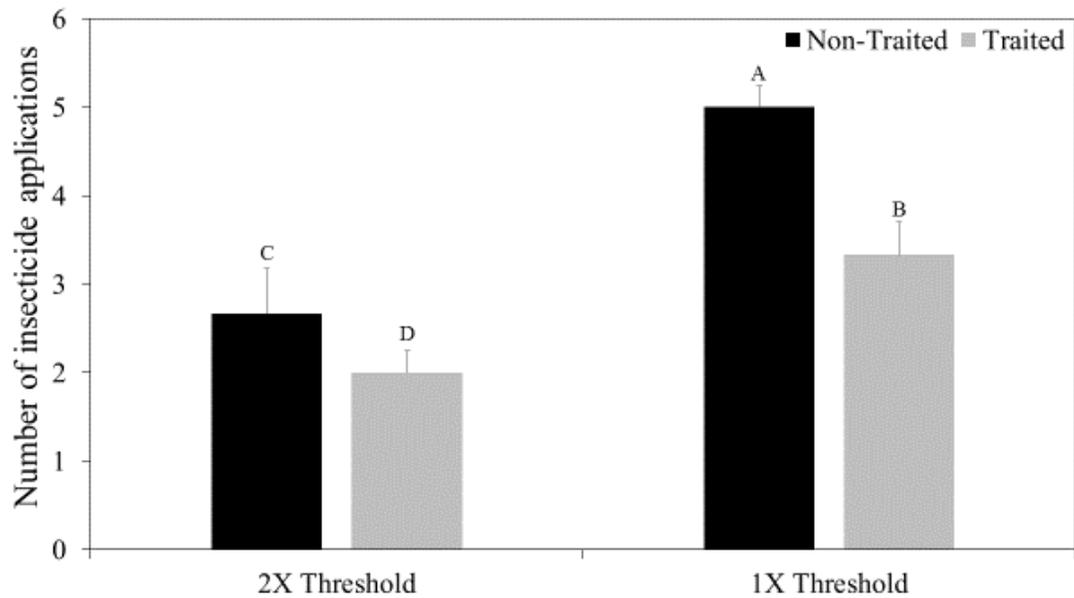


Figure 2.3 Mean number of insecticide applications needed to manage tarnished plant bug in traited and non-traited cotton using a 1X threshold and 2X threshold averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

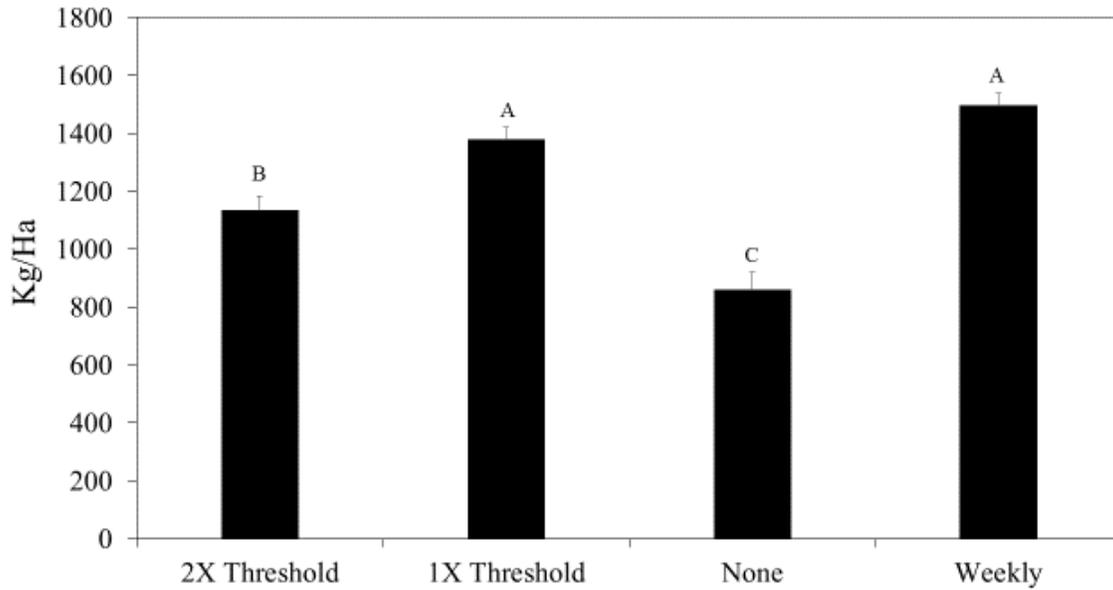


Figure 2.4 Impact of threshold spray treatment on cotton yields averaged across traited and non-traited lines in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

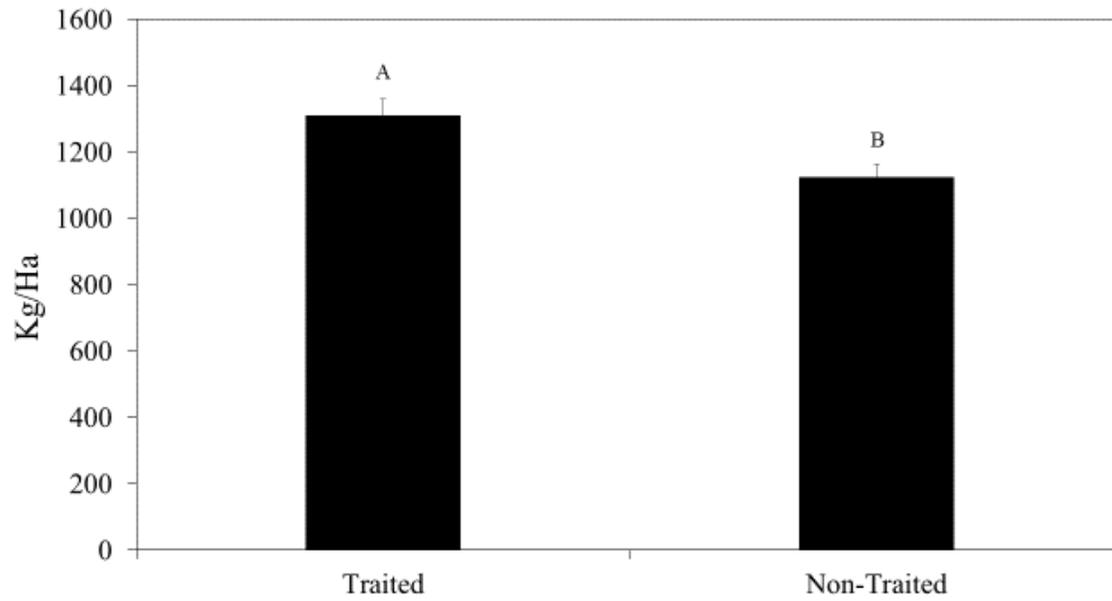


Figure 2.5 Impact of trait on cotton yields averaged across threshold treatments in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

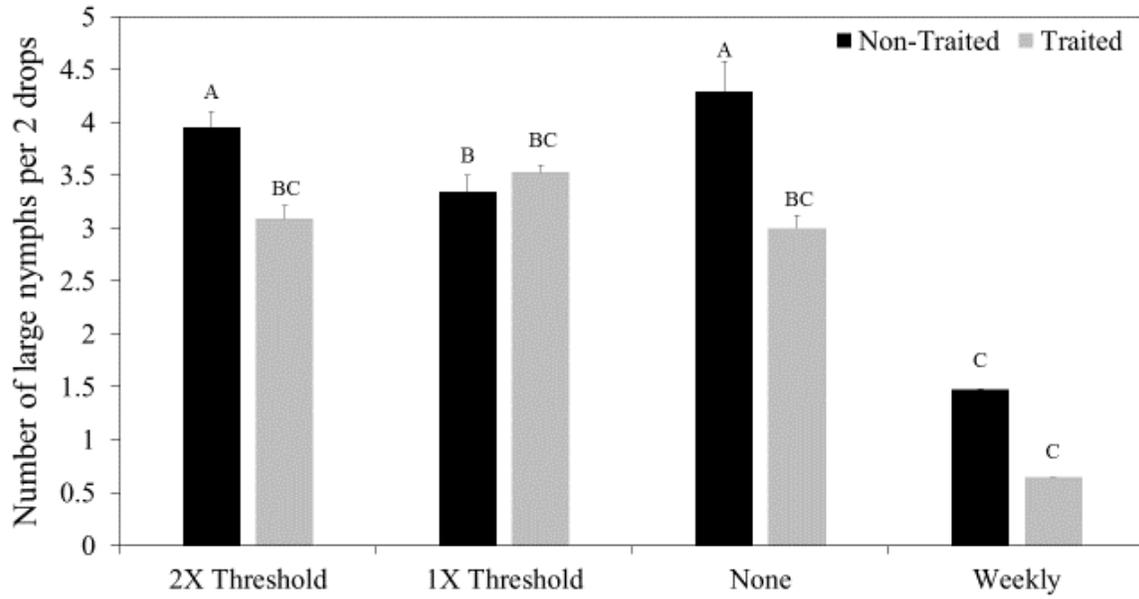


Figure 2.6 Interaction between trait and threshold treatment on the mean number of large nymphs per two drops in blooming cotton averaged across all sample dates in Sidon, MS in 2017.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

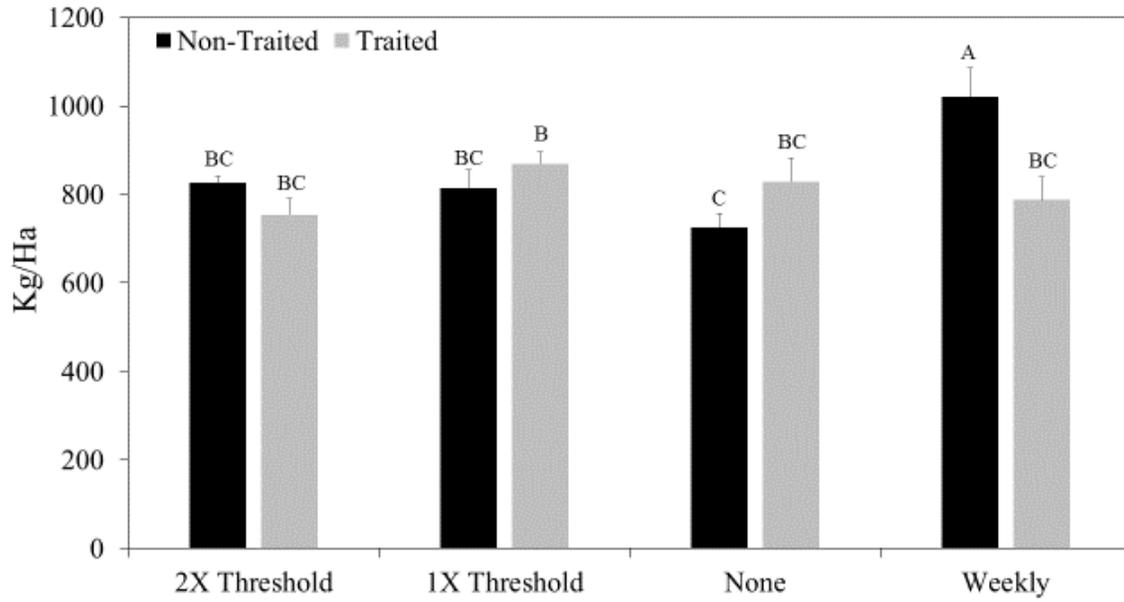


Figure 2.7 Interaction between trait and threshold treatment on mean cotton yields in Sidon, MS in 2017.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

References

- Adams, B., A. Catchot, J. Gore, D. Cook, F. Musser, & D. Dodds. 2013. Impact of planting date and varietal maturity on tarnished plant bug (Hemiptera: Miridae) in cotton. *J. Econ. Entomol.* 106:2378-2383.
- Bachman, P.M., A. Ahmad, J.E. Ahrens, W. Akbar, J.A. Baum, S. Brown, T.L. Clark, J.M. Fridley, A. Gowda, J.T. Greenplate, P.D. Jensen, G.M. Mueller, M.L. Odegaard, J. Tan, J.P. Uffman, and S.L. Levine. 2017. Characterization of the activity spectrum of Mon 88702 and the plant-incorporated protectant Cry51aa2. 834_16. *PLoS one* 12.1: e0169409.
- Bourland, F. M., N. P. Tugwell, and D. M. Oosterhuis. 1992. Concept for monitoring the growth and development of cotton plants using the mainstem node counts. *J. Prod. Agric.* 5: 532-538.
- Gore, J., A. Catchot, F. Musser, J. Greene, B.R. Leonard, D.R. Cook, G.L. Snodgrass, and R. Jackson. 2012. Development of a plant-based threshold for tarnished plant bug (Hemiptera: Miridae) in cotton. *J. Econ. Entomol.* 105: 2007-14.
- Graham, S.H. 2015. Best management strategies to manage the tarnished plant bug (heteroptera: miridae) in cotton. M.S. Thesis. Mississippi State University. Mississippi State, MS.
- Gutierrez, A. P., T. F. Leigh, Y. Wang, and R. D. Cave. 1977. An analysis of cotton production of cotton production in California: *Lygus hesperus* (Heteroptera: Miridae) injury- an evaluation. *Can. Entomol.* 109: 1375-1386.
- Hanny, B., T.C. Cleveland, and W.R. Meredith, Jr. 1977. Effects of tarnished plant bug, (*Lygus lineolaris*), infestation on presquaring cotton (*Gossypium hirsutum*). *Environ. Entomol.* 6.3: 460-62.
- Layton, M. 1995. Tarnished plant bug: biology, thresholds, sampling, and status of resistance, pp. 131-134. *In Proc. Beltwide Cotton Res. Conf., National Cotton Council, Memphis, TN.*
- Pack, T.M., and P. Tugwell 1976. Clouded and tarnished plant bugs on cotton: a comparison of injury symptoms and damage on fruit parts. Agricultural Experiment Station Report Series 227, Division of Agriculture, University of Arkansas, Fayetteville.
- Musser, F., S. Stewart, R. Bagwell, G. Lorenz, A. Catchot, E. Burriss, D.R. 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: 1916-23.

- NASS. 2017. Cotton production data.
https://www.nass.usda.gov/Quick_Stats/Ag_Overview/stateOverview.php?state=MISSISSIPPI. Accessed: 2/18/2018.
- Riley, J.M., A. Catchot, D. Dodds, D. Reynolds, J. Bond, and G. Andrews. 2010. Cotton 2011 Planning Budgets.
<http://www.agecon.msstate.edu/what/farm/budget/pdf/10/MSUCOT10.pdf>.
- Russell, J. S., B. R. Leonard, J. Gore and G. E. Church 1999. Cotton boll abscission influenced by tarnished plant bug feeding. *In Proc. Beltwide Cotton Res. Conf., National Cotton Council, Memphis, TN.* 2. 1046-1048.
- Scales, A. L. and R. E. Furr 1968. Relationship between the tarnished plant bug and deformed cotton plants. *J. Econ. Entomol.* 61: 114-118.
- Snodgrass, G. 1996. Glass-vial bioassay to estimate insecticide resistance in adult tarnished plant bugs (Heteroptera: Miridae). *J. Econ. Entomol.* 89: 1053-59.
- Snodgrass, G. L. 1994. Pyrethroid resistance in a field population of the tarnished plant bug in cotton in the Mississippi delta, p. 1186. *In Proc Beltwide Cotton Conference, New Orleans, LA.* 2. 1186-1188.
- Snodgrass, G. L., J. Gore, R. Jackson and C. A. Abel 2009. Acephate resistance in populations of the tarnished plant bug(Heteroptera: Miridae) from the Mississippi River Delta. *J. Econ. Entomol.* 102: 699-707.
- Snodgrass, G. and W. Scott 1988. Tolerance of the tarnished plant bug to dimethoate and acephate in different areas of the Mississippi delta. pp. 294-295. *In Proc. Beltwide Cotton Res. Conf., National Cotton Council, Memphis, TN.*
- Tugwell, P., S. C. Young, B. A. Dumas, and J. R. Phillips. 1976. Plant bugs in cotton: importance of infestation time, types of cotton injury, and significance of wild hosts near cotton. Arkansas Agricultural Experiment Station Report. Report Series 227. University of Arkansas, Fayetteville, AR.
- Williams, M. R. 2014. Cotton insect losses. Proc Beltwide Cotton Conference, New Orleans, LA. 798-812.
- Williams, M. R. 2012. Cotton insect losses.
<http://www.entomology.msstate.edu/resources/tips/cotton-losses/>. Accessed: 2/21/2018.

CHAPTER III
TO DETERMINE THE VALUE OF EARLY SEASON PROTECTION COMPARED
TO MID-LATE SEASON PROTECTION ON COTTON MODIFIED TO BE
RESISTANT TO LYGUS (MON 88702)

Abstract

Field trials were conducted in Stoneville, MS and Sidon, MS during 2016 and 2017 to compare the interactions between Bt cotton (MON 88702, Cry51Aa2.834_16) and insecticide spray timing for *Lygus* control. Spray treatments were applied either during the early season (pre-flowering) or late season (flowering). Cotton with MON 88702 had fewer later instar tarnished plant bug nymphs, but little to no effect on smaller nymphs or adults. MON 88702 also reduced the number of dirty squares compared to those in conventional cotton. For cotton sprayed in the early season there were no differences in the number of insecticide applications triggered between cotton with MON 88702 and conventional cotton. However, cotton with MON 88702 produced greater yield compared to conventional cotton when insecticide applications were made early in the season. Cotton with MON 88702 reduced late season insecticide applications while also providing increased yield compared to conventional cotton.

Introduction

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), was once considered a secondary pest of cotton, *Gossypium hirsutum* (L.), (Luttrell and King

2014). The eradication of the boll weevil, *Anthonomus grandis grandis* (Boheman), as well as, the introduction of *Bacillus thuringiensis* (Berliner) (Bt) technology to control *Helicoverpa zea* (Boddie) and *Heliothis virescens* (F.), elevated the status of the tarnished plant bug into being the primary insect pest of cotton in the midsouthern U.S. Increased issues from tarnished plant bug were caused by the absence of coincidental control from insecticide applications intended for the boll weevil, bollworm, and tobacco budworm. This may have also been an important factor in the tarnished plant bug's development of resistance to organophosphates and pyrethroids (Snodgrass and Scott 2003).

Tarnished plant bug causes significant damage to cotton over a long period of time. Tarnished plant bug can cause damage to cotton as early as emergence and can continue to cause damage until early lint development of the last harvestable bolls (Black 1973). However, the majority of economic damage caused by tarnished plant bug occurs from first-square through early bloom (Black 1973). Early season damage by tarnished plant bug is caused by feeding on cotton terminals. This feeding causes "crazy cotton", identified by the formation of multiple secondary terminals (Scales and Furr 1968). Early season damage on cotton can result in reduced plant height and weight, in conjunction with malformed leaves, swollen nodes, and delayed maturity, all of which are capable of causing a yield loss (Scales and Furr 1968, Hanny et al. 1977).

Tarnished plant bug feeding can cause two forms of damage to cotton. Mechanical damage to plant cells occurs at the feeding site, while the salivary enzymes injected into the plant by tarnished plant bug feeding has disruptive effects and is likely more important (Layton 1995). Tarnished plant bug prefers to feed on small squares (flower buds), approximately 3.2 mm in diameter, which typically results in the

abscission of those squares within a few days (Layton 1995). Tarnished plant bug will also feed on larger squares, although this usually does not cause the square to abscise. Damage caused during the squaring growth stage may be observed on the open bloom as dark brown anthers. Flowers with discolored anthers are often referred to as “dirty blooms”. Tarnished plant bug will continue to feed on cotton during the flowering period and can cause significant yield losses during this growth stage. Pack and Tugwell (1976) found little to no effect on yield when < 30% of anthers of an individual flower are damaged from tarnished plant bug feeding. However, when the level of anther damage rose above 30% in an individual flower, the level of malformed bolls and percent of bolls shed increased (Layton 2000). This high level of anther damage can most likely be contributed to insufficient pollination. Tarnished plant bug will also feed on small bolls (fruit) (Pack and Tugwell 1976). Tarnished plant bug feeding on cotton bolls results in sunken lesions on the exterior of the boll wall that eventually will turn black and necrotic (Pack and Tugwell 1976). Damage caused by boll feeding can occur up to eight days after anthesis (Greene et al. 1999), although lint yield is considered safe once the boll has accumulated 250-300 heat units after anthesis (Horn et al. 1999).

The ability of tarnished plant bug to cause damage at any point during the growing season is a major factor in its importance as a pest of cotton in the Mid-South. Cotton growers typically deal with insect pests for relatively short time frames, such as thrips being an early season pest and stink bugs being a late season pest on bolls. In contrast, the tarnished plant bug has an unusually long window in which it can cause damage, resulting in several problems for cotton growers. One problem this extended window causes is a large amount of time needed to scout for tarnished plant bug.

Because the tarnished plant bug can cause damage beginning at emergence, scouting for tarnished plant bug must begin shortly after emergence. Another problem this extended window causes is a large number of insecticide applications to manage this pest. The longer an insect pest can cause damage to a cotton plant, the more likely it is to trigger multiple insecticide applications throughout the year.

The large number of insecticide applications required throughout a single season has resulted in tarnished plant bug developing resistance to several major classes of insecticides, such as organophosphates, pyrethroids, and carbamates. Cleveland and Furr (1979) first documented insecticide resistance in tarnished plant bug to methyl parathion in the Mississippi Delta during the late 1970's. Snodgrass (1988) documented tolerance to dimethoate in the same region. As early as the mid-1990's tarnished plant bug resistance to pyrethroid, organophosphate, and cyclodiene insecticides were reported in the Mississippi Delta (Snodgrass 1996). Widespread resistance to acephate has been reported in the region as well (Snodgrass and Gore 2007, Snodgrass et al. 2009). Although tarnished plant bug has developed widespread resistance to a growing number of insecticides, scouting and use of foliar insecticides remain the most important components of tarnished plant bug management in cotton. This resistance has led to strides being taken to find new strategies to manage tarnished plant bug in cotton in the Mid-South. One potential new management tool is cotton that expresses the MON 88702 trait by Monsanto Company. Transgenic crops expressing insecticidal proteins derived from the entomopathogenic bacterium *Bacillus thuringiensis* against lepidopteran and coleopteran insect pests have become a critical tool in insect pest management, with over 58 million hectares planted to transgenic crops in 2010 worldwide (Huesing et al. 2004

and James 2010). The Cry51Aa2.834_16 protein was discovered to have insecticidal activity toward both tarnished plant bug, and the Western tarnished plant bug, *Lygus hesperus* (Knight) (Baum et al. 2012; Gowda et al. 2016). The event MON 88702 (Cry51Aa2.834_16) targets hemipteran and thysanopteran pests, particularly plant bugs and thrips, *Frankliniella* spp. (Bachman et al. 2017). The utilization of MON 88702 presumably will reduce the number of insecticide applications needed for tarnished plant bug management, which should also extend the useful life of other management strategies and technologies. Little research has been conducted with this technology, therefore it is important to understand its full capabilities and weaknesses so that it can be utilized in the most efficient and effective way possible. Using appropriate sampling methods based on the point of the growing season can be a major factor in properly using this new technology (Musser et al. 2009). Understanding how this new technology will impact tarnished plant bug populations throughout the year must be understood in order to properly utilize MON 88702. Therefore, the purpose of this study was to determine the value of early season (pre-flowering) tarnished plant bug protection compared to mid-late season (flowering) tarnished plant bug protection on cotton modified to be resistant to *Lygus* (MON 88702).

Materials and Methods

To determine the value of early season (pre-flowering) protection compared to mid-late season (flowering) protection on cotton modified to be resistant to *Lygus*, experiments were conducted at the Delta Research and Extension Center in Stoneville, MS and on Sidon Plantation in Sidon, MS in 2016 and 2017. The Sidon trial was planted on 17 May while the Stoneville trial was planted on 23 May in 2016. Trials at both

locations were planted on 17 May in 2017. Trials were conducted in four row plots with dimensions of 12.19 m long by 3.87 m wide. Seed were planted into raised conventional tilled beds with a 1.02 m row spacing at a plant population of ca.120,000 seed per hectare. A commercial premix of thiodicarb, imidiclopid, trifloxystrobin, triadimenol, and metalaxyl (Aeris Trilex Advanced, Bayer CropScience, Raleigh, NC) was applied to the cotton seed in order to reduce the impact of thrips, nematodes, and seedling disease. For the control of summer annual weeds within the trial area a preemergence application of herbicide was applied. Seed were planted approximately 2.5 cm below the soil surface. When necessary, furrow irrigation was applied to the trial area.

This experiment was conducted using a two by four factorial arrangement of treatments within a randomized complete block design. Factor A consisted of a traited cotton variety (MON 88702, Cry51Aa2.834_16) versus a non-traited cotton variety (Delta Pine 393). Factor B consisted of tarnished plant bug protection with insecticides during certain periods of plant development which included protection prior to flowering, protection after flowering had begun, protection during the entire season (automatic weekly applications), and a non-treated control. The current Mississippi square retention threshold is to treat when less than 80% of first position squares remain on the plant prior to first bloom (Catchot 2018). The current Mississippi sweep net threshold is eight tarnished plant bugs per 100 sweeps during the first two weeks of squaring and fifteen tarnished plant bugs per 100 sweeps from the third week of squaring through bloom (Catchot 2018). The current Mississippi drop cloth threshold is one tarnished plant bug per 1.524 row meters during the first two weeks of squaring and three tarnished plant bugs per 1.524 row meters from the third week of squaring through bloom (Catchot

2018). The current Mississippi threshold for dirty squares is when 10% of the squares are dirty, looking at medium sized squares with exposed buds that have been discolored by plant bug feeding (Catchot 2018). Other states within our region, such as Arkansas and Louisiana, have the same thresholds.

The most efficient sampling method for tarnished plant bug varies depending on the developmental stage of cotton. Adults are typically more common during the pre-bloom period while nymphs become more of a problem during bloom (Musser et al. 2009). Sweep net samples were taken during the first three weeks of squaring. Samples were taken with a 38 cm diameter sweep net twice per week. A total of 15 sweeps per plot were taken, alternating sampling on row one and four in different weeks. Square retention was taken once per week within each plot during the first three weeks of squaring. To determine square retention, a sample of 25 plants per plot were examined, looking for the presence of squares at first position fruiting sites from the upper three nodes. Any square missing was considered abscised. An indicator for a missing square included the presence of an abscission scar at the first position on each fruiting branch. Additionally, squares were considered abscised if evidence of tarnished plant bug damage was observed as either blasted squares or if the bracts were flared. The percentage of retained squares was then recorded. Beginning in the flowering stages, plots were sampled twice per week. One sample was taken with a 1.52 m black drop cloth, taking two drops per plot. The drop cloth was positioned between the second and third row near the center of each plot and plants within the length of the drop cloth were vigorously shaken from each row onto the cloth. All tarnished plant bug samples were separated into four categories: adults, small nymphs (first and second instars), medium nymphs

(third and fourth instars), and large nymphs (fifth instars). The second sample per week was a plant based sampling technique which is often utilized during the flowering period, called “dirty square” samples. “Dirty square” samples were taken once per plot weekly. A total of 25 squares per plot were visually observed to determine if there was any tarnished plant bug damage.

The non-treated plots were not treated for tarnished plant bugs at any point in the growing season. Season long protection was achieved with weekly automatic insecticide applications from first-square until cutout. Early season and late season protection was achieved with insecticide applications based on economic thresholds for the appropriate sampling methods for that period. For the purposes of this research, early season was defined as the first three weeks of cotton squaring and late season was defined as the fourth week of squaring until cutout. Insecticides utilized for management of tarnished plant bug included sulfoxaflor (Transform WG, DOW AgroSciences, Indianapolis, IN), thiamethoxam (Centric 40 WG, Syngenta Crop Protection, Greensboro, NC), acephate (Orthene 90S, Valent Corporation, Walnut Creek, CA), and acephate plus bifenthrin (Brigade® 2EC, FMC Corporation, Princeton, NJ). Once cotton reached five nodes above white flower (NAWF) plus 350-400 heat units (DD60s) tarnished plant bug sampling was terminated. Main stem nodes above the uppermost first position white flower were counted to calculate nodes above white flower (Bourland et al. 1992). Tarnished plant bug cannot damage cotton bolls once they have accumulated at least 300 heat units (Russell et al. 1999). Therefore, when cotton plants average five nodes above white flower plus 300 heat units, the latest harvestable bolls are considered safe from tarnished plant bug damage. At harvest, rows two and three of every plot were harvested

mechanically with a cotton picker modified for small plot harvest and seedcotton weights were recorded. Lint yield was determined by taking the weight of the harvest sample and multiplying by 39%. All data were analyzed using analysis of variance using PROC GLIMMIX. Trait and cotton protection period were considered fixed effects, while site year was considered a random effect. Due to low tarnished plant bug populations throughout the 2017 growing season in Sidon, MS, a separate analysis was conducted for this location. Response variables included tarnished plant bug densities, number of dirty squares, number of insecticide applications, percent square retention, and yield. Degrees of freedom were calculated using the Kenwood-Rogers method for all analyses. Means were calculated using the LSMEANS statement and separated based on Fisher's protected least significant difference ($\alpha=0.05$).

Results

All Sites except Sidon 2017

Pre-Bloom Samples

For small nymphs, there was a significant effect of cotton protection period ($F=6.8$; $df=3, 14$; $P<0.01$), but no significant effect of trait ($F=3.71$; $df=1$; $P=0.07$) and the interaction was not significant ($F=0.68$; $df=3$; $P=0.58$). The non-treated control protection (0.039 ± 0.02) and the late season control treatment (0.18 ± 0.049) had significantly more small nymphs than the early season (0.008 ± 0.008) and season long control plots (0.016 ± 0.011). For medium nymphs, there was a significant trait by protection period interaction ($F=5.61$; $df=3, 14$; $P<0.01$). The non-treated cotton with late season tarnished plant bug protection only had significantly more medium nymphs than all other cotton trait-protection period combinations (Fig. 3.1). For large nymphs

($F=4.59$; $df=3, 16$; $P=0.02$) and adults ($F=5.08$; $df=3, 14$; $P=0.01$) there was a significant effect of protection period. In general, the season long protection resulted in fewer tarnished plant bugs than the non-treated control (data not shown).

Square Retention

For square retention, there was a significant effect of trait ($F=14.55$; $df=1, 278$; $P<0.01$) and protection period ($F=8.73$; $df=3, 278$; $P<0.01$), but the interaction was not significant ($F=0.95$; $df=3$; $P=0.42$). The season long protection (89.94 ± 1.20) and early season control spray treatments (88.33 ± 1.40) had significantly higher square retention than the late season (83.00 ± 1.42) and non-treated control (82.89 ± 1.61) protection periods. Additionally, the cotton with MON 88702 ($88.39\pm .90$) had significantly higher square retention than the conventional cotton (83.69 ± 1.11).

Bloom Samples

For small nymphs, there was a significant effect of protection period ($F=11.15$; $df=3, 14$; $P<0.01$) but not for trait ($F=3.59$; $df=1$; $P=0.08$) nor was the interaction significant ($F=0.71$; $df=3$; $P=0.56$). The season long protection resulted in significantly fewer small nymphs than protection during the early or late periods and the non-treated control plots. For medium nymphs, there was a significant trait by protection period interaction ($F=3.53$; $df=3, 14$; $P=0.04$). For the early season and non-treated control protection periods, there was a significant difference between the cotton with MON 88702 and conventional cotton but not for the other protection periods (Fig. 3.2). For large nymphs, there was a significant effect of trait ($F=5.07$; $df=1, 14$; $P=0.04$) and protection period ($F=15.44$; $df=3, 14$; $P<0.01$), but the interaction was not significant

($F=0.63$; $df=3$; $P=0.60$). The non-treated control (2.28 ± 0.36) and early season control (1.94 ± 0.33) protection periods had significantly more large nymphs than the late season (0.69 ± 0.13) and season long control (0.19 ± 0.065) protection periods. Additionally, the cotton with MON 88702 (0.96 ± 0.17) had significantly fewer large nymphs than the conventional cotton (1.59 ± 0.20). For adults, there was a significant effect of trait ($F=17.35$; $df=1, 14$; $P<0.01$) and protection period ($F=12.36$; $df=3, 14$; $P<0.01$), but the interaction was not significant ($F=2.25$; $df=3$; $P=0.13$). The non-treated control (1.48 ± 0.16) and early season control (1.26 ± 0.20) protection periods had significantly more adults than the late season (0.69 ± 0.096) and season long control spray treatments (0.49 ± 0.081). Additionally, the cotton with MON 88702 (0.69 ± 0.07) had significantly fewer adults than the conventional cotton (1.26 ± 0.13).

Dirty Squares

For dirty square data, there was a significant trait by protection period interaction ($F=3.09$; $df=3, 438$; $P=0.03$). For the early season and non-treated control protection periods, there was a significant difference between the cotton with MON 88702 and conventional cotton, but not for the other protection periods (Fig. 3.3). The early season and non-treated control protection periods had significantly more dirty squares than the late season protection period. The late season control treatment had significantly more dirty squares than the season long protection plots. Cotton with MON 88702 (1.33 ± 0.12) had significantly fewer dirty squares than conventional cotton (1.89 ± 0.15).

Insecticide Applications

For number of insecticide applications, there was a significant trait by protection period interaction ($F=10.23$; $df=1, 42$; $P<0.01$). Regardless of cotton trait, late season protection required significantly more insecticide applications than early season protection. The use of MON 88702 traited cotton did not reduce the number of insecticide applications needed for early season tarnished plant bug protection compared to conventional cotton. However, cotton expressing the MON 88702 trait required significantly fewer insecticide applications for late season tarnished plant bug protection compared to cotton without the MON 88702 trait (Fig. 3.4).

Yield

For yield, there was a significant trait by protection period interaction ($F=2.77$; $df=3, 86$; $P=0.05$). For the early season control, late season control, and non-treated control spray treatments, there was a significant difference between the cotton with MON 88702 and conventional cotton, with the cotton with MON 88702 significantly out-yielding the conventional cotton (Fig. 3.5).

Sidon 2017

Pre-Bloom Samples

No significant differences were observed for small nymphs, medium nymphs, large nymphs, or adults ($P>0.05$).

Square Retention

For square retention, there was a significant effect of trait ($F=17.82$; $df=1, 88$; $P<0.01$) and protection period ($F=3.37$; $df=3, 88$; $P=0.02$), but the interaction was not

significant ($F=0.75$; $df=3$; $P=0.52$). The season long control protection period (85.00 ± 1.88) had significantly higher square retention than the late season (77.17 ± 2.41) and non-treated control (75.67 ± 2.10) protection periods. Cotton with MON 88702 (84.00 ± 1.27) had significantly higher square retention than conventional cotton (74.58 ± 1.92).

Bloom Samples

For small nymphs, there was a significant effect of protection period ($F=10.42$; $df=3, 152$; $P<0.01$). The late season (1.35 ± 0.35) and non-treated control (1.80 ± 0.33) protection periods had significantly more small nymphs than the early season (0.40 ± 0.10) and season long protection period (0.10 ± 0.060). For medium nymphs, there was a significant effect of protection period ($F=8.55$; $df=3, 152$; $P<0.01$). The non-treated control protection had significantly more medium nymphs than the other protection periods (data not shown). For large nymphs, there was a significant trait by protection period interaction ($F=4.07$; $df=3, 152$; $P=0.01$). For the non-treated control spray treatment, there was a significant difference between the cotton with MON 88702 and conventional cotton but not for the other protection periods (Fig. 3.6). No significant differences were observed for adults ($F=1.46$; $df=3$; $P=0.23$).

Dirty Squares

No significant differences were observed for dirty squares for trait ($F=0.81$; $df=1$; $P=0.37$), protection period ($F=0.81$; $df=1$; $P=0.37$), nor their interaction ($F=0.03$; $df=1$; $P=0.86$).

Insecticide Applications

For number of insecticide applications, there was a significant effect of trait ($F=63$; $df=1, 14$; $P<0.01$), while no significant differences were observed for protection period ($F=1.4$; $df=1$; $P=0.26$). Conventional cotton (2.0 ± 0.0) triggered significantly more insecticide applications than cotton with MON 88702 (0.5 ± 0.19).

Yield

For yield, there was a significant trait by protection period interaction ($F=3.43$; $df=3, 24$; $P=0.03$). For the season long protection plots, there was a significant difference between the cotton with MON 88702 and conventional cotton, with the conventional cotton significantly out-yielding the cotton with MON 88702 (Fig. 3.7). No significant differences were observed for the other spray treatments.

Discussion

These data suggest that the implementation of MON 88702 can provide significant benefits to cotton growers in the Mid-South, particularly in regards to yield protection and a reduction in insecticide applications for tarnished plant bug. Similar to what Bachman et al. (2017) observed, a significant reduction in number of medium to large nymphs were observed with MON 88702 expressing cotton compared to non-traited DP 393 without supplemental tarnished plant bug management. Also these data demonstrate MON 88702 has no significant impact on small tarnished plant bug nymphs or adult tarnished plant bugs, which has been observed in other studies (Bachman et al. 2017). Early season tarnished plant bug protection produced similar results with both traited and non-traited cotton. This was to be expected because early season, or pre-

bloom, tarnished plant bug populations are primarily migrating adults, on which MON 88702 has little impact. However, cotton expressing MON 88702 with early season protection yielded significantly more than DP 393 with early season protection. The combination of cotton expressing the MON 88702 trait with late season tarnished plant bug protection yielded significantly greater and required significantly fewer insecticide applications than DP 393 cotton with late season tarnished plant bug protection. As opposed to the early season where tarnished plant bug populations are primarily adults, tarnished plant bug nymphs typically establish a healthy population during the late season. Since MON 88702 has been shown to have a significant impact on later instar tarnished plant bug nymphs, it is to be expected that there will be a reduction in insecticide applications during the late season when these susceptible life stages are more prevalent within the population (Bachman et al. 2017).

When evaluating the value of early season (pre-flowering period) tarnished plant bug protection to the value of late season (flowering period) tarnished plant bug protection on cotton modified to be resistant to *Lygus* (MON 88702), the current study demonstrates that protection during both periods is important. Unlike some other *Bt* technologies (ex. Cry1AC against tobacco budworm) this technology is not 100%, rather closer to 60-70% effective. The early season traited treatment triggered 1.2 fewer insecticide applications compared to the late season traited treatment. Insecticide costs for tarnished plant bug in cotton in the Mississippi Delta averaged \$32.27 per hectare per application, averaging seven applications (Williams 2012). This equates to approximately \$240 per hectare of total cost for controlling tarnished plant bug. By multiplying this with the 1.2 difference in insecticide applications, on average the early

season treated treatment may possibly save cotton growers \$38.72 per hectare on insecticide application costs compared to the late season treated treatment, not taking into consideration the differences in yield. When looking at differences in lint yield, data showed the late season treated treatment out-yielded the early season treated treatment by 206 pounds per hectare. By multiplying this by \$0.32, the current cost of cotton per kg, on average the late season treated treatment would bring in \$65.92 per hectare more than the early season treated treatment, not taking into consideration the differences in insecticide application costs. These economics suggest that when comparing early season protection compared to late season protection, late season protection is \$27.20 more valuable per hectare to cotton growers on average than early season protection.

These data also suggest that with the implementation of MON 88702 into cotton production in the Mid-South that it may be feasible to entirely eliminate early season (pre-flowering) insecticide sprays for tarnished plant bug. MON 88702 cotton with late season protection produced similar yields compared to MON 88702 cotton with season long protection, and significantly higher yield than MON 88702 cotton with only early season tarnished plant bug protection. This allows for the possible elimination of early season insecticide applications for tarnished plant bug, which would help dramatically decrease the cost per acre of growing cotton in this region. However, these experiments were conducted on small plot research plots and it is not known how this trait will perform when planted on a commercial production scale. Further research, perhaps on a larger scale, will be necessary before the elimination of early season insecticide sprays can be formally recommended with this technology. These data, along with research conducted by Baum et al. (2012), Gowda et al. (2016), and Kos et al. (2009) highlight

that while this technology should provide a significant benefit to cotton producers by providing insecticidal control against *Lygus*, it is not expected to be a 100% effective control strategy for tarnished plant bug, but rather incorporated into an established IPM program. It will be vital for farmers, local extension agents, and crop consultants to make timely, educated application decisions in order to maximize the potential of this new technology.

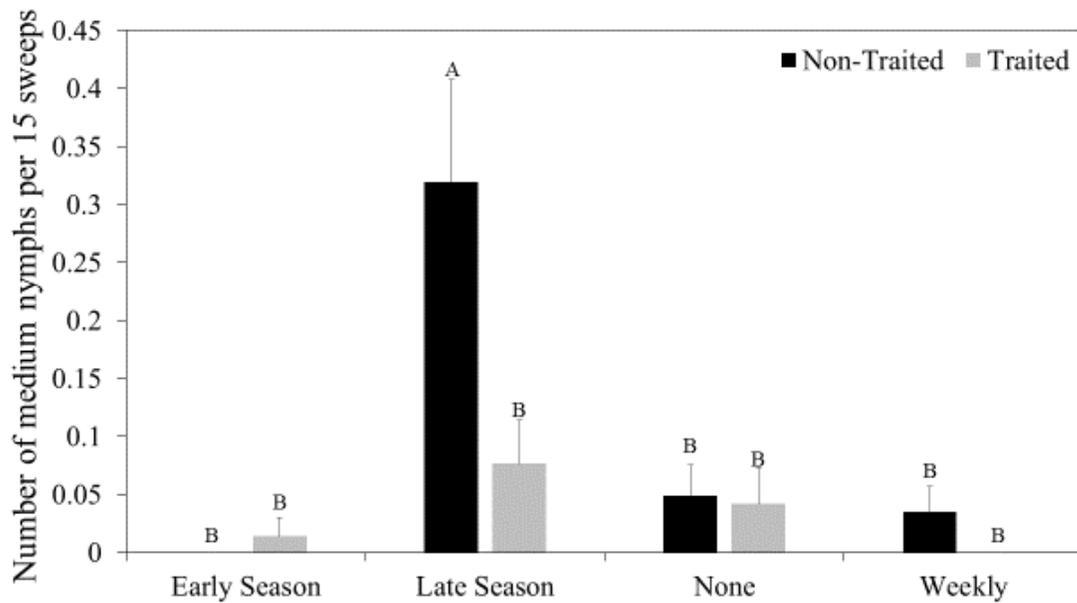


Figure 3.1 Interaction between trait and threshold treatment on the mean number of medium nymphs per 15 sweeps in pre-bloom cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

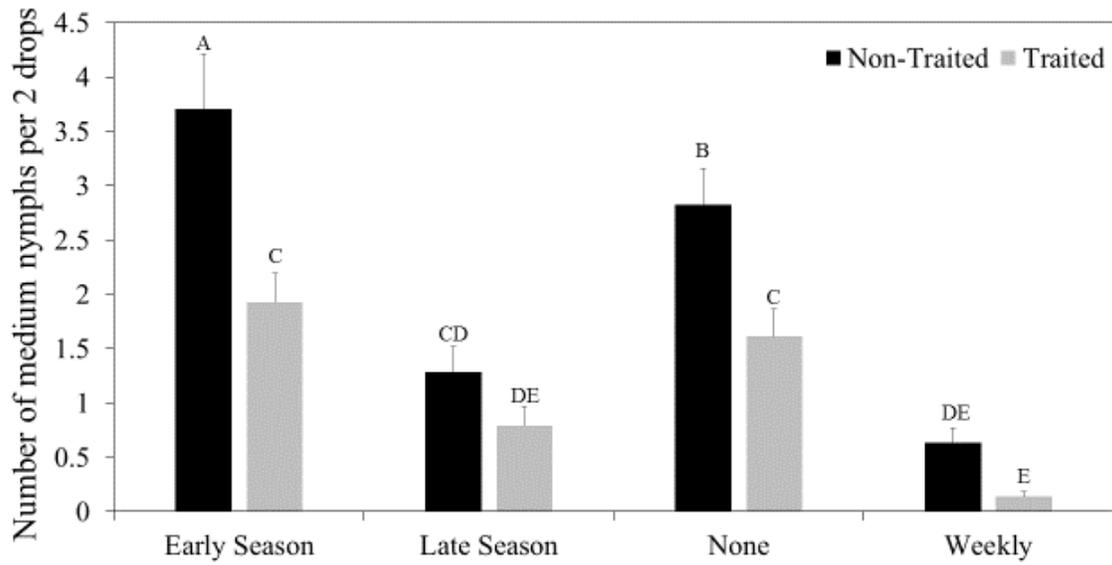


Figure 3.2 Interaction between trait and threshold treatment on the mean number of medium nymphs per 2 drops in blooming cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

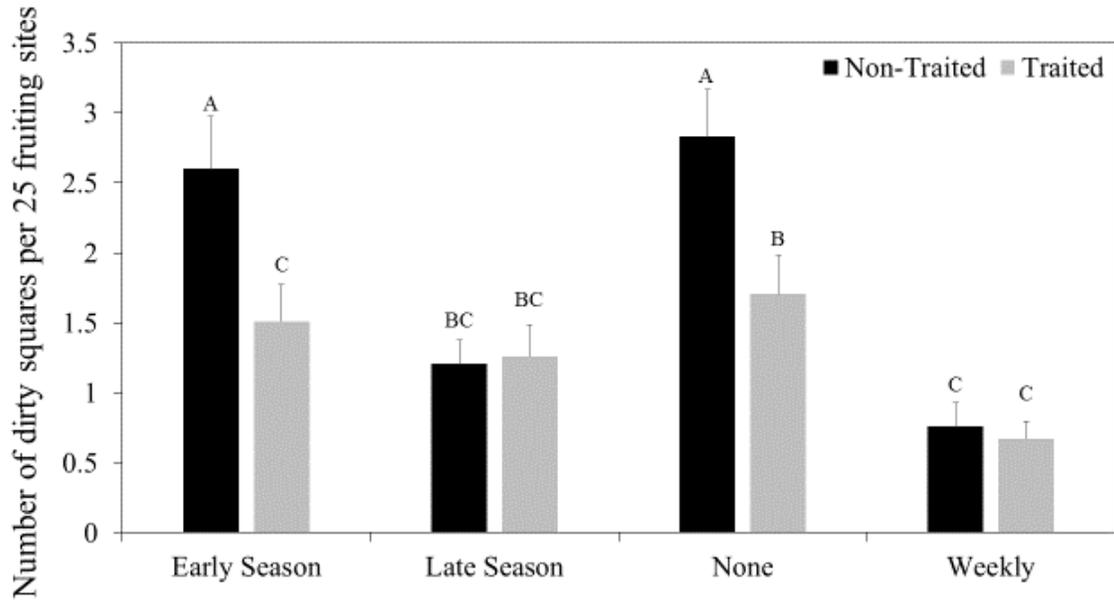


Figure 3.3 Interaction between trait and threshold treatment on the mean number of dirty squares per 25 fruiting sites in blooming cotton averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

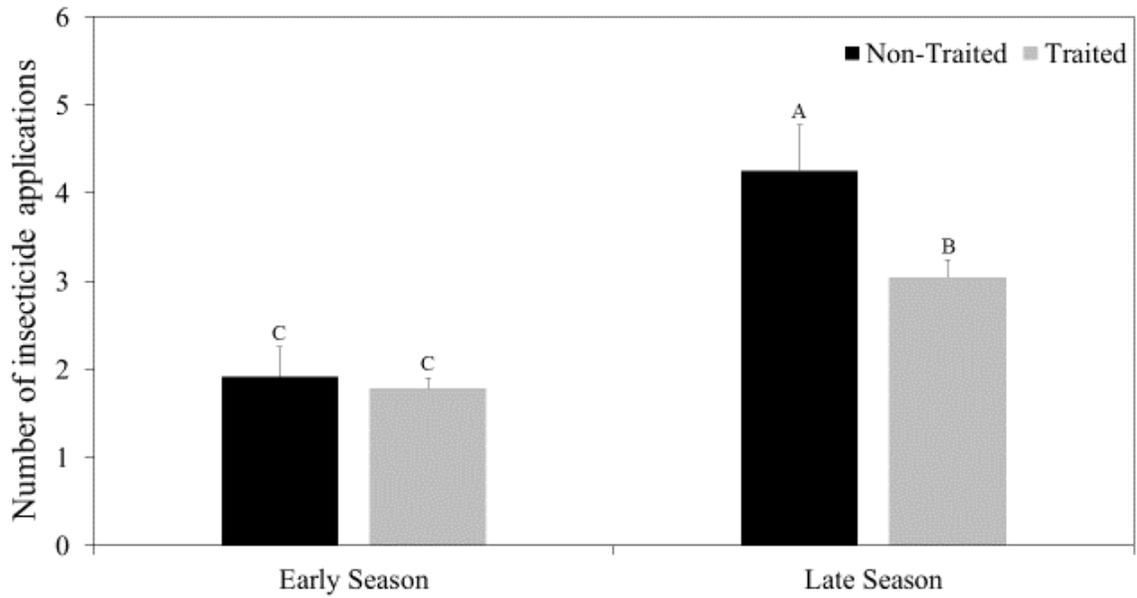


Figure 3.4 Mean number of insecticide applications needed to manage tarnished plant bug in traited and non-traited cotton for the early season and late season treatments averaged across all sample dates in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

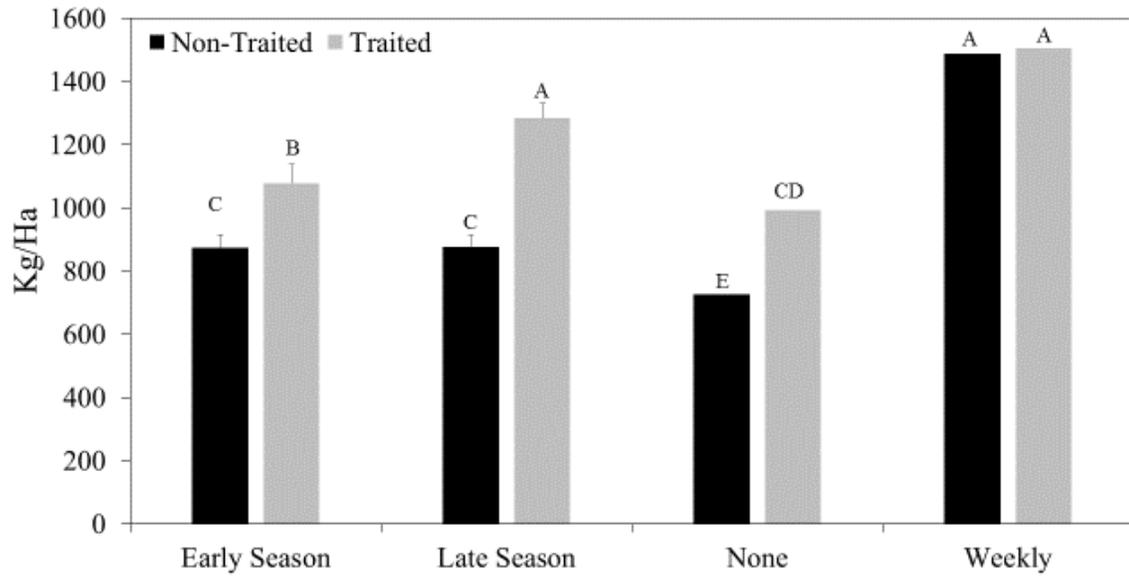


Figure 3.5 Interaction between trait and spray treatment on mean cotton yields in Stoneville, MS in 2016 and 2017, and Sidon, MS in 2016.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

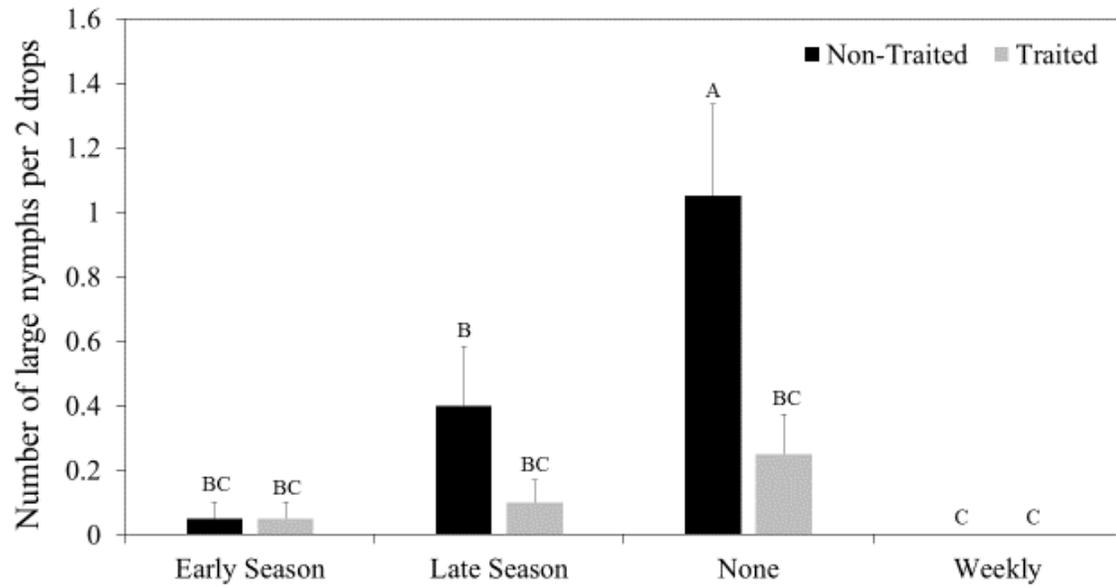


Figure 3.6 Interaction between trait and threshold treatment on the mean number of large nymphs per 2 drops in blooming cotton averaged across all sample dates in Sidon, MS in 2017.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

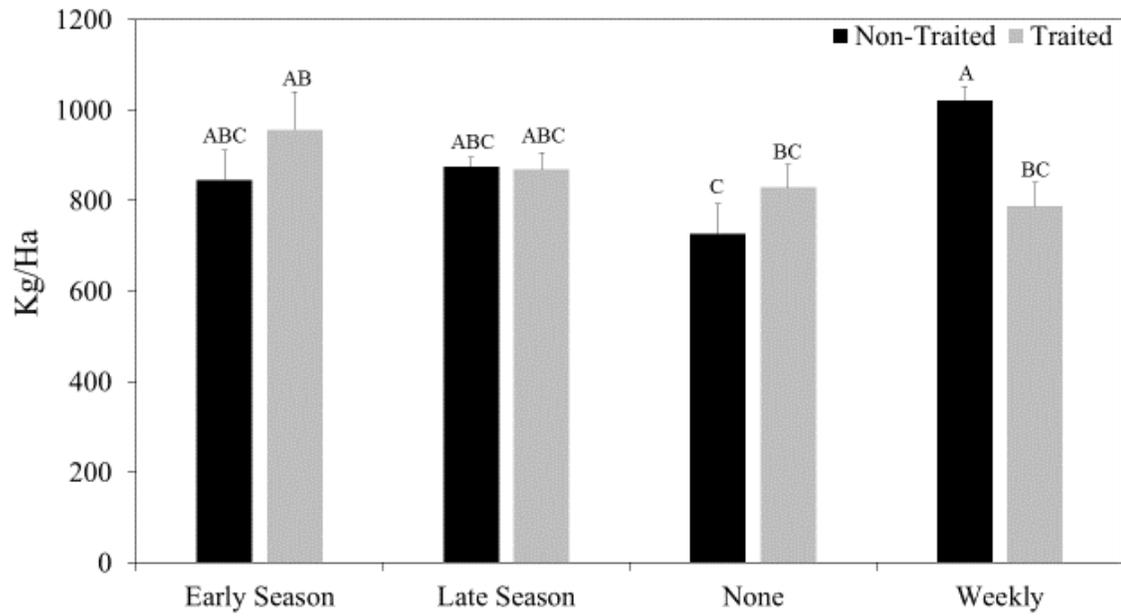


Figure 3.7 Interaction between trait and spray treatment on mean cotton yields in Sidon, MS in 2017.

Means followed by the same letter are not significantly different ($\alpha=0.05$). Error bars indicate standard error of the mean.

References

- Bachman, P.M., A. Ahmad, J.E. Ahrens, W. Akbar, J.A. Baum, S. Brown, T.L. Clark, J.M. Fridley, A. Gowda, J.T. Greenplate, P.D. Jensen, G.M. Mueller, M.L. Odegaard, J. Tan, J.P. Uffman, and S.L. Levine. 2017. Characterization of the activity spectrum of Mon 88702 and the plant-incorporated protectant Cry51aa2. 834_16. PloS one 12.1: e0169409.
- Baum, J. A., U.R. Sukuru, S. R. Penn, S. E. Meyer, S. Subbarao, X. Shi, S. Flasiński, G. R. Heck, R. S. Brown, and T. L. Clark. 2012. Cotton plants expressing a hemipteran-active *Bacillus thuringiensis* crystal protein impact the development and survival of *Lygus hesperus* (Hemiptera: Miridae) nymphs. J. Econ. Entomol. 105, no. 2: 616-624.
- Black, E. R. 1973. Economic threshold studies of the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), in cotton. 108 pp. Ph.D. Dissertation, Department of Entomology, Mississippi State University. Mississippi State, MS.
- Bourland, F. M., N. P. Tugwell, and D. M. Oosterhuis. 1992. Concept for monitoring the growth and development of cotton plants using the mainstem node counts. J. Prod. Agric. 5: 532-538.
- Cleveland, T. and R. Furr 1979. Toxicity of methyl parathion applied topically to tarnished plant bugs. J. Georgia Entomol. Soc. 15: 304-307.
- Gowda, A., T.J. Rydel, A.M. Wollacott, R.S. Brown, W. Akbar, T.L. Clark, S. Flasiński, J.R. Nageotte, A.C. Read, X. Shi, and B.J. Werner. 2016. A transgenic approach for controlling *Lygus* in cotton. Nature communications, 7, p.12213.
- Greene, J. K., S. G. Turnipseed, M. J. Sullivan and G. A. Herzog. 1999. Boll damage by southern green stink bug (Hemiptera: Pentatomidae) and tarnished plant bug (Hemiptera: Miridae) caged on transgenic *Bacillus thuringiensis* cotton. J. Econ. Entomol. 92: 941-944.
- Hanny, B. W., T. C. Cleveland and J. W.R. Meredith 1977. Effects of tarnished plant bug, *Lygus lineolaris*, infestation on presquaring cotton, *Gossypium hirsutum*. Environ. Entomol. 6: 460-462.
- Horn, T. O., F. A. Harris, J. T. Robbins and R. E. Furr, Jr. 1999. Influence of boll age on susceptibility to tarnished plant bug injury. In Proc. Beltwide Cotton Conference, Orlando, FL. 1044-1045.
- Huesing, J. and L. English. 2004. The impact of Bt crops on the developing world. AgBioForum 7:84-95.
- James, C. 2010. Global status of commercialized biotech/GM crops: 2010. ISAAA, Ithaca, NY.

Kos, M., J.J. van Loon, M. Dicke, and L.E. Vet. 2009. Transgenic plants as vital components of integrated pest management. *Trends in biotechnology* 27, no. 11: 621-627.

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

Layton, M. 1995. Tarnished plant bug: biology, thresholds, sampling, and status of resistance, pp. 131-134. *In Proc. Beltwide Cotton Res. Conf., National Cotton Council, Memphis, TN.*

Luttrell, R. and E. King. 2014. Importance of tarnished plant bug as a USDA ARS Research Priority. *Midsouth Entomol.* 7.2: 89-98.

Pack, T.M., and P. Tugwell 1976. Clouded and tarnished plant bugs on cotton: a comparison of injury symptoms and damage on fruit parts. *Agricultural Experiment Station Report Series 227, Division of Agriculture, University of Arkansas, Fayetteville.*

Musser, F. R., A. L. Catchot, S. D. Stewart, R. D. Bagwell, G. M. Lorenz, K. V. Tindall, G. E. Studebaker, B. R. Leonard, D. S. Akin, D. R. Cook and C. A. Daves. 2009. Tarnished plant bug (Hemiptera: Miridae) thresholds and sampling comparisons for flowering cotton in the midsouthern United States. *J. Econ. Entomol.* 102: 1827-1836.

Russell, J. S., B. R. Leonard, J. Gore and G. E. Church 1999. Cotton boll abscission influenced by tarnished plant bug feeding. *In Proc. Beltwide Cotton Res. Conf., National Cotton Council, Memphis, TN.* 2. 1046-1048.

Scales, A. L. and R. E. Furr 1968. Relationship between the tarnished plant bug and deformed cotton plants. *J. Econ. Entomol.* 61: 114-118.

Snodgrass, G. L. 1996. Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi Delta. *J. Econ. Entomol.* 89: 783-790.

Snodgrass, G. and J. Gore 2007. Status of insecticide resistance for the tarnished plant bug, pp. 56-59. *In Proc. Beltwide Cotton Res. Conference. National Cotton Council, Memphis, TN.*

Snodgrass, G. L., J. Gore, R. Jackson and C. A. Abel 2009. Acephate resistance in populations of the tarnished plant bug (Heteroptera: Miridae) from the Mississippi River Delta. *J. Econ. Entomol.* 102: 699-707.

Snodgrass, G. and W. Scott 1988. Tolerance of the tarnished plant bug to dimethoate and acephate in different areas of the Mississippi delta. pp. 294-295. *In Proc. Beltwide Cotton Res. Conf., National Cotton Council, Memphis, TN.*

Snodgrass, G. and W. Scott. 2003. Effect of ULV malathion use in boll weevil (Coleoptera: Curculionidae) eradication on resistance in the tarnished plant bug (Heteroptera: Miridae). *J. Econ. Entomol.* 96: 902-908.