1-1-2016

Best Management Strategies to Manage the Tarnished Plant Bug (Heteroptera: Miridae) in Cotton

Scott Hester Graham

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By

Scott Hester Graham

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

Mississippi State, Mississippi

May 2016

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By

Scott Hester Graham

Approved:

Angus L. Catchot
(Co-Major Professor)

Jeffrey Gore
(Co-Major Professor)

Don Cook
(Committee Member)

Darrin Dodds
(Committee Member)

Fred Musser
(Committee Member)

Michael Caprio
(Graduate Coordinator)

George M. Hopper
Dean
College of Agriculture and Life Sciences

Name: Scott Hester Graham

Date of Degree: May 6, 2016

Institution: Mississippi State University

Major Field: Agricultural Life Science

Major Professors: Dr. Angus L. Catchot and Dr. Jeffrey Gore

Title of Study: Best management strategies to manage the tarnished plant bug (heteroptera: miridae) in cotton

Pages in Study: 65

Candidate for Degree of Master of Science

Experiments were conducted to determine the impact of combining multiple best management practices to manage the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), on cotton. The implementation of this program reduced the amount of insecticide applications needed, significantly increased both square retention during the three weeks of squaring, and yield in the Delta region. Another study was conducted to explore behavioral responses of tarnished plant bug nymphs to several classes of insecticides. There appeared to be both avoidance and attraction depending on insecticide class. The presence of an insecticide did not affect the fruiting structure tarnished plant bugs were found on in the field, there were significantly more tarnished plant bugs found in the middle of the plant than the top or bottom at 3 DAT. At 7 DAT there were significantly more tarnished plant bugs found on the top of the plant followed by the middle then bottom.

DEDICATION

I would like to dedicate this research to my family. First, my parents Chip and Gwen Graham. Words cannot express how much your support and guidance have helped me throughout all of my life. Your steady support in my life, work, and faith in Jesus Christ mean more to me than I can ever repay. Next my sister Whitney and her husband Travis Woods. You two have always been there for me as someone listen to me and give advice on my "problems" in life. I would also like to dedicate this research to my grandparents, Jeanette and the late Charles Tiffin Graham, Sr. and Janice and Bobby White. You have always gone above and beyond the responsibilities of grandparents. I don't know what I would do without the love you have shown me my entire life. I would also like to thank Bobby White for taking a lazy kid and instilling the value of hard work. Without those Saturday mornings and summer days working with you, I do not think I would be where I am today. Lastly, I would like to dedicate this research to my late great uncle, Ed Hester. It was riding in the truck to Miss. State football, basketball, and baseball games my entire life that lead to my interest in agriculture. Without your generosity in my entire life, I do not know if I would have pursued a degree in agriculture. I hope this research can help the great farmers of the Mississippi Delta, just like Uncle Ed.

ACKNOWLEDGEMENTS

First I want to thank my Lord and Savior, Jesus Christ. Without Him nothing is possible. I would to thank my major advisors, Dr. Angus Catchot and Dr. Jeff Gore for their support and guidance throughout my graduate school experience. A special thanks to Dr. Catchot for allowing me to work in his program as a student worker throughout my undergraduate degree. The experience gained and friendships made during those three years are invaluable. I would also like to thank my committee members, Dr. Darrin Dodds, Dr. Don Cook, and Dr. Fred Musser for their expertise and support. Also a special thanks to Dung Bao, not only for his work in the field (he is the best in the business) but also for his friendship. You will not meet a more genuine person than Dung.

I would like to thank Cotton Incorporated for funding this research project. I would like to thank Mr. Boise Stokes and Mr. Chris Dobbins for their help at the Delta Research and Extension Center. I also give my most sincere thanks to Mrs. Beverly Catchot, Emily Jacks, and Lauren Sanders for their help with insect rearing. The lab portion of this project would not have been possible without them. I would like to thank all of the graduate students that I had the pleasure of working with for their help and friendship: Andrew Adams, Brian Adams, Nick Bateman, Whitney Crow, Chelsie Darnell, Taylor Dill, Ben Von Kanel, Brittany Lipsey, John North, Jeff Ramsey, Ben Thrash, and Adam Whalen. Lastly, I would like to thank all of the student works for all of their help and hard work.

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CHAPTER I

INTRODUCTION

Cotton

Cotton, *Gossypium hirsutum* L., is an important agronomic crop throughout Mississippi. Over the last 10 years, the amount of cotton grown in the state has decreased significantly. This is due to several reasons including decreased commodity prices of cotton and an increase in commodity prices of other row crops, such as corn, *Zea mays* L., and soybean, *Glycine max* (L.) Merrill. Another reason is an increase in the inputs needed to produce the crop. In 2004, 445,154 hectares of cotton were planted in Mississippi, with an insect control cost of $232.06 per hectare. A decade later, the area planted to cotton declined to 171,991 hectares with insect controls costs of $477.80 per hectare (Williams 2015).

Cotton fiber is an important commodity that is used to make products such as linens, paper, and money. Cottonseed produces meal or oil that can be used to feed livestock as well as humans. Until the 1940's, cottonseed oil was the top vegetable oil produced in the United States. It now ranks third with a yearly average of 1 billion pounds produced, making up around 6% of the total domestic fat and oil supply for the U.S. (http://cottonseed.com/pages/20facts).

Cotton is believed to have been planted for the first time in the U.S. in Florida in 1556 (http://www.cotton.org/pubs/cottoncounts/story/). There are two species of cotton produced in the United States, upland cotton, *Gossypium hirsutum*, and extra-long staple cotton, *Gossypium barbadense*. Upland cotton is the primary type grown in the mid-south, due to its ability to adapt to various growing conditions (Robertson and Roberts 2010).

Cotton is a perennial shrub grown as an annual crop (Ritchie et al. 2007). Although cotton's early season growth above ground appears to be slow, root growth is rapid. The radicle, which becomes the taproot, can reach depths up to 25.4 cm during the cotyledon stage (Oosterhuis 1990, Ritchie et al. 2007). Any conditions that hinder early season root growth, such as soil temperature, soil aeration, and soil moisture can result in reduced yield potential of the crop (McMichael 1986, Ritchie et al. 2007). The taproot and lateral roots can be found up to 1.2 m deep, although most are found between 0.3 and 1 m deep. Roots continue to grow along with the plant, flowering begins and the plant switches its energy to boll production (Oosterhuis 1990, Ritchie et al. 2007).

Heat unit estimation is a fairly consistent and predictable method to predict cotton growth and development (Oosterhuis 1990). The base temperature needed for cotton growth is 15.5^{0}C. Daily heat units are calculated by adding the daytime high and low temperatures, dividing by 2, and then subtracting the base temperature (Oosterhuis 1990). Understanding heat units can help to predict what stage the cotton is in, as well as how much time is needed until the next stage (Robertson et al., 2007).

Cotton has five general growth stages: germination and emergence, seedling establishment, leaf area and canopy development, flowering and boll development, and maturation (Oosterhuis 1990). Germination and emergence requires between 50 and 60 heat units (Robertson et al. 2007). Mutsaers (1983b) suggests that water uptake is the limiting factor for seedling cotton growth and development. Cotton plants produce two types of branches, monopodial (vegetative) and sympodial (fruiting). Reproductive branches grow from the base of vegetative leaves, causing the rate of fruiting branches to be dependent on vegetative growth (Mauney 1986). Overall branch growth is dependent on temperature. Hesketh et al. (1972) found it takes leaves three times longer to develop at 18° C than at 30° C. Typically between 45 and 60 heat units are needed for production of each additional main stem node (Robertson et al 2007). Vegetative growth is also dependent on water availability. As the plant matures, vegetative leaf growth slows. At a certain point, the plant produces enough leaves that the ability of the root system to seek out further moisture or absorb and transfer enough water becomes too great to continue exponential growth (Mauney 1986). In a glasshouse study, Mauney et al. (1978) observed a reduction of vegetative production beginning two weeks before flowering in cotton plants under no physiological stress, suggesting at this point the plant switches primary energy sources to reproduction.

Growth of sympodial branches is characterized by internodal elongation behind the square (floral bud), resulting in fruiting structures moving away from the main stem as the plant develops (Oosterhuis 1990). The length of time between planting, first square, and first bloom depend on the node where the first sympodial branch occurs (Mauney 1986). The node where the first sympodial branch is grown is dependent on both day and night time temperatures. Multiple researchers have found that the first fruiting branch is lower at cooler temperatures than at high temperatures (Mauney 1966; Hesketh et al. 1972). According to Oosterhuis (1990), the first fruiting branch on cotton in the mid-south is typically found on the fifth or sixth node. New nodes are produced roughly every three days (Ritchie et al. 2007, Oosterhuis 2008). According to Jenkins et al. (1990), a cotton plant will produce from 16 to 18 fruiting branches with two to five fruiting positions per branch. In favorable conditions, 35 days after planting are generally required for first-square (floral bud). The square undergoes several stages before reaching a flower. As soon as a square becomes recognizable, it is referred to as a pinhead square, followed by a match-head square, which is roughly one-third grown. Finally, just before flowering, the square is referred to as a candle square (Ritchie et al. 2007).

Flowering begins approximately 21 days after first-square and lasts six weeks. At this point, nutrient uptake increases while nodal growth slows and fruiting structure maturation begins (Ritchie et al. 2007, Oosterhuis 2008, 2013). Flowers typically begin to open on the first position of the sixth or seventh node (Oosterhuis 2008). White flowers will be pollinated within hours of opening. Flowers turn pink the day after pollination, and red on the third day. After five to seven days, bloom tags fall off of the newly developing boll (Ritchie et al. 2007). Three days are typically needed for flowers to open on the branches one node above, while six days are needed for flowers to open on the next position of the same node (Oosterhuis 2008). In the Mid-South, harvestable crop maturity occurs at five nodes above white flower. At this point the crop will continue to flower, but subsequent bolls will not have time to accumulate enough heat units to fully mature into harvestable bolls (Bourland 1992).

Chemical defoliants are used to help growers with harvest. Because of cotton's perennial growth habit, these harvest aids defoliate cotton, assist with boll opening, and reduce plant regrowth to improve harvest efficiency. Different harvest aids in two classes, herbicidal and hormonal, can be used. Selection of a harvest aid depends on several factors including temperature, plant condition, spray coverage, and the rate of product used (Ritchie et al. 2007).

Insect Pests of Cotton

In the United States, there are over 100 species of arthropods that can cause damage to cotton throughout the growing season. Less than 25 of these species cause economic losses on a yearly basis when left uncontrolled. Several of these economically important insects can be found in the mid-south (Leigh et al. 1996). From emergence to fourth true leaf, thrips belonging to the genus *Franklinella* are important pests. The tobacco thrips, *Franklinella fusca* (Hinds), and the western flower thrips, *Franklinella occidentalis* (Pergande), are the most prevalent in Mississippi cotton (Stewart et al 2013). Damage from thrips injury is characterized by silvering of lower leaf surfaces as well as deformed leaves (Leigh et al. 1996). Injury from thrips typically results in delayed maturity and may result in yield losses.

From first-square to first bloom, the bollworm, *Helicoverpa zea* (Boddie), and the tobacco budworm, *Heliothis virescens* (F.), are pests that feed directly on the fruiting structures. First and second instar larvae feed on terminals and small squares. As larvae grow, they begin to feed on larger squares, and chew holes into the square, causing the square to “flare” and abscise from the plant (Leigh et al. 1996).

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is an important pest of cotton. It can feed on terminals, squares, flowers, and even bolls causing severe damage (Leigh et al, 1996). Stink bugs, in the family Pentatomidae, are occasional pests of cotton. Stink bugs feed by sticking their stylet directly into bolls, causing warts on the inside of the boll resulting in stained lint or shrunken seeds. Feeding on smaller bolls can result in the abortion of the boll (Leigh et al. 1996).

Tarnished Plant Bug

Biology

Tarnished plant bug is taxonomically in the order Heteroptera and family Miridae (Triplehorn 2005). This family of small, soft-bodied true bugs is characterized by having piercing and sucking mouthparts, four segmented antennae, four segmented proboscis, and lack ocelli. The adult tarnished plant bug is a brown bug with a characteristic yellow-brown Y shaped mark on its scutellum, with reddish-brown antennae (Triplehorn 2005, Leigh et al. 1996). First and second instar nymphs are characterized by a greenish body color, similar to aphids. The early nymphs are distinguished from aphids because they move faster, have reddish tips on their antennae, and lack cornicles. Older nymphs are a green to light brownish color with five characteristic black dots on their dorsum (Leigh et al. 1996).

The tarnished plant bug is a polyphagous insect known to have a wide host plant range of up to 385 species or subspecies in 55 plant families across 39 states in the United States (Young 1986). In Mississippi alone, Young (1986) recorded 159 plant hosts. Although there are a few monocotyledonae plants that serve as hosts, most are dicotyledonae, particularly in the subclasses Rosidae and Asteridae. The variety of host plants of the tarnished plant bug has allowed the insect to develop a natural tolerance to many of the chemical defensive compounds found in plants (Young 1986). In Mississippi, henbit, *Lamium amplexicaule* L., is an important host for overwintering because it flowers during the winter (Snodgrass et al. 1984). Sour dock, *Rumex crispus*

L., and crimson clover, *Trifolium incarnatum* L., are also good winter hosts and important for reproduction in the spring (Snodgrass et al. 1984). Annual fleabane, *Erigeron annus* L., is thought to be the most important spring host of tarnished plant bug in the Mississippi Delta (Cleveland 1982). These hosts are important because they allow tarnished plant bug populations to build in the spring, and host many of the pests that will invade cotton fields. The tarnished plant bug will typically complete one or two generations per year on wild early season weed hosts (Fleischer and Gaylor 1987) before moving into available agronomic crops after these weedy hosts begin to senesce (Layton 1995, Leigh et al. 1996).

The tarnished plant bug has three distinct life stages: egg, nymph, and adult. The life cycle begins as an adult overwintering in leaf trash (Cleveland 1982). The tarnished plant bug life cycle takes from 22 to 46 days, depending on temperature (Fleischer and Gaylor 1988, Snodgrass et al. 1984). Over the course of the female life cycle, egg production can reach 175 eggs at an average temperature of 27^{0}C (Ugine 2012). As temperature increases, however, total egg production is reduced, even though the maximum amount of eggs laid per day is the highest at 30^{0}C. This is because adults live significantly fewer days at 30^{0}C than they do at temperatures less than 27^{0}C (Ugine 2012). At 25^{0}C an average of 7.6 days are needed to incubate tarnished plant bug eggs. At the same temperature, 19.7 days are required to go through the five nymphal instars. Roughly 5 days are required to complete the first instar stage, around 3 days each are required to complete the second, third, and fourth instar stages and roughly 5 days are required to complete the fifth instar stage (Ridgway and Gyrisco 1960). Multiple generations can be completed in the southeastern United States in one year (Leigh et al. 1996).

Damage to Cotton

Tarnished plant bug is an important pest of cotton in the Mid-South. It can feed on cotton at any growth stage from emergence to the last maturing bolls (Layton 2000). Feeding typically occurs on leaf buds and reproductive structures, such as squares, flowers, and fruit (Pack and Tugwell 1976). Early season feeding can result in 'crazy cotton' by causing reductions of plant height and weight, swollen nodes, deformed leaves, and can also lead to a delay in fruiting maturity (Scales and Furr 1968, Hanny et al. 1977). Feeding on flower buds by large infestations of tarnished plant bug in early season cotton can result in blank squares and significant yield losses. (Scales and Furr 1968, Scott et al. 1985). Most economic damage occurs from first-square to early bloom (Black 1973). Feeding on small pinhead squares (<0.32 cm diameter) can lead to the abscission of the squares, resulting in major yield loss (Layton 1995). Gutierrez et al. (1997) showed that a single tarnished plant bug can cause the abscission of 0.6 to 2.1 squares per day. As the crop matures, the tarnished plant bug can still cause damage. Feeding on late season bolls (≥ 300 HU) can potentially lead to lint or seed damage, but is highly unlikely to cause boll abscission (Russell et al. 1999).

Tarnished plant bug injects digestive salivary enzymes into plant tissue that breaks the tissues down and assists in the ingestion of nutrients (Pack and Tugwell 1976, Layton 2000). This feeding damages the plant in two ways. The first is mechanical breakdown of the cells at the site of feeding. Secondly, enzymes disrupt plant tissue and is thought to be the more critical aspect of the damage (Layton 1995). Damage from the enzymes injected with saliva are localized and do not appear to be systemic (Layton 2000).

Sampling

The tarnished plant bug is primarily controlled with foliar insecticides. Due to this, much research has been done to determine economic thresholds of tarnished plant bug at different growth stages of cotton (Musser et al. 2009). Snodgrass (1993) looked at the density of nymphs in cotton using both drop-cloths and sweep nets. Second and fourth instar nymphs were placed at four locations on cotton plants: leaves, mainstem, terminal, and inside square bracts. Drop-cloths captured more nymphs than the sweep net in every trial, regardless of plant height, release position, or size of the nymph. This is similar to research done by Young and Tugwell (1976) that found the drop cloth to capture 65% of the actual nymph population compared to 16% of the nymph population with the sweep net. Further research by Musser et al. (2007) comparing the drop-cloth and sweep net found similar results to the previous research. The drop-cloth caught more tarnished plant bug nymphs than the sweep net did in both years of the study. The sweep net caught more total tarnished plant bugs in one year than the drop-cloth (Musser et al. 2007). The sweep-net also caught more total insects than the drop-cloth (Musser et al. 2007).

When comparing indirect sampling methods, the most damage was seen when scouting dirty-blooms and the outside of bolls (Musser et al. 2007). The dirty-bloom method was the fastest indirect scouting method, but damage shown is considered to be old damage because it cannot be detected until around a week after feeding. Scouting for dirty-squares is the most efficient method of indirect sampling when considering damage found relative to when the damage occurred and the amount of time needed to scout fields. While time of day was not important for total tarnished plant bug or nymph counts for any sampling method, more adults were found later in the day when using whole-plant counts (Musser et al. 2007). Musser et al. (2007) compared drop-cloth fabric colors and although adult counts were not significantly different, there was a 22% increase in the number of nymphs found on black drop-cloths compared to white drop-cloths. This has led to the use of a black drop-cloth when sampling for tarnished plant bug.

Threshold

Tarnished plant bug population densities and square retention proved to be important factors for lint yield in pre-bloom cotton. Musser et al. (2009b) found that economic losses were minimized at thresholds of eight tarnished plant bugs per 100 sweeps and 80 to 90% square retention. Percent dirty squares and squares with yellow frass were the most promising predictor of yield reduction in flowering cotton. The use of drop-cloths and sweep-nets were not good predictors of yield reduction due to the stage of the crop (Gore 2005). When scouting fields once per week, the economic threshold for tarnished plant bug in blooming cotton should be between 1.6 to 2.6 plant bugs per 1.5 m of row (Musser et al. 2009). A threshold of 5-10% dirty squares is equivalent to the threshold given by Catchot (2015) of three tarnished plant bugs per 1.5 m of row with a black drop cloth and provides a similar economic return in blooming cotton (Gore et al. 2007).

Control Methods

Chemical insecticides are currently the most widely used method of control for tarnished plant bug in cotton in the mid-south. Several classes of insecticides are needed to control this pest. Due to growing resistance to organophosphates and pyrethroids, neonicotinoid insecticides are becoming more prevalent for control in both pre-bloom and blooming cotton. Imidicloprid (Admire Pro, Bayer Crop Science, Raleigh, NC) and thiamethoxam (Centric, Syngenta Crop Protection, Greensboro, NC) are the most commonly used neonicotinoids for control of tarnished plant bug (Gore et al. 2007). The insect growth regulator novaluron (Diamond® 0.83EC, ADAMA USA, Raleigh, NC), and the pyridine carboxamide, flonicamid (Carbine, FMC Corportation, Philadelphia, PA) are newer classes of insecticides targeted at tarnished plant bug control in cotton (Gore et al. 2007). Appropriate timing of both initial applications and subsequent applications is key to satisfactory control of tarnished plant bug. To avoid further resistance selection, the 2015 Insect Control Guide for Agronomic Crops from Mississippi State University offers an insecticide rotation strategy for tarnished plant bug management. From first-square to first bloom, the use of neonicotinoids or pyridine carboxamide are recommended. From first flower to peak flower, organophosphates (OP), OP + pyrethriod, or OP + novaluron are recommended. After peak flower the same recommendations as first flower to peak flower, plus neonicotinoids are given (Catchot et al. 2015). Fields should be scouted twice a week to ensure populations are controlled as soon as threshold is reached (Gore et al. 2007).

With the declining efficacy of most insecticides against tarnished plant bug, a multi-tactical approach is needed to obtain economical control. Adams (2012) showed that early planting dates combined with an early maturing cotton variety can significantly reduce the number of insecticide applications needed to control tarnished plant bug throughout the growing season. Reducing plant height and opening the plant canopy with plant growth regulators, such as mepiquat chloride, can potentially increase the effectiveness of insecticides needed late in the growing season (Graham 1985). Applying selective herbicides to areas such as turn rows, ditches, and roadsides for control of early season hosts can effectively help to reduce the cost of control later in the growing season (Snodgrass 2003, Snodgrass et al. 2006, Gore et al. 2010). Nectariless varieties have significantly fewer nymphs when compared to nectaried varieties (Schuster et al. 1976, Bailey et al. 1984). This could be due to reduced tarnished plant bug populations as well as reduced fecundity of plant bug females on nectariless varieties (Schuster et al. 1976). Over the years, cotton was bred to have frego bracts to deter boll weevil ovipostion. Unfortunately, this characteristic is attractive to plant bugs (Jenkins and Parrot 1971). The combination of early planting and nectariless varieties shows resistance to plant bugs (Milam et al. 1985).

Best Management Practices

Due to the polyphagous nature of the tarnished plant bug, it is able to survive and reproduce on several cultivated plants, including corn, *Zea mays* L., and soybean, *Glycine max* (L.) Merrill. Early season soybeans provide an important food source for tarnished plant bug with little exposure to insecticides. Tarnished plant bugs infest indeterminate soybeans and eventually move into cotton (Snodgrass et al. 2010). Research also has shown that corn is a good host for tarnished plant bug until the brown silk stage, when cotton becomes more preferable (Abel and Snodgrass 2003, Kumar and Musser 2009, Abel et al. 2010). Because of this, cotton should be planted in contiguous blocks away from early season soybeans and corn to avoid or minimize the “edge effect” from tarnished plant bugs moving into cotton.

Variety selection and planting date have been researched for their impact on tarnished plant bug management. Variety selection is a pivotal decision for growers. Although yield potential is the primary factor for variety selection, growers in areas where tarnished plant bug is the primary pest should also consider leaf pubescence (Wood 2015) and varietal maturity (Adams 2012) when selecting their varieties to minimize the impact of tarnished plant bug. Along with varietal maturity and planting date, there has been considerable research with cotton variety pubescence and how it affects various insects. Pubescence refers to the trichomes growing from the epidermis of leaves, shoots, and roots. Cotton varieties are described as glabrous (no trichomes), hirsute (moderate pubescence), or pilose (dense pubescence) (Nawab et al. 2011). Trichome density has been shown to have an effect on the ability of sucking pests to feed (Laster and Meredith 1974, Meredith and Schuster 1979, Bailey et al. 1980, Wilson and George 1986, Meagher et al. 1997). Laster and Meredith (1974) and Meredith and Schuster (1979) noted that a smooth leaf variety was significantly more sensitive to tarnished plant bug compared to hirsute varieties. Studies have shown glaborous varieties to have significantly more square damage than pilose varieties with similar plant bug populations (Meredith and Schuster 1979, Bailey et al. 1980). Wood (2015) reported that a hirsute variety with plant bug numbers two times threshold retained more squares than a smooth variety, with fewer plant bug numbers. When choosing cotton varieties, pubescence should be taken into consideration, and a hirsute to pilose variety should be considered.

Planting date and varietal maturity should also be taken into consideration. Early planting, from mid-April to early-May, required fewer insecticide applications to keep tarnished plant bug populations below threshold in a two year study in Mississippi (Adams 2012). Of the four planting dates used in the study, the first had the highest yield with each successive planting date having less than the previous planting date. This shows both an agronomic advantage to early planting, and affords the crop the chance to advance beyond the most susceptible stage before the tarnished plant bug population peaks. Although planting date showed no significant impact on plant bug densities, later planting dates required more insecticide applications for tarnished plant bug than earlier planting dates. In sprayed and unsprayed plots, the early maturing variety yielded significantly more than the later maturing variety.

Fertilization is another important factor for tarnished plant bug management. Nitrogen is important for cotton growth, development and yield. Tarnished plant bugs are attracted to lush cotton, which can result from excess nitrogen. Varco et al. (1990) reported that excessive nitrogen applications tend to cause an increase in plant height, vegetative growth, and can alter crop maturity. Samples (2014) showed that yield was maximized at 90 kg of nitrogen per hectare and 1 to 1.5 fewer insecticide applications were needed for tarnished plant bug management when compared to 134 kg N ha and 179 kg N ha, respectively. These results suggest the potential for growers to be able increase profitability by reducing fertilizer inputs while minimizing the impact of tarnished plant bug.

With the decline of insecticide efficacy, it is important to optimize application procedures to increase control. Hollow-cone nozzles show greater efficacy compared to air-induction nozzles for control of tarnished plant bug (Gore et al. 2010). The application of a single insecticide will seldom reduce the number of tarnished plant bugs below threshold. Research has shown that sequential applications can increase efficacy. Spray intervals of four to five days have been shown to give greater efficacy than six to seven day intervals (Gore et al. 2010, Bateman et al. 2014). Rotating insecticide chemistries on sequential applications can also improve efficacy. Research has shown that rotation of chemistries can maintain tarnished plant bug numbers below threshold for a longer period of time (Gore et al. 2010).

Much research has been conducted on the insect growth regulator, novaluron (Diamond® 0.83EC, ADAMA USA, Raleigh, NC). Lab trials have shown that Diamond is much more effective on 1st instar nymphs compared to 2nd and 5th instars, and can have significant detrimental effects on fecundity (Owen et al. 2011). Field research has shown a significant increase in yield when Diamond is part of a tank mix with other insecticides targeted at tarnished plant bug. Further research, in Mississippi, shows that the application of Diamond before the first week of flowering can significantly improve yields (Gore et al. 2010, Owen et al. 2011, Dobbins et al. 2014). Determining when to terminate tarnished plant bug applications is the final consideration needed for tarnished plant bug management. Making sprays that do not effectively protect yield both waste money and add unwarranted insecticide selection pressure on populations. Research has shown no benefit of tarnished plant bug applications made after the fourth week of flowering (Wood 2015). Applications targeted at tarnished plant bug in Mississippi should be terminated at five nodes above white flower plus 350 – 400 heat units (Catchot et al. 2015).

The development of these best management practices can help cotton growers combat the tarnished plant bug and reduce their reliance on chemical insecticides. These practices can help reduce the detrimental effects tarnished plant bug has on yields and, potentially, reduce the number of insecticide applications needed for control.

Justification

Economic damage from tarnished plant bug feeding was relatively low before the introduction of transgenic cotton varieties in 1996. These varieties contain proteins from the bacterium *Bacillus thuringiensis* Berliner (Bt) for control of the heliothine complex. The heliothine complex consists of two insect caterpillars that feed on fruiting structures of cotton, the tobacco budworm and bollworm, (Siebert et al. 2008). Bt cotton, combined with the eradication of the boll weevil, *Anthonomus grandis grandis* Boheman, has caused the tarnished plant bug to go from a secondary pest to a primary pest, because applications targeted at these pests, which provided management of the tarnished plant bug, are no longer required (Musser et al. 2009). Target and non-target applications made for tarnished plant bug have begun to select for resistance. Tarnished plant bug resistance to methyl parathion in the Mississippi River Delta was first documented in a 1979 study by Cleveland and Furr (1979). Resistance to dimethoate was reported in the Mississippi Delta by Snodgrass and Scott (1988), but there was little tolerance to acephate found. Resistance to pyrethroids, organophosphates, and cyclodiene insecticides in the Mississippi Delta were reported in 1996 (Snodgrass 1996). Widespread resistance to pyrethroid insecticides has been reported in the Mississippi River Delta regions of

Mississippi, Arkansas, and Louisiana (Pankey et al. 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2000, Snodgrass 2006). Acephate resistance was documented in one county of the Mississippi Delta in 2005 (Snodgrass 2006), and was widespread across the region by 2006 (Snodgrass and Gore 2007a, Snodgrass et al. 2009).

Due to the reduced efficacy of many insecticides, it is important to develop an integrated pest management program to reduce the reliance on chemical insecticides to manage this pest. To address the problem concerning tarnished plant bug in Mississippi, the following objectives were proposed:

Objective 1: Combine multiple best management practices to determine if the reliance on chemical insecticides to manage TPB can be reduced.

Objective 2 A: To determine the behavioral response of TPB to several classes of insecticides.

Objective 2 B: To determine the behavioral response of TPB to several classes of insecticides in the field.

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CHAPTER II

IMPACT OF BEST MANAGEMENT PRACTICES ON TARNISHED PLANT BUG MANAGEMENT

Abstract

An experiment was conducted in Starkville, MS and Stoneville, MS during 2014 and 2015 to determine the benefits of combining multiple best management practices into a tarnished plant bug integrated pest management program (TPB-IPM) for the tarnished plant bug in cotton. Two approaches were used in the study, a TPB-IPM approach and a standard approach. The TPB-IPM approach included combining an early planting date with a medium maturing, hirsute variety as well as 90 kg N/ha and an application of an insect growth regulator at peak tarnished plant bug adult migration. The standard approach utilized a late planting date combined with a late maturing, glabrous variety with 134 kg N/ha. In the hills, there was no difference in yield between cotton planted early managed using the TPB-IPM approach and cotton planted late managed using the standard approach, although cotton managed using the standard approach averaged 0.5 fewer insecticide applications per acre. There were relatively low tarnished plant bug densities during the study in the Hills. In the Delta, where tarnished plant bug densities were much higher, early planted cotton managed with the TPB-IPM approach resulted in significantly higher yields than late planted cotton managed using the standard approach.

Introduction

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is the most important pest of cotton in Mississippi. It has a known host range of nearly 330 plant species or subspecies in 55 plant families in the United States (Young 1986). The tarnished plant has a paurometabolous life cycle that takes from 30-40 days (Fleischer and Gaylor 1988). It was the primary pest of cotton in the Mississippi Delta in 2014, with a total loss of 47,616 bales. Growers in the region averaged 5 insecticde applications targeting the tarnished plant bug over the growing season, costing $133.13 per hectare (Williams 2014).

Although tarnished plant bug can feed on cotton at any stage, cotton is most susceptible to damage starting at squaring and lasting through mid-bloom (Scales and Furr 1968). Early season feeding in terminals can result in 'crazy cotton,' characterized by profuse secondary terminals due to the loss of apical dominace. High infestations in early season cotton can result in reductions of plant height and weight, swollen nodes, deformed leaves, and delayed maturity (Scales and Furr 1968, Hanny et al. 1977). Feeding typically occurs on leaf buds and reproductive structures, such as flowers, squares (flower buds), and bolls (Pack and Tugwell 1976). Feeding on squares can result in blank squares and significant yield reductions (Scales and Furr 1968, Scott et al. 1985). Typically, economic damage occurs from first-square through early bloom (Black 1973). Feeding on pinhead squares (<0.32 cm diameter) can result in abscission and cause significant yield losses if greater than 20% abscission occurs. (Layton 1995). A single tarnished plant bug can cause the abscission of 0.6 to 2.1 squares per day (Gutierrez et al 1997).

The pest status of the tarnished plant bug has increased in the past decade. Prior to the eradication of the boll weevil, *Anthonomus grandis grandis* (Boheman), and the introduction of *Bacillus thuringiensis* (Berliner) (Bt) crops for bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), direct losses caused by the tarnished plant bug were not as obvious as they presently are because much more focus was placed on these other pests (Luttrell and King 2014). Insecticide applications targeted at these pests over the years also selected for resistance in tarnished plant bug (Snodgrass et al. 2009), causing it to become a major pest of Mississippi cotton (Musser et al. 2009). In 1978, resistance to methyl parathion was documented in five counties of Mississippi and three counties of Arkansas along the Mississippi River (Cleveland and Furr 1979). This was the first confirmed resistance in the area Populations of the tarnished plant bug have since shown resistance to several classes of insecticides throughout the Mississippi Delta, including the pyrethroid, organophosphate, cyclodiene, and carbamate insecticides (Snodgrass and Scott 1988, Pankey 1996, Snodgrass 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2000, Snodgrass 2006, Snodgrass and Gore 2007, Snodgrass et al. 2009).

Control of the tarnished plant bug has become increasingly difficult in the past decade. A multi-tactical approach of best management practices is needed to combat this pest. Considerable research has been conducted on individual cultural practices and insecticide use strategies to improve management of tarnished plant bug. Variety selection and planting date are important practices for this pest. Adams et al. (2012) found that combining early planting dates, mid-April to early-May, with an early maturing variety provides a higher yield potential and reduces the number of insecticide applications needed to manage tarnished plant bug populations. Varietal pubescence also plays a role in combating tarnished plant bug. Smooth leaf varieties have been shown to be significantly more sensitive to tarnished plant bug feeding than hairy varieties (Laster and Meredith 1974, Meredith and Schuster 1979). Wood (2014) showed that a hairy variety with plant bug numbers twice as high as a smooth variety had higher square retention rates than the smooth variety. Proper timing of novaluron (Diamond® 0.83EC, ADAMA USA, Raleigh, NC) application has shown to aid in controlling tarnished plant bug nymphs. In Mississippi, applying novaluron at the third week of squaring improved tarnished plant bug control and significantly increased yields (Gore et al. 2010, Owen et al. 2011, Dobbins et al. 2014). Because tarnished plant bugs are attracted to lush cotton, it is important to properly manage nitrogen. Samples (2014) reported similar yields and fewer plant bug applications at 90 kg of N per hectare compared to both 134 kg and 179 kg N per hectare. Combining these best management practices can help combat the tarnished plant bug and reduce the reliance on chemical insecticides to achieve adequate control. As noted previously, considerable research has been conducted on individual management practices. However, comprehensive research that combines all of these practices into an overall integrated pest management approach has not been compared to a standard approach of managing this pest with insecticides. The objective of this research as to evaluate an overall TPB-IPM program for tarnished plant bug management in cotton.

Materials and Methods

Integrated Pest Management

To determine the benefits of the integrated pest management approach for tarnished plant bug in cotton, experiments were conducted in the hills region of Mississippi at the R.R. Foil Plant Science Research Facility in Starkville, MS and at the Delta Research and Extension Center in Stoneville, MS in 2014 and 2015. Treatments were in a split-plot arrangement in a randomized complete block design with four replications. Plots were 14 m long by 4 m wide. The main plot factor was planting date and included an early and late planting date. The subplot factor was management strategy and included a tarnished plant bug integrated pest management (TPB-IPM) approach and a standard approach. Early planting dates in 2014 were April 21 at Starkville and April 24 at Stoneville. Late planting dates in 2014 were May 27 at Starkville and June 4 at Stoneville. In both regions in 2014, plots for the IPM approach were only planted at early planting dates while plots for the standard approach were planted only at late planting dates. Two separate trials were conducted at different field locations within each region in 2015. Early planting dates in 2015 were May 5 for all trials and late planting dates were May 27 for all trials. Plots for both approaches were planted at early and late planting dates in all trials in 2015.

For the IPM approach, a medium maturity hirsute variety (Stoneville 5288 B2RF; Bayer CropScience, Raleigh, NC) was used. Ninety kg N/ha was applied to the TPB-IPM approach at the four-leaf stage. Novaluron was applied during the third week of squaring. The insecticide application strategy for the TPB-IPM approach was applying the highest labeled rates of each insecticide and using tank mixtures to ensure the best possible control of this pest regardless of cost.

For the standard approach, a late maturing glabrous variety (Deltapine 1050 B2RF; Monsanto Co., St. Louis, MO) was used. Nitrogen in this approach was applied during the four-leaf stage at a rate of 134 kg N/ha. Novaluron was not applied at any time during the season. The insecticide application strategy utilized lower rates and products that would give satisfactory control for the lowest possible price.

Both approaches were planted into conventionally tilled beds at a seeding rate of ~120,000 seeds per hectare. The seed were treated with a commercial premix of thiodicarb, imidicloprid, trifloxystrobin, triadimenol, and metalaxyl (Aeris Trilex Advanced, Bayer CropScience, Raleigh, NC) to reduce impacts from thrips, nematodes, and disease. Stoneville 5288B2F and Deltapine 1050B2RF have dual gene Bt protection against lepidopteran pests. Plots were sprayed with a preemerenge herbicide to control summer annual weeds. Planting depth was set to 2.54 cm below the soil surface. All plots were managed for high yield potential and scouted for disease and insect pests throughout the season.

Tarnished plant bug population estimates and damage assessments were sampled as appropriate to the cotton growth stage. Weekly tarnished plant bug sampling began at first-square and continued through bloom. During the first two weeks of squaring, samples were taken with a 38.1 cm diameter sweep net and square retention rates were monitored. Square retention was monitored by examining the first position fruiting sites on the top three nodes of cotton plants until 25 fruiting sites were examined in each plot. A square was considered abscised if it was missing. Also, squares were considered abscised if evidence of tarnished plant bug damage was observed as either blasted (dried) squares or if the bracts were opened (flared). Missing squares were determined by the presence of an abscission scar at the first position on each fruiting branch. The percentage of retained squares was recorded. From the third week of squaring through bloom, tarnished plant bug densities were determined using a black drop cloth. Samples were taken by laying the cloth between two cotton rows near the center of the plot and vigorously shaking all of the plants from each row onto the cloth. One sample resulted in 1.52 m of row being sampled. All tarnished plant bug numbers were recorded separately as nymphs and adults. Sampling was terminated when cotton reached five nodes above white flower plus 350-400 heat units (DD60s). Insecticide applications were made at threshold levels published in the 2014 and 2015 Insect Control Guides for Agronomic Crops (Catchot et al. 2015). During the first two weeks of squaring, the threshold was defined as 8 bugs/100 sweeps or when square retention rates fell below 80%. From the third week of squaring through bloom, threshold levels were 3 bugs per 1.52 row m (Catchot 2015). The center two rows of each plot were harvested and seed cotton weights were recorded. Lint percentage was determined by taking 40 percent of the total seed cotton weight.

Data Analysis

All data were analyzed with a general linear mixed model analysis of variance PROC GLIMMIX of SAS (Version 9.3, SAS Institute, Cary, NC). Region, planting date, and management program were designated as fixed effects in the initial analysis. Year, replication nested in year, and replication by region nested in year were designated as random effects in the model. Degrees of freedom were estimated using the Kenwood-

Rogers method. Means were estimated using LSMEANS and separated based on Fisher's protected least significant difference (α=0.05).

The overall goal of each of these approaches was to maintain tarnished plant bug densities below the current threshold (3 per 1.52 row m) and maintain the currently recommended level of square retention (>80%). The initial analysis included sample date as a fixed effect, but it was removed from the model because insecticides were applied to each treatment independently and affected each of these factors. As a result, each treatment was sprayed a different number of times and at different times during the season making interpretation of tarnished plant bug numbers and square retention difficult. Cumulative tarnished plant bug density and mean square retention was analyzed across all sample dates to show the impact of the overall management strategy on those variables. The analyses for tarnished plant bug densities and square retention included region, planting date, treatment and their interactions as fixed effects in the model. Year, replication nested in year, replication by trial nested in year, and replication by planting date nested in year were considered random effects in the model. Degrees of freedom were estimated using the Kenwood-Rogers method. Means were estimated using LSMEANS and separated based on Fisher's protected least significant difference (α=0.05).

Results and Discussion

Integrated Pest Management Program

The total number of applications for tarnished plant bug management ranged from 0 to 6 across all years, regions, and locations (Table 2.1). In general, more insecticide applications were needed in the delta than in the hills. Additionally, later planting dates and the standard approach required more applications than earlier planting dates and the TPB-IPM approach in the delta.

In terms of cumulative numbers of tarnished plant bug, there was a significant region by planting date interaction ($F=25.11$; $df=1, 45.2$; $P<0.01$). Significantly more tarnished plant bugs were recorded in the delta region compared to the hill region (Table 2.2). Additionally, more tarnished plant bugs were observed at the early planting date compared to the late planting date in the delta. In a recent study, tarnished plant bug survival and development times of populations from the delta region were greater on cotton than populations from the hills region (Adams et al. 2014). Additionally, adults from the delta region laid more viable eggs than populations from the hills region.

Similar to tarnished plant bug densities, there was a significant region by planting date interaction for mean square retention ($F=4.8$; $df=1, 26.3$; $P=0.04$). As would be expected based on tarnished plant bug densities, mean square retention was significantly lower in the delta compared to the hills (Table 2.3). Square retention at the early planting date was significantly lower than the later planting date in the hills. The region by treatment interaction was also significant for mean square retention ($F=42.1$; $df=1, 24.7$; $P<0.01$). No differences in mean square retention were observed between treatments in the hills (Table 2.2). In the delta, the TPB-IPM approach resulted in significantly higher mean square retention than the standard approach. The difference in square retention among treatments in the delta is interesting because there was no statistical difference in number of plant bugs for plots managed with either approach. Plots managed using the standard approach required more insecticide applications than plots managed using the TPB-IPM approach. This suggests that some other component of the TPB-IPM approach, most likely varietal pubescence (Meredith and Schuster 1979), is impacting tarnished plant bug feeding injury allowing the cotton to retain higher levels of square retention. Mean square retention in the hills and in the TPB-IPM program in delta were above the current 80% threshold.

There were significant region by planting date by treatment ($F=14.95$; $df=1, 90.30$; $P=0.02$), region by planting date ($F=7.93$; $df=1, 73.34$; $P=0.01$), and region by treatment ($F=63.9$; $df=1, 73.34$; $P<0.01$) interactions for yield. This was expected because of the relative differences in the intensity of tarnished plant bug infestations between the two regions. Because of the differences in tarnished plant bug pressure and square retention between the hills and delta and because of the interactions with region, yield data were analyzed by region. Within each region, there were no trial by planting date by treatment, trial by planting date, or trial by treatment interactions for cotton yields (Table 2.4). Therefore, trial was designated as a random effect in the model for analysis of yield.

Hills Region

Yield data for the hills region were analyzed by year. Because planting date was not a factor in 2014, yields among treatments were compared with a t-test. There was a significant difference among treatments in 2014 ($t=2.44$, $df=6$, $P=0.05$). The TPB-IPM approach resulted in greater yields than the standard approach (Table 2.5).

There was a significant planting date by treatment interaction for yield in 2015 ($F=4.42$; $df=1, 23.8$; $P=0.05$). Cotton at the early and late planting dates using the standard approach had significantly higher yields than the TPB-IPM approach at the early planting date (Table 2.5). Late planted cotton using the standard approach was not significantly different from late planted cotton using the TPB-IPM approach. Early planted cotton utilizing the TPB-IPM approach had significantly lower yields than cotton utilizing the standard approach at either planting date (Table 2.5). The significant increase in yield for early planted cotton grown with the standard approach could be explained by a number of things. Cotton was planted in wet conditions in 2015, which could have led to shallow root growth and delayed maturity. Full season cotton varieties are better equipped to overcome early season stress than mid to short season varieties. Also, variety trials conducted in 2015 on the same farm in Starkville, MS showed that late season varieties had higher yields on average than medium maturing varieties. This suggests that growers in the hills region of Mississippi should place less emphasis on varietal selection based on tarnished plant bug management and more on overall yield potential in their area.

Delta Region

In the delta region, trends among treatments were similar when yield data were analyzed by year. Therefore, data were analyzed across years. There was a significant planting date by treatment interaction for yield ($F=9.96$; $df=1, 21$; $P<0.01$). Cotton planted at the early planting date and managed utilizing the TPB-IPM approach had significantly greater yields than cotton planted at the early planting date and managed using the standard approach and cotton planted at the later date and managed using either approach (Fig. 2.6). No differences in yield were observed between cotton planted at the late planting date utilizing the TPB-IPM approach and cotton planted at the early planting date utilizing the standard approach. Cotton planted at the late planting date utilizing the standard approach had significantly lower yields than cotton planted at the late planting date utilizing the TPB-IPM approach and cotton planted at the earlier planting date managed using either approach.

In the hills region, cotton managed utilizing the TPB-IPM approach yielded significantly higher than cotton managed utilizing the standard approach in 2014, while in 2015 the early planted cotton utilizing the standard approach yielded significantly higher than cotton utilizing the TPB-IPM approach. On average, Cotton utilizing the TPB-IPM approach yielded higher than cotton utilizing the standard approach in the delta at both the early and late planting date. This can be attributed to the differences in tarnished plant bug pressure in the delta region compared to the hills region. The fact that cotton grown using the standard approach yielded higher than cotton grown using the TPB-IPM approach in the hills region during 2015 with very low tarnished plant bug pressure suggests that the increase in yields in the delta region is not attributed to cotton variety yield potential. These data suggest that there should be an emphasis placed on cotton TPB-IPM based on regions of Mississippi. Growers in regions with historically low tarnished plant bug densities should focus on yield potential when choosing verities rather than tarnished plant bug IPM. This program was not as effective when cotton was not subjected to large tarnished plant bug populations that were difficult to control with more conventional approaches. Growers in regions with historically high tarnished plant bug densities, on the other hand, should strongly consider tarnished plant bug IPM along with yield potential when selecting cotton varieties.

This study combined several researched tactics into an overall tarnished plant bug integrated pest management program. Integrating tactics to manage the earliness of the cotton crop (Adams 2012) with a hirsute cotton variety (Meredith and Schuster 1979, Bailey et al. 1980, Wilson and George 1986, Wood 2014), using reduced rates of nitrogen (Samples 2014), and an application of novaluron (Gore et al. 2010) reduced the number of insecticide applications required in the Mississippi Delta and resulted in greater yields.

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Table 2.1 Number of foliar insecticide applications needed to manage tarnished plant bugs in cotton grown in the hills and delta regions of Mississippi.

	2014		**2015**			
	Early	**Late**	**Early**		**Late**	
	TPB-IPM	**Standard**	**TPB-IPM**	**Standard**	**TPB-IPM**	**Standard**
Hills	3	1	1	1	1	0.50
Delta	3	6	4	5	5	6

Table 2.2 Interaction between region and planting date on the mean (SEM) cumulative number of tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), nymphs in cotton averaged across 2014 and 2015.

Cumulative Number per 3.04 row m			
Delta		**Hills**	
Early Planted	**Late Planted**	**Early Planted**	**Late Planted**
54.4 (5.4)a	36.9 (4.9)b	9.2 (1.6)c	10.8 (1.6)c

Means separated by a common letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Table 2.3 Interaction between region and planting date, and region and treatment on mean (SEM) percent square retention in cotton averaged across 2014 and 2015.

Percent			
Delta		**Hills**	
Early Planted	**Late Planted**	**Early Planted**	**Late Planted**
79.2 (2.0)c	74.8 (1.8)c	91.8 (0.8)b	94.9 (0.9)a
TPB-IPM	**Standard**	**TPB-IPM**	**Standard**
84.0 (0.8)b	69.9 (1.5)c	93.5 (1.1)a	93.3 (0.7)a

Means separated by a common letter within a row are not significantly different according to Fisher's Protected LSD (α=0.05).

Table 2.4 Analysis of variance for the impact of trial interactions on cotton yields in the hills and delta regions of Mississippi in 2014 and 2015.

Hills Region	F	df	**P**
Trial*planting date*treatment	1.64	1, 24.8	0.21
Trial*planting date	0.09	1, 24.8	0.77
Trial*treatment	0.34	1, 24.8	0.57
Delta Region			
Trial*planting date*treatment	0.48	1, 24.8	0.49
Trial*planting date	0.36	1, 24.8	0.55
Trial*treatment	1.76	1, 24.8	0.19

Table 2.5 Mean (SEM) cotton lint yields in 2014 and 2015 for each tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), management program at two planting dates (Early and Late) in the hills region of Mississippi.

2014		2015			
Early	Late	Early		Late	
IPM	Standard	IPM	Standard	IPM	Standard
999 (51) a	823 (51) b	933 (44) c	1314 (62) a	1087 (83) bc	1207 (54) ab

Means within a year separated by a common letter are not significantly different according to Fisher's Protected LSD (α=0.05).

Table 2.6 Mean (SEM) cotton lint yields averaged across 2014 and 2015 for each tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), management program at two planting dates (Early and Late) in the delta region of Mississippi.

Weight (kg/Ha)			
Early		Late	
TPB-IPM	Standard	TPB-IPM	Standard
1400 (86) a	729 (53) b	973 (41) b	720 (29) c

Means separated by a common letter are not significantly different according to Fisher's Protected LSD (α=0.05).

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CHAPTER III

TARNISHED PLANT BUG BEHAVIORAL RESPONSE TO CHEMICAL INSECTICIDES

Abstract

Laboratory experiments were conducted during 2014 and 2015 to study the behavioral response of tarnished plant bug nymphs to several classes of insecticides. Twenty, third-instar nymphs were placed in individual dishes divided into four quadrants with 5 green bean pieces in each quadrant (10 treated and 10 untreated green beans in each dish). Dishes were checked at 1, 4, 8, and 24 hours. Tarnished plant bug nymphs appeared to avoid green beans treated with IGR, pyrethroid, organophosphate or carbamate insecticides, while there appeared to be an attraction to green bean pieces treated with sulfoxamine and pyridine carboxamide insecticides. No relationship was observed with neonicotinoid insecticides within 24 hours. Field experiments were conducted in Glendora, MS during 2014 and 2015 to study behavioral responses in the field. Insecticides representative of several classes used for tarnished plant bug control in cotton were evaluated. Twenty total plants were sampled per treatment. Sampling each individual plant began at the terminal and moved down each node and across to each fruiting structure on the respective nodes. Plots were also sampled with a black drop cloth to determine the efficacy of each treatment. There did not appear to be any behavioral

response to insecticide treatments in the field trials at 3 days after treatment or 7 days after treatment.

Introduction

The tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is the primary pest of cotton in the Mid-South. Prior to the eradication of the boll weevil, *Anthonomas grandis grandis* (Boheman), and the introduction of *Bacillus thuringiensis* (Berliner) (Bt) crops to manage bollworm, *Helicoverpa zea* (Boddie), and tobacco budworm, *Heliothis virescens* (F.), the pest status of tarnished plant bug was less obvious than it is now (Luttrell and King 2014). Insecticide applications targeting the boll weevil, bollworm, and tobacco budworm provided coincidental control of tarnished plant bug and may be an important cause for resistance to organophosphates and pyrethroids in tarnished plant bug (Snodgrass and Scott 2003). The first documentation of insecticide resistance in tarnished plant bug was to methyl parathion in populations from the Mississippi Delta during the late 1970's (Cleveland and Furr 1979). Subsequently, Snodgrass (1988) reported tolerance to dimethoate in the same region. By the mid-1990's, resistance to pyrethroid, organophosphate, and cyclodiene insecticides was reported in the Mississippi Delta (Snodgrass 1996). Resistance to pyrethroids have been reported in most counties along the Mississippi River in Arkansas, Louisiana, and Mississippi (Pankey et al. 1996, Hollingsworth et al. 1997, Snodgrass and Scott 2000, Snodgrass 2006). More recently, widespread resistance to acephate has been reported in the region (Snodgrass and Gore 2007, Snodgrass et al. 2009). Despite widespread resistance, insecticides remain an important component of tarnished plant bug management in cotton.

The distribution of tarnished plant bug in cotton following an insecticide spray is a topic that needs further research. A study by Fye (1971) reported that approximately 85% of insects recorded in untreated cotton plants were found in the upper 0.61 m of plants. Snodgrass (1998) found that 75% of adult and nymph tarnished plant bugs were found on the upper six nodes of the main stem in untreated cotton. In the same study, a strong preference for fruiting structures by nymphs was observed from squaring throughout the season. Adults were found in high numbers on vegetative structures during the first three weeks of squaring, then moving to reproductive structures towards the end of the season. A study on *Lygus hesperus* Knight showed this pest to be found mainly on the upper five to seven nodes (Wilson et al. 1984). This study also reported that nymphs prefer to feed on squares and adults on bolls. Pack and Tugwell (1976) showed that tarnished plant bugs prefer pinhead squares over larger squares or bolls.

The impact of insecticide classes on the behavior and distribution of tarnished plant bug in cotton is also important. Fontenot (2009) reported significantly more tarnished plant bug nymphs on squares than on white flowers or bolls in acephate treated cotton at various sampling periods ranging from 24 to 120 hours after treatment. The only difference between the distribution of tarnished plant bug on acephate treated and untreated cotton was observed at 24 hours after treatment. Significantly fewer nymphs were observed on acephate treated squares than on untreated squares. Fontenot (2009) also studied the vertical distribution of nymphs on acephate treated and untreated plants. Significantly more nymphs were observed in the upper and middle thirds than the lower thirds of untreated plants, while the greatest number of nymphs were observed in the middle third of the canopy after treatment with acephate and in the upper third of the canopy in the untreated cotton. The authors speculated that this could be because the highest mortality rate occurred in the upper third of the canopy directly after application. As the lethal residual decays, the upper level could be re-infested by nymphs migrating up the plant or by newly hatched nymphs (Fontenot 2009). The behavioral response of tarnished plant bug to other insecticides has not been investigated. The objectives of these experiments were to determine the behavioral response of tarnished plant bug to several classes of insecticides in the laboratory and in the field on cotton plants.

Materials and Methods

Laboratory Behavior

Experiments were conducted at the Clay Lyle Entomology Complex in Starkville, MS in 2014 and 2015 to determine the response of tarnished plant bug to selected insecticides representing the organophosphate, carbamate, pyrethroid, neonicotinoid, insect growth regulator, and pyridine carboxamide classes. These experiments were conducted using third instar nymphs from a laboratory colony. Twenty nymphs were aspirated into 1.5 mL scintillation tubes. Green beans, *Phaseolus vulgaris,* were washed in a mixture of water and bleach, rinsed with clean water, and placed under a vent hood to dry. Green beans were then cut into 1.27 cm long pieces and submerged into mixtures of insecticides for three seconds using a stainless steel mesh strainer. After treatment, the green bean pieces were placed on paper towels and allowed to air dry. The insecticides were mixed in 4.64 L stainless steel sprayer tanks. The insecticides and mix rates used in 2014 were sulfoxaflor (Transform WG™, Dow AgroSciences, Indianapolis, IN) at 8.6 g ai per ha, acephate (Orthene 90S WSP, Valent USA, Walnut Creek, CA) at 148.6 g ai per ha, bifenthrin (Brigade® 2EC, FMC Corporation, Princeton, NJ) at 18.3 g ai per ha, oxamyl (Vydate® C-LV, DuPont Crop Protection, Wilmington, DE) at 52.3 g ai per ha, flonicamid (Carbine™ 50WG, FMC Corporation, Wilmington, DE) at 16.3 g ai per ha, thiamethoxam (Centric®, Syngenta Crop Protection, Inc., Greensboro, NC) at 13.6 g ai per ha, and novaluron (Diamond® 0.83EC, ADAMA USA, Raleigh, NC) at 57 g ai per ha. The untreated control consisted of a tank with water only. In 2015, the treatments included imidicloprid (Admire® Pro, Bayer Crop Science, Raleigh, NC) at 7.4 ml ai per ha, permethrin (Ambush®, AgNova Technologies, Box Hill North Vic, Australia) at 36.7 ml ai per ha, bifenthrin (Brigade® 2EC, FMC Corporation, Princeton, NJ) at 18.3 g ai per ha, thiamethoxam (Centric®, Syngenta Crop Protection, Inc., Greensboro, NC) at 13.6 g ai per ha, and water for an untreated control. All concentrations were calculated based on a 112 L per ha spray volume.

The assay arenas consisted of individual 245mm square polystyrene bioassay dishes (Corning™ Product Number 431272) that were divided into four quadrants. Five green bean pieces were placed into each quadrant, for a total of 20 green beans per dish. Two of the quadrants contained treated green beans and the other two quadrants contained untreated green beans. Assays were conducted a total of seven times over the two year period. Insecticides were grouped based on IRAC MoA chemical sub-group or exemplifying active ingredient (Table 3.1). Treatments were not evaluated at every assay (Table 3.1). In each assay, treatments were replicated four times, with one dish per replication for a total of four dishes per treatment. Green bean pieces were randomly assigned (treated or untreated) to each quadrant within each replication. Twenty green beans were chosen to allow for a 1:1 ratio of green beans to plant bugs to ensure that there were enough green beans to host the plant bugs and minimize overcrowding. The dishes were wrapped in parafilm to ensure no plant bugs were able to escape. Plant bugs were emptied from the scintillation tubes in the center of the dishes and every attempt was made to avoid placing them closer to the treated or untreated green beans. The dishes were checked at intervals of 1 hour, 4 hours, 8 hours, and 24 hours. The number of alive and dead plant bugs were recorded by date, replication, treatment, and time interval. The location (treated or untreated) of live plant bugs was also recorded. Tarnished plant bugs that were found dead were not counted in the analysis for the interval found or any intervals after, because behavior cannot be monitored for dead insects.

Field Behavior

An experiment was conducted in Glendora, MS in 2014 to determine the response of tarnished plant bugs to certain insecticides in the field. Treatments were arranged in a randomized complete block with four replications. Each plot consisted of four rows on 1.02 m centers that were 12.19 m in length. Replications were separated by 1.52 m of bare soil to reduce movement of tarnished plant bug between replications.

Treatments were applied when tarnished plant bug populations reached threshold based on recommendations from the 2014 Insect Control Guide for Agronomic Crops (Catchot et al. 2014). Insecticides were sprayed with a high-clearance sprayer (MudMaster Multi-Purpose Sprayer, Bowman Manufacturing, Newport, AR) with 4.64 L tanks calibrated to deliver 93.5 L per ha at 4.82 kph. The insecticides and rates tested included sulfoxaflor (Transform WG™, Dow AgroSciences, Indianapolis, IN) at 8.6 g per ai ha, acephate (Orthene 75 S90 WSP, Amvac, Walnut Creek, CA) at 148.6 g ai per ha, bifenthrin (Brigade® 2EC, FMC Corporation, Princeton, NJ) at 18.3 g ai per ha, oxamyl (Vydate® C-LV, DuPont Crop Protection, Wilmington, DE) at 52.3 g ai per ha, flonicamid (Carbine™ 50WG, FMC Corporation, Wilmington, DE) at 16.3 g ai per ha, thiamethoxam (Centric®, Syngenta Crop Protection, Inc., Greensboro, NC) at 13.6 g ai per ha, and novaluron (Diamond® 0.83EC, ADAMA USA, Raleigh, NC) at 57 g ai per ha, dicrotophos (Bidrin® 8, Amvac Chemical Chemical Company, Walnut Creek, CA), and an untreated control.

All plots were sampled three days after treatment. Each sample consisted of visually examining 20 plants looking for plant bugs. Visual sampling of each individual plant began at the terminal and moved down each node and across to each fruiting structure on the respective nodes. Numbers of tarnished plant bug adults and nymphs were recorded by the node, position of fruiting structure, and type of fruiting structure (square, flower, or boll). Two samples per plot were also taken with a 0.76 m black drop cloth sampling 1.52 row m. Drop cloth samples were taken by laying the cloth between two cotton rows near the center of the plot and vigorously shaking all of the plants within the 0.76 m on each row onto one cloth. An average plant node count for each plot was determined. Plant heights and nodes above white flower were also recorded from each plot. Nodes above white flower were determined by counting the number of plant nodes above the upper-most first position white flower on an individual plant.

A separate experiment was conducted in Glendora, MS in 2015. The experimental procedures were the same as the experiment conducted in 2014 except that different insecticides were evaluated and plots were sampled at 3 and 7 days after treatment. The insecticides included, imidicloprid (Admire® Pro, Bayer Crop Science, Raleigh, NC) at 7.4 g ai per ha, bifenthrin (Brigade® 2EC, FMC Corporation, Princeton, NJ) at 18.3 g ai per ha, a tank mixture of imidicloprid and bifenthrin (at the same respective rates), and an untreated control.

Data Analysis

For laboratory bioassays, the proportions of live nymphs on treated green beans were calculated at each rating interval for each insecticide. Laboratory bioassay data at the 24 hour rating were analyzed with a general linear mixed model analysis of variance for repeated measures (PROC GLIMMIX, Little et al. 1996). Insecticide class was designated as a fixed effect in the model. Time was the repeated term. Test and replication nested in test were designated as random effects in the model. Degrees of freedom were estimated using the Kenward-Roger method. Means were estimated using LSMEANS and separated based on Fisher's Protected Least Significant Difference (α=0.05). Additionally, the relationship between the proportions of nymphs on treated green beans over time was analyzed with regression analysis for each insecticide group.

Field behavioral data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, Littell et al. 1996). Treatment was considered a fixed effect in 2014, and treatment, days after treatment and their interaction were considered fixed effects in 2015. Plant and plant nested in replication were considered random in both years. Degrees of freedom were estimated using the Kenward-Roger method. Means were estimated using LSMEANS and separated based on Fisher's protected least significant difference (α=0.05).

Drop cloth data were analyzed with a mixed model analysis of variance (PROC GLIMMIX, Littell et al. 1996). In 2014, treatment was considered a fixed effect and replication was considered the random effect. In 2015, treatment and days after treatment were considered fixed effects and replication was considered the random effect. Degrees of freedom were estimated using the Kenward-Roger method for both years. Means were estimated using LSMEANS and separated based on Fisher's Protected Least Significant Difference ($\alpha=0.05$).

Results and Discussion

Laboratory Behavior

Tarnished plant bug nymphs were attracted to green beans treated with sulfloxamine and pyridine carboxamide insecticides. There was a positive quadratic relationship ($F=5.24$; $df=1, 61$; $P=0.03$) for the proportion of tarnished plant bug nymphs on sulfoxamine treated green beans over time (Fig. 3.1). In contrast, there was a positive linear relationship ($F=4.52$; $df=1, 61$; $P=0.04$) for the proportion of nymphs on pyridine caboxamide treated green beans over time. For the sulfoxamine insecticide, attraction to the treated green beans occurred rapidly and attraction to the pyridine carboxamide occurred gradually over time (Fig. 3.1).

Tarnished plant bug nymphs appeared to avoid green beans treated with IGR, organophosphate and carbamate insecticides. There were significant negative linear relationships for the IGR insecticide ($F=6.04$; $df=1, 46$; $P=0.02$), and the organophosphate insecticides ($F=8.92$; $df=1, 62$; $P<0.01$) for the proportion of nymphs observed on treated green beans over time (Fig. 3.1). There was a significant negative quadratic relationship ($F=3.06$; $df=1, 76$; $P=0.08$) for the proportion of nymphs observed on treated green beans for the carbamate insecticides. This would suggest that nymphs rapidly avoid carbamates and slowly avoid the IGR and organophosphate insecticides over time. As treatments were rated at the 1, 4, and 8 hour intervals, it was apparent that the tarnished plant bug nymphs were gaining a stronger attraction or avoidance to each respective treatment leading up to the 24 hour rating (Table 3.2), when the tarnished plant bug nymphs had ample time to make behavioral responses.

No relationship ($F=1.56$; $df=1, 155$; $P=0.21$) was observed for the proportion of nymphs observed on neonicotinoid treated green beans over time in these studies (Fig. 3.1). In the case of the neonicotinoid insecticides, there appeared to be a slight increase over time in the proportion on treated green beans. The attraction to neonicotinoid treated sucrose solutions has been shown with honeybees, *Apis melifera,* and bumblebees, *Bombus terrestris,* (Kessler et al. 2015). The active ingredients used in that study were the two used in this study, imidacloprid and thiamethoxam. We were not able to show a behavioral response of tarnished plant bug to neonicotionoids within the 24 hour period evaluated. It is not known what may have happened after the 24 hour period and we cannot conclude that there is no attraction.

Similar to neonicotinoids, there was no relationship ($F=1.58$; $df=1, 142$; $P=0.21$) for the proportion of nymphs on pyrethroid treated green beans over time (Fig. 3.1). Although the relationship was not significant, tarnished plant bug nymphs appear to avoid pyrethroid insecticides. At the initial rating interval (1 hour), the number of nymphs on pyrethroid treated green beans was already less than 40%. By 24 hours, this proportion had declined to 31% (Table 3.2). It appears that the behavioral response of tarnished plant bug to pyrethroids occurred very rapidly in these studies. In a field study observing the behavioral response of honey bees, the presence of permethrin caused honey bees to avoid entering treated fields (Pike et al. 1982). The results found by Pike et al. (1982) were similar to results with the tarnished plant bug in this study, which observed third instar tarnished plant bug nymphs avoiding green beans treated with permethrin.

With the widespread occurrence of resistance to multiple classes of insecticides (Snodgrass 1988, Snodgrass 1996, Snodgrass 2003, Snodgrass 2006, Snodgrass and Gore 2007, Snodgrass 2009), understanding the behavioral response of tarnished plant bugs to those to insecticide classes may improve our understanding of control and control failures. Spraying insecticides that invoke behavioral responses could help improve efficacy or lead to control issues. Using insecticides that tarnished plant bugs tend to avoid could cause them to potentially move out of protected structures of the plant, such as square bracts, and put them in direct contact with the insecticide. Similarly, using insecticides that tend to attract tarnished plant bugs could cause them to move into better contact with the insecticide. Obviously, if tarnished plant bugs do have an avoidance behavior to insecticides, this could also cause them to flee to lower parts of the cotton canopy, where there is little to no insecticide coverage, leading to control failures. Further research needs to be conducted to better understand the behavioral responses of the tarnished plant bug to chemical insecticides.

Field Behavior

There was no significant effect of treatment on the distribution of tarnished plant bugs in cotton in 2014 ($F= 1.12$; $df = 8, 145$; $P=0.35$). Therefore, a separate analysis was done to compare all insecticide treatments to the untreated control. In that analysis, the presence of an insecticide had a significant effect on the average location of tarnished plant bugs in cotton ($F= 4.12$; $df = 1, 149.7$; $P=0.04$). Tarnished plant bugs in untreated plots were a mean (SE) of 7.21 (0.38) nodes below the terminal compared to 6.36 (0.34) nodes below the terminal in the insecticide treated plots.

There was no interaction between treatment and days after treatment ($F= 0.87$; $df= 3, 204.1$; $P=0.46$) or no effect of treatment ($F= 1.83$; $df = 3, 204.8$; $P=0.14$) on the distribution of tarnished plant bugs in the canopy of cotton in 2015. There was a significant effect of days after treatment on the distribution of tarnished plant bugs in the canopy ($F= 16.49$; $df = 1, 196.2$; $P<0.01$). Tarnished plant bugs were a mean (SE) of 13.27 (0.46) nodes below the terminal at three days after treatment compared to 15.26 (0.29) nodes below the terminal at seven days after treatment.

Treatment had a significant effect ($F=2.85$; $df= 3,27$; $P=0.02$) on mean number of tarnished plant bugs found per treatment in 2014. All treatments were significantly different than the untreated control, except for dicrotophos that was not significantly different from either (Table 3.4).

In 2015, there was no interaction between treatment and days after treatment ($F=0.53$; $df= 3,24$, $P=0.66$) or no effect of days after treatment ($F=1.53$; $df=1,24$; $P=0.23$) on the number of tarnished plant bugs found per treatment. There was a significant effect of treatment on number of tarnished plant bugs found per treatment ($F=4.40$; $df=3,24$; $P=0.01$). Imidacloprid at both days after treatment and bifenthrin at three days after treatment were not significantly different from the untreated control. Bifenthrin at seven days after treatment and the tank mixture of imidicloprid plus bifenthrin were significantly lower than the check (Table 3.5).

Based on these data, use of insecticide did not appear to affect the distribution of tarnished plant bugs in the canopy of cotton plants. However, in a study by Fontenot

(2009) evaluating tarnished plant bug behavioral responses to acephate, significantly more tarnished plant bugs were found in the middle third of the cotton canopy compared to the untreated control, which had significantly more tarnished plant bugs in the upper third. The differences found in these two studies could be attributed to scouting method. Fontenot scouted plants until 20 tarnished plant bugs were found. In this study, 20 plants were observed. Not all plots in this study found 20 tarnished plant bugs leading to a smaller and uneven data set. This potentially effected distribution since there were not as many data points to evaluate.

There were no observable differences in field trials for either avoidance or attraction. This could be caused by a difference in tarnished plant bug populations used. Native (wild) populations evaluated most likely have higher resistance to some of the insecticides used when compared to laboratory colonies. Sample intervals could have also been a factor. All laboratory samples were observed within a 24 hour period, while field trials were observed at 72 and 168 hours after treatment. More research needs to be conducted to further explore tarnished plant bug behavioral responses to insecticides.

Table 3.1 List of insecticide treatments used for the impact of tarnished plant bug behavior in laboratory bioassays in 2014-2015.

Insecticide	Class	Tests	Reps
Oxamyl	1A	4	4
Dicrotophos	1B	1	4
Acephate	1B	4	4
Permethrin	3A	3	4
Bifenthrin	3A	6	4
Imidacloprid	4A	3	4
Sulfoxaflor	4C	4	4
Thiamethoxam	4A	7	4
Flonicamid	9C	4	4
Novaluron	15	3	4

Table 3.2 Proportion of tarnished plant bugs nymphs attracted to treated green beans 24 HAT in laboratory bioassays during 2014 and 2015.

Treatment	Proportion Attracted
Sulfloxamines	0.71 (0.04) a
Pyridine Carboxamides	0.60 (0.04) ab
Neonicotinoids	0.57 (0.05) ab
Insect Growth Regulators	0.36 (0.05) bc
Organophosphates	0.34 (0.04) bc
Pyrethroids	0.31 (0.03) bc
Carbamates	0.19 (0.05) c

Table 3.3 Mean (SEM) number of tarnished plant bugs observed per drop in insecticide efficacy trial in Glendora, MS in 2014.

Treatment	3 DAT
UTC	19.75 (5.06) A
Dicrotophos	12.25 (4.27) AB
Bifenthrin	6.75 (1.93) B
Flonicamid	7.5 (1.94) B
Thiamethoxam	7.0 (0.91) B
Novaluron	6.25 (0.85) B
Acephate	5.75 (1.38) B
Sulfloxaflor	7.75 (1.44) B
Oxamyl	8.75 (2.29) B

Means followed by the same letter are not significantly different at alpha = 0.05.

Table 3.4 Mean (SEM) number of tarnished plant bugs observed per drop in insecticide efficacy trial in Glendora, MS in 2015.

Treatment	3 DAT	7 DAT
UTC	29.00 (5.72) A	25.00 (4.67) AB
Imidicloprid	21.25 (5.85) ABCD	19.25 (1.60) ABCD
Bifenthrin	24.00 (1.74) ABC	15.50 (2.02) BCD
Imidicloprid + Bifenthrin	12.50 (2.90) D	13.503.59) DC

Means followed by the same letter are not significantly different at alpha = 0.05.

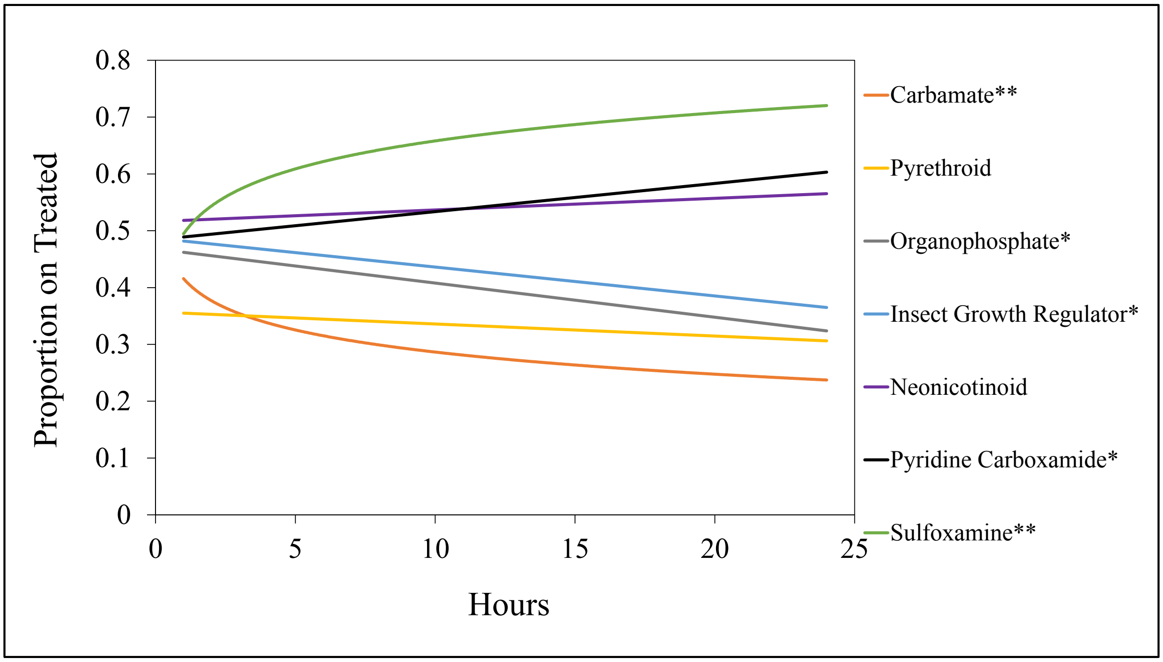

Figure 3.1 Relationship between the proportion of tarnished plant bugs on treated green beans over time for various insecticide groups based on laboratory bioassays

*Significant linear relationship, **Significant quadratic relationship.

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CHAPTER IV

SUMMARY

The purpose of these studies was to determine the effects of combining multiple best management practices to manage the tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), in cotton and to study the behavioral response of the tarnished plant bug to several classes of insecticides. The tarnished plant bug was the primary pest of cotton in the Mississippi Delta in 2014, causing a total loss of 47,616 bales in the region. On average, five insecticide applications were required to manage this pest over the season, costing $133.13 per hectare (Williams 2014). Development of an integrated pest management (IPM) program to help manage the tarnished plant bug and increase the profitability of cotton in areas with high tarnished plant bug populations is needed.

Chapter two focused on determining the benefits of an IPM approach to tarnished plant bug management (TPB-IPM) in cotton. Each tarnished plant bug approach was planted at an early and late planting date. The TPB-IPM approach included planting a hirsute early maturing variety with 90 kg N ha, a prophylactic application of novaluron before the first week of flowering, and an aggressive insecticide application strategy. The standard approach consisted of a late maturing smooth variety with 134 kg N ha. There was no application of novaluron and an insecticide application strategy that used lower rates and products that would give satisfactory control for the lowest possible price.

On average, the implementation of the TPB-IPM program reduced the total amount of insecticides needed for tarnished plant bug management by 0.66 applications in both regions during 2014 and 2015. In the Hills region, cotton utilizing the TPB-IPM approach required a total of three times more (Table 2.1). This can be attributed to the prophylactic application of novaluron the week before flowering. In the Delta region, cotton grown utilizing the TPB-IPM approach reduced the total number of insecticide applications required for tarnished plant bug (Table 2.1).

Tarnished plant bug densities were similar in plots for both approaches in the hills during both years (Table 2.2) Also, both approaches resulted in similar square retention in the hills during for both years (Table 2.3). This would be expected because of the low tarnished plant bug populations observed. Tarnished plant bug infestation levels were much higher in the delta region, but no differences were observed among treatments. Cotton grown using the TPB-IPM approach resulted in greater square retention than cotton grown using the standard approach (Table 2.3). This is consistent with other reports with a hirsute variety (Meredith and Schuster 1979, Bailey et al. 1980, Wood 2014).

Cotton grown utilizing the standard approach resulted in higher yields than cotton grown using the TPB-IPM approach in the hills region in both years (Table 2.5), while cotton grown using the TPB-IPM approach had higher yields than cotton using the standard approach in the Delta (Table 2.6). The fact that using the standard approach resulted in higher yields in the hills region, which experienced very low tarnished plant bug infestation levels, demonstrates that the increased yields with the TPB-IPM approach in the delta region can be attributed to the effects of the tarnished plant bug IPM program and not the yield potential of each variety.

Chapter three focused on studying the behavioral response of tarnished plant bug to several classes of insecticides. The increasing resistance to several classes of insecticides has led to difficulty managing this pest (Snodgrass and Scott 1988, Pankey 1996, Snodgrass 1996, Snodgrass and Gore 2007, Snodgrass 2009). There has been work done to determine the distribution of tarnished plant bug in cotton (Fye 1971, Pack and Tugwell 1976, Snodgrass 1998). Fontenot (2009) studied the behavioral response of tarnished plant bug to acephate, but no research has been conducted to determine the behavioral response to other insecticides.

In laboratory studies, tarnished plant bug nymphs showed a preference for green beans treated with carbamates and pyridine carboxamides (Table 3.2). There were significantly more tarnished plant bug nymphs on untreated green beans than treated green beans for insect growth regulators, organophosphates, and pyrethroids (Table 3.2). This finding is consistent with Pike et al. (1982) who found honey bees to have a similar response to a pyrethroid. Having a better understanding of tarnished plant bug behavioral responses to insecticides can help to better understand control issues.

The presence of an insecticide did not have an effect on the behavior of tarnished plant bug in the field. This could have been because there were little differences in control of the insecticides compared to the untreated check (Table 3.3 and Table 3.4). Further research needs to be conducted to determine the behavioral response of tarnished plant bug to chemical insecticides.

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