An Evaluation of Hemipteran Pests and Cotton in Alabama

by

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Abstract

Hemipterans have been major pests in cotton since the end of the Boll Weevil Eradication Program and the advent of transgenic cotton. The objective of this study is to provide better insights into integrated resistance management strategies in tarnished plant bugs and the addition of a new class of insecticide to help provide residual control of stink bugs in cotton. An evaluation of insecticide resistance to five common insecticides used in cotton on tarnished plant bug was tested in six distinct regions of Alabama. Field collections were made in the non-crop reservoirs daisy fleabane and crimson clover prior to the growing season and a lab colony of tarnished plant bug was obtained from Mississippi State University to serve as a standard to measure insecticide resistance. A glass scintillation vial bioassay was performed using technical grade formulations for acephate, bifenthrin, dicrotophos, imidacloprid, and thiamethoxam. Distinct regions of Alabama were shown to have resistances that were specific to that area with bifenthrin being the most commonly resistant insecticide. We evaluated the effects of the insect growth regulator novaluron in southern green stink bug, *Nezara viridula*. Sublethal effects on adult fecundity were tested using a bean dip assay. We found no significant effect on egg masses, eggs, or egg hatch rate. Direct mortality was tested using a bean dip assay on second through fifth instar southern green stink bug nymphs. Mortality was significantly greater in second, third and fifth instars and approaching significance in fourth instars. Field trials were conducted using a randomized complete block design; we found no significant differences for mean internal boll damage or mean yield across the three site-years.

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Table of Contents

Abstract 2

Acknowledgments 3

List of Tables 6

List of Figures 7

List of Abbreviations 8

Chapter 1: Literature Review of the Tarnished Plant Bug and Stink Bug Spp. in Alabama Cotton 9

Tarnished Plant Bug: Life History and Biology 10

Tarnished Plant Bug: Insect-Host Interactions 12

Tarnished Plant Bug: Evolution of Insecticide Resistance 13

Tarnished Plant Bug: Overview of Insecticides Used… 14

Tarnished Plant Bug: Novaluron… 14

Tarnished Plant Bug: Thresholds and Sampling Methods 15

Stink Bug Spp.: Life History. 16

Stink Bug Spp.: Insect-Host Interactions. 18

Stink Bug Spp.: Threshold and Sampling Methods 18

References. 20

Chapter 2: Bioassay to Evaluate Resistance Levels in Tarnished Plant Bug (Hemiptera:Miridae) Populations in Alabama to Common Cotton Insecticides 28

Abstract. 28

Introduction. 28

Materials and Methods 30

Results. 33

Discussion. 34

References. 41

Chapter 3: An Evaluation of Novaluron on Southern Green Stink Bug (Hemiptera: Pentatomidae) Nymphs and Sublethal Effects on Fecundity in Adults 44

Abstract. 44

Introduction. 45

Materials and Methods 46

Sublethal Effects of Diamond on SGSB Adults 47

Effects of Diamond on Nymphal Mortality. 48

Effects of Diamond on SGSB Damage and Lint Yield. 48

Data Analysis. 50

Results. 50

Sublethal Effects of Diamond on SGSB Adults 50

Effects of Diamond on Nymphal Mortality. 51

Effects of Diamond on SGSB Damage and Lint Yield. 52

Discussion 60

References. 65

List of Tables

Table 2.1..... 36

Table 2.2..... 37

Table 2.3.....38

Table 2.4..... 39

Table 2.5..... 40

List of Figures

Figure 3.1 Mean Number of Egg Masses..... 54

Figure 3.2 Mean Number of Eggs Laid..... 55

Figure 3.3 Percent Hatch Rate 56

Figure 3.4 Percent Mortality at 96h 57

Figure 3.5 Mean Yield 58

Figure 3.6 Mean Internal Boll Damage..... 59

Figure 3.7 63

Figure 3.8 64

List of Abbreviations

TPB	Tarnished plant bug
Bt	*Bacillus thuringiensis*
SGSB	Southern green stink bug
PARU	Prattville Agricultural Research Unit
WGREC	Wiregrass Research and Extension Center
DAA	Days after application
IRAC	Insecticide Resistance Action Committee
MoA	Mode of action
ACES	Alabama Cooperative Extension System

Chapter 1: Literature Review of the Tarnished Plant Bug and Stink Bug Spp. in Alabama Cotton

Introduction

Upland cotton (*Gossypium hirsutum* L.) has historically been one of the three main crops produced in Alabama. Cotton is produced in 60 of the 67 counties in Alabama, with more than 420,000 acres of cotton planted during the 2022 growing season (Cook et al. 2022). Cotton is used mainly in the production of textiles such as fiber for various types of clothing. In addition, home furnishings, medical supplies, industrial thread, and tarps comprise a sizable proportion of the remaining lint consumption (National Cotton Council of America 2023). Cottonseed is also a valuable commodity as feed for livestock and the use of cottonseed oil in cooking, margarine, salad dressing, and other industrial uses such as in medicines, soaps, and cosmetics (National Cotton Council of America 2023).

Effects of Boll Weevil Eradication on the Emergence of Hemipteran Pests in Alabama

The boll weevil (*Anthonomus grandis* (Boheman) was first detected in Alabama in 1910, and as a result, cotton production fell by over 70 % in the following decade (Smith 2007). The pest's impact shifted human populations and areas of cotton production. Major cotton producing areas were historically concentrated in south Alabama, but Limestone County in the north became the

largest production area in Alabama by 1917 (Smith 1998; Smith 2007). The boll weevil would continue to wreak havoc on the cotton industry for the next 80 years (Smith 2007). By the late-1970's, the National Cotton Council had collected research from entomologists across the cotton belt and acquired partial funding from the federal government to initiate the Boll Weevil Eradication Program (Raszick 2021). Area-wide applications of first chlorinated hydrocarbons and then organophosphates played a major role in control of both weevils and Lepidopteran pests like bollworm (*Helicoverpa zea* (Boddie)) and the tobacco budworm (*Chloridea virescens* F.) (Smith 1998). By 1995, the boll weevil had been eradicated in Alabama and most southeastern states. Boll weevil eradication, and the introduction of *Bacillus thuringiensis* (*Bt*) transgenic cotton varieties led to a reduction in the number of broad-spectrum insecticides, and this led to the emergence of two key Hemipteran pests, the tarnished plant bug, *Lygus lineolaris L.*, and various stink bug species (Pentatomidae) (Snodgrass 1996; Snodgrass & Scott 2000; Snodgrass & Scott 2003; Greene et al. 2001; Musser et al. 2007; Musser et al. 2009; Parys et al. 2018; Dorman et al. 2021).

Tarnished Plant Bug (*Lygus lineolaris*)

Life History and Biology

Tarnished plant bug (TPB), *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is a highly prolific plant-feeding insect with over 700 host species, including over 100 crops (Esquivel et al. 2007; George et al. 2021). Unlike most members of the family Miridae, *Lygus* species overwinter as adults in leaf litter (Crosby and Leonard 1914; Kelton 1980). Several non-crop hosts serve as reservoirs for TPB populations during the winter months. The most important of these hosts in the southeastern United States are henbit (*Lamium amplexicaule* L.) and shepherd's purse (*Capsella bursa-pastoris* L.) (Snodgrass et al. 1984). Prior to the growing season, non-crop hosts like daisy fleabane (*Erigeron anuus* L.) and crimson clover (*Trifolium incarnatum* L.) serve as hosts for a few generations before they move into production areas (Fleischer and Gaylor 1987).

Adult TPB are brown, oval shaped, and approximately five millimeters in length. They are characterized dorsally by a "V"-shaped yellow marking on the scutellum (directly behind the pronotum) and by two light spots on either side of the wings known collectively as the cuneus (Springer 2023; George et al. 2021; Stewart 2023). Tarnished plant bug nymphs develop through five instars, each lasting three to four days (George et al. 2021). The early instar nymphs are small and green. They can be confused with aphids, but they lack cornicles and move more quickly than aphids do when disturbed (Graham 2021). The last two instars have five black spots on their dorsum; the last instar can be distinguished by the presence of wing buds (George et al. 2021). Although abiotic conditions can play a major role in life span, the average TPB life cycle lasts approximately 30 - 40 days (Kelton 1975). Given acceptable conditions, females will lay an average of 100 - 120 eggs in their lifetime (Capinera 2001).

Insect-Host Interactions

Tarnished plant bugs are among the most economically important cotton pests in Alabama cotton, as well other cotton producing states in the southeastern United States (Cook et al. 2022). Alabama farmers planted over 420,000 acres of cotton in 2022 and 95 % of those acres received foliar insecticide sprays for TPB control (Cook et al. 2022).

Tarnished plant bugs cause direct damage to the flower bud and developing meristematic tissue in cotton (Tingey & Pillemer 1977). In some cases, TPB will feed in the terminal of seedling cotton plants, leading to the loss of apical dominance in these plants. Once apical dominance is lost, the lateral meristems and vegetative structures will continue to grow, causing what is referred to as "crazy cotton" which has significantly fewer bolls than undamaged cotton (George et al. 2021). Flower buds, called "squares," are damaged by the piercing and sucking mouthparts which inject enzymes into the developing fruit. These enzymes liquefy the plant tissue so it can be taken in through their proboscis (Layton 2000). The resulting damage of the carpels and the introduction of this saliva-like liquid creates an entry point for other pathogens to enter the developing squares (George et al. 2021). This early damage causes small squares to abscise, resulting in delayed maturity and yield loss (Cleveland & Smith 1968). Larger squares often remain on the plant after being fed on, but subsequent blooms may be damaged. This results in discoloration of the flower, typically referred to as "dirty blooms," and can damage the reproductive structures (Layton 2000). Damage to the flower may cause incomplete fertilization resulting in malformed bolls which are called "hawk-billed" bolls. Plant bugs may also feed on small developing bolls causing stained lint. Damage to developing bolls can also cause boll rot and an inability of the locules to fully or properly open; these are called "hardlocked bolls"

(Dorman et al. 2021). Developing bolls are susceptible to TPB damage until they have accumulated 250 - 300 degree-days (Greene et al. 1999).

Evolution of Insecticide Resistance

Tarnished plant bugs are primarily controlled with foliar insecticides in cotton. Common active ingredients used include acephate [Insecticide Resistance Action Committee Mode of Action (IRAC MoA)] 1B, bifenthrin/other pyrethroids (IRAC MoA 3A), dicrotophos (IRAC MoA 1B), neonicotinoids like imidacloprid and thiamethoxam (IRAC MoA 4A), insect growth regulators like novaluron (IRAC MoA 15) and sulfoximines (IRAC MoA 4C). During the Boll Weevil Eradication Program, many broad-spectrum insecticide applications were used for control of the boll weevil. Due to the broad nature of the insecticides, these sprays also controlled hemipteran pests. More recently, the introduction of *Bt* insecticidal proteins to control Lepidopteran pests in the 1990's significantly reduced foliar pesticide usage. Successful boll weevil eradication and the resulting reduced pesticide use, paired with that of increased *Bt* adoption, led to TPB becoming a major pest in Alabama cotton systems (Musser et al. 2007). TPB quickly developed insecticide resistance and has been well documented since the eradication of the boll weevil and adoption of *Bt* technologies (Hollingsworth et al. 1997, Snodgrass & Scott 2000; Snodgrass & Scott 2003; Snodgrass et al. 2009; Parys et al. 2018; Dorman et al. 2020; Catchot et al. 2022).

Snodgrass (1996) first found resistance in TPB to pyrethroids in the mid-southern United States. Snodgrass et al. (2009) found that TPB populations were resistant to the organophosphate acephate which resulted in cross-resistance to other organophosphate active ingredients.

Snodgrass and Scott (2000) found that TPB resistance increases throughout the season. Possible non-chemical control strategies include early planting dates, varietal selection, and management of field borders. These can help mitigate some of the damage from TPB, but chemical control is still needed to keep TPB within economically damaging levels (Adams et al. 2013). The exact mechanism by which resistance occurs in TPB is not fully known, although resistant populations have higher levels of metabolic detoxification molecules like esterases, cytochrome P450's, glutathione S-transferases, and carboxylesterases (Zhu and Snodgrass 2003; Zhu and Luttrell 2012; Fleming et al. 2016).

Overview of Insecticides Used

The organophosphates acephate and dicrotophos insecticides work on the nervous system through acetylcholinesterase inhibition. This enzyme relaxes muscular activity through the binding of acetylcholine (US EPA 2001; US EPA 2002). Bifenthrin is a pyrethroid insecticide, a synthetic compound similar to pyrethrum, a natural insecticide produced by chrysanthemum (*Chrysanthemum indicum* L.). Bifenthrin also works on the nervous system as a sodium channel modulator, which plays a key role in the ability to send nerve impulses through a nerve cell (Johnson et al. 2010). Imidacloprid and thiamethoxam are both in the neonicotinoid class of insecticides which act upon nicotinic acetylcholine receptors to block signals from one nerve cell to another, causing paralysis much like organophosphates (Hayenga 2009).

Novaluron

Novaluron is a class of benzophenyl urea insecticides that inhibit the production of chitin, a crucial component of the insect exoskeleton. It can be ingested orally or through contact and is effective in some species of juvenile insects. Novaluron inhibits the production of a new exoskeleton after molting and has significant activity in economically important pests of humans, cattle, and crops (Elia-Amira et al. 2022; Lohmeyer et al. 2014). Novaluron has insecticidal activity against TPB nymphs and is especially toxic to first instars (Owen et al. 2011). Novaluron has also shown some sublethal effects in adults through modification of the ovaries or transovarial action (Catchot et al. 2021; Kim et al. 2011). Catchot et al. (2021) performed microdissections on the ovaries of healthy TPB and determined that those exposed to novaluron had malformations in the ovaries, especially those treated within 24 hours of molting into the adult stage. Mann et al. (2023) compared a tank mixture of a contact adulticide with novaluron versus just contact spray. They reported significant decreases in TPB populations with tank mixtures (novaluron plus a contact adulticide) as compared to the contact material alone.

Literature concerning the effects of novaluron on stink bug is limited. Novaluron is considered a reduced-risk insecticide due to its low toxicity to birds and mammals (Barazani 2001) but does have off-target effects on bees and other beneficial insects (Hodgson et al. 2011; Jamil et al. 2019; Amarasekare and Shearer 2013).

Thresholds and Sampling Methods

The Alabama Cooperative Extension System (ACES) advises that pre-bloom cotton should be sampled with a sweep net at a threshold of two plant bugs per 25 sweeps (Smith et al. 2007). Pre-bloom cotton should also be scouted for first position square retention in the three uppermost

nodes of the plant, with a goal of maintaining at least 80 % of these squares prior to bloom (Musser et al. 2009). After the third week of squaring and into bloom, cotton should be sampled with a black drop cloth. The threshold for drop cloth sampling is three plant bugs per 1.5 m row (Smith et al. 2007). At these later stages of plant growth, the sweep-net is a less reliable way to sample TPB nymphs since plants are too large for efficient sweeping. Gore et al. (2012) explored using a plant-based post-bloom approach to TPB sampling that involves counting the number of dirty squares rather than using a drop cloth. They found that sampling for dirty squares using an economic threshold of 10 % gave similar insights into TPB populations and could be used for insect control decisions in Arkansas, Louisiana, and Mississippi.

Stink Bug Spp.

Life History

The most common stink bug spp. that infest cotton in the southeastern U.S. are the southern green stink bug (*Nezara viridula* L.), green stink bug (*Chinavia hilaris* (Say)), brown marmorated stink bug (*Halyomorpha halys* (Say)), and brown stink bug (*Euchistus servus* (Stal)) (Greene et al. 2001; Reay-Jones 2009; Hebert & Toews 2011). Adult stink bugs are characterized by their shield-like shape, five-segmented antennae and by the presence of scent glands (Squitier 1997). Our study will focus on the southern green stink bug (SGSB)

since it has a worldwide distribution and is an important pest of crops on a global scale (Panizzi & Slansky 1991). Although very similar in appearance to the green stink bug, the SGSB has amber colored stripes on its antennae rather than the black stripes of green stink bug. Another distinctive characteristic is the size of the scent glands on SGSB, which are much smaller than those on green stink bugs (Squitier 1997).

Southern green stink bug males are slightly smaller than the females. Female SGSB can produce eggs four to five weeks after molting into adults (Squitier 1997). A female can produce up to two egg masses in her life but usually produces only one. One egg mass can contain more than 120 eggs that are deposited on the underside of leaves for protection (Squitier 1997). The eggs begin pale white to yellowish and transition to a pink-orange color as they near eclosion. In summer, the incubation time for eggs averages five days (Squitier 1997). The nymphs emerge together in a window of approximately 1.5 hours and aggregate on and around the now empty egg masses. This aggregation is thought to be a deterrence against predation, via a pooling of their individual chemical defenses (Squitier 1997). First instars were long believed to not feed; however, recent studies have shown that they will feed on green beans in a laboratory environment (Esquivel & Medrano, 2014). First instars are yellowish-orange and molt in approximately three days. Second instars are completely black on the dorsum, but the abdomen can be reddish. This life stage lasts approximately five days (Squitier 1997). Third and fourth instars are characterized by white spots on the abdomen and an increase in the amount of green on the dorsum, with each stage lasting approximately seven days. The fifth and final instar is almost all green except for a row of pink segments on the wing margins and two rows of white spots on the abdomen dorsally. Fifth instars also have wing pads (Squitier 1997). Southern green stink bug is multivoltine in the southern U.S. with an average of four generations per year. They overwinter as adults in tree bark and leaf litter on

field borders (Squitier 1997).

Insect-Host Interactions

Stink bugs cause direct damage to cotton bolls through feeding with piercing and sucking mouthparts, targeting the developing seed inside the boll (Roberts et al. 2007). Huang and Toews (2012) found that both brown stink bug and SGSB prefer to feed on bolls that are 2 to 2.5 cm in diameter. A study by Bommireddy et al. (2007) reported that the damage done to bolls is largely contingent on boll maturity when feeding occurs. Adults and nymphs that fed on bolls with less than 280 DD60's after flowering caused complete boll abscission. Feeding by adults with less than 500 DD60's after flowering produced a significant reduction in seed cotton weight, lint yield, and the quality of lint produced (Bommireddy et al. 2007). In addition to lint reduction, SGSB may also vector for the boll rot disease caused by *Pantoea agglomerans* strain Sc 1-R (Esquivel 2011). Peak populations of stink bugs coincide with peak bloom, which is the third, fourth, and fifth weeks of bloom (Greene et al. 2008), the time when all stages of developing bolls are present in cotton (Bundy and McPherson 2000).

Threshold and Sampling Methods

Internal boll damage is used to determine control measures in post-bloom cotton due to an

uneven distribution of stink bug densities across any given field and the difficulty of visible counts (Reay-Jones et al. 2010a). The optimal threshold for a 20-boll sample was found to be less than 20 % internal boll damage (Reay-Jones et al. 2010b). Due to their proclivity for aggregation, sample sets should be taken no less than 150 m apart (Pulakkatu-Thodi et al. 2014). A dynamic threshold approach using boll damage as an indicator is the recommended method for scouting for post-bloom stink bug damage in Alabama cotton. For the first and eighth week of bloom, the threshold for control is 50 % bolls damaged, 30 % for weeks two and seven of bloom, and for weeks three through six, 10 % damage is the recommended threshold (Bacheler et al. 2009). These varying thresholds consider the number of susceptible bolls present and increasing numbers of susceptible bolls warrant a decrease in the economic threshold. Sweep net sampling for stink bugs shows a bias towards adults. While drop cloth sampling gives a better picture of the population densities present, internal boll damage is the most effective way to scout for stink bugs (Greene and Herzog 1999; Toews et al. 2008). Although evaluating internal boll damage is the recommended method of sampling for stink bug population densities, the process can be time consuming. Toews et al. (2009) looked at using external boll lesions as a metric by which stink bugs could be sampled. They found that more bolls must be sampled than when evaluating internal damage as an indicator for control decisions but showed some promise as being a quicker way to evaluate stink bug densities, albeit with less precision.

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Chapter 2: Bioassay to Evaluate Resistance Levels in Tarnished Plant Bug (Hemiptera: Miridae) Populations in Alabama to Common Cotton Insecticides

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Abstract

The tarnished plant bug, *Lygus lineolaris*, has emerged as the major insect pest of cotton in the mid-southern United States following the eradication of the boll weevil and the introduction of genetically modified *Bt* cotton for caterpillar pests. The objective of this study is to evaluate tarnished plant bug resistance to the five most common insecticides used for control across six distinct growing regions. Glass-vial bioassays were used to evaluate resistance of field populations in a laboratory setting. Elevated levels of resistance of tarnished plant bug to bifenthrin and, to a lesser degree, imidacloprid have been reported in various regions of Alabama when compared to a susceptible lab population. There is a limited number of chemical classes available for insect control, therefore further resistance monitoring is necessary to inform management strategies and to slow the development of resistance. This will contribute to the overall goal of establishing and maintaining the most cost efficient and efficacious control programs for tarnished plant bug in Alabama.

Introduction

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae), is consistently the most damaging single pest of cotton in the mid-southern United States (Musser et al. 2009). Tarnished plant bug (TPB) is a highly polyphagous insect with over 700 plant hosts (Esquivel et al. 2007). In the 2022 growing season in Alabama, TPB caused $16.6 M in damage, over $6 M more than the next most damaging pest. Of the 426,458 acres of cotton planted in Alabama, 100 % of the acreage was infested and 95 % of the acres were treated an average of 2.4 times for TPB (Cook et al. 2022). This pest causes direct damage and yield loss in cotton through feeding and subsequent abscission of pinhead squares (flower buds) as well as feeding on larger squares and small bolls (Cleveland & Smith 1968). TPB feed by piercing the developing buds and injecting saliva that breaks down plant tissue so it can be ingested (Layton 2000). Yield losses can be directly attributed to the loss of first position pinhead squares and the introduction of pathogens into fruiting structures. These pathogens cause further damage to seed and lint in bolls that are not abscised (George et al. 2021). Furthermore, TPB feeding on medium or larger squares results in flowers that are damaged; the resulting injury, which is referred to as "dirty blooms," manifests in the reproductive parts of the cotton flower and results in misshapen bolls due to incomplete fertilization. Such misshapen bolls are described as "hawk-billed" (Layton 2000).

Prior to the introduction of *Bt* (*Bacillus thuringiensis*) transgenic cotton and the completion of boll weevil eradication in Alabama, TPB were not major pests of cotton. With the decrease in broad-spectrum insecticide sprays associated with boll weevil eradication and the genetic control of Lepidopteran pests with *Bt*, TPB emerged as an opportunistic economic pest (Musser et al. 2007). Rix and Cutler (2017) showed that chronic, multigenerational exposure to these insecticides as they break down and become less lethal in the environment can cause an

increase in the likelihood of future resistant populations. Resistance of TPB populations to insecticides has been well documented over the last few decades in the mid-southern U.S. following these changes (Snodgrass 1996; Hollingsworth et al. 1997; Snodgrass & Scott 2000; Snodgrass & Scott 2003; Snodgrass et al. 2009; Parys et al. 2018; Dorman et al. 2020; Catchot et al. 2022).

Snodgrass (1996) first identified pyrethroid resistance in TPB and later found resistance to the organophosphate acephate which gave rise to some populations of TPB with cross-resistance to additional organophosphates (Snodgrass et al. 2009). Non-chemical strategies such as varietal selection, planting date, and field border management provide some control but chemical management is still needed for adequate control of TPB (Adams et al. 2013). Given the growing problem with insecticide resistance and cross-resistance in TPB, resistance monitoring is an important tool in TPB pest management. This information helps to highlight the importance of rotating insecticide chemistries and modes of action both during the season and from year to year to reduce the selective pressure applied to these insect pest populations. The objective of this study is to evaluate TPB resistance to the five most common insecticides used for control across six distinct cotton producing regions in Alabama. We hypothesize that geographically distinct populations will have region-specific resistance with changes in resistance from region to region.

Materials and Methods

TPB populations were collected in six distinct cotton-producing regions of Alabama:

southwest (Monroe County), southeast (Henry County), central west (Dallas County), central east (Macon County), northwest (Limestone County), and northeast (Cherokee County). These populations were obtained from uncultivated hosts daisy fleabane (*Erigeron annuus*) and crimson clover (*Trifolium incarnatum*) between May-June 2023. These collections were made prior to the growing season to get a baseline resistance in the population prior to the introduction of insecticides. Insects were collected using a sweep net (38 X 38 cm) and aspirator. Each collection was placed in a plastic container (Plastic Screw-Top Canister (1 Gallon), Mainstays; Bentonville, AR) with shredded copy paper as a substrate to increase the surface area available inside the container. TPB populations remained in the lab for 24 hours after collection to acclimate the insects to the laboratory environment following field collection and remove any dead or moribund insects. No less than 100 individuals were tested for each location (N = 3517).

A laboratory colony of adult TPB was obtained from Mississippi State University (Mississippi State, MS, USA) for use as a susceptible baseline for calculating resistance ratios. This colony has been maintained for >10 years following details outlined by (Cohen 2000; Musser 2012). The insects were reared under controlled conditions (27°C and 16:8 (L:D) h).

Bioassays were performed using the glass vial method described by Snodgrass et al. (1996, 2009) and can indicate technical resistance in a laboratory setting. Scintillation vials (20-mL, VWR Scientific; Radnor, PA) were submerged in a 10% Clorox solution for at least two days prior to testing, triple rinsed with tap water and heated until dry on a hot dog roller (Great Northern Commercial 1650-Watts 30-Hot Dog 11-Roller Grilling Machine; Lorain, OH).

The technical grade insecticides bifenthrin, acephate and dicrotophos (VWR Scientific; Radnor, PA) were prepared using a coated vial technique as described by Snodgrass (1996). Technical grade thiamethoxam and imidacloprid were prepared using a floral foam method as described by Teague and Tugwell (1996). These methods were chosen according to the mode of

action of the respective insecticides. Bifenthrin, acephate and dicrotophos are contact insecticides, whereas thiamethoxam and imidacloprid are most effective when ingested (Snodgrass et al. 2008). Each insecticide was prepared using a serial dilution of a stock solution of insecticide/acetone in concentrations of 0.1, 0.316, 1.0, 3.16, 10.0, and 31.6 ug/vial for the coated vial assays. Coated vials of pure acetone were used as a negative control. The same concentrations of a 10 % honey water and insecticide solution were used for floral foam treated vials. A 10 % honey water solution was used as a negative control in these assays.

All vials were prepared the day of the test. For the coated vials, 0.25 mL of solution was pipetted into each vial and the vials were rolled until dry under a fume hood on the unheated hot dog roller. The drying process allowed the acetone to evaporate, and the insecticide was left as a residue on the inner surface of the vial (Snodgrass 1996). When dry, a 1.3 cm piece of green bean was placed inside each vial as a food source. Green beans, were soaked in a 10 % Clorox solution for five minutes for surface sterilization, then rinsed with tap water for five minutes and allowed to dry prior to being cut into pieces and placed in vials. Two adult TPB were used per vial, with a minimum of nine replicates for each concentration per insecticide per location. Each vial was closed with a cotton ball to prevent insect escape. Vials were kept at room temperature and mortality assessed and recorded after 24 hours. Adults were considered dead if they could not right themselves in five seconds or did not move when gently prodded.

All vials were prepared the day of each respective test. For each floral foam vial, a disk of wettable floral foam (Oasis Floral Products; Kent, OH) measuring approximately 12 mm X 12 mm was obtained using a coring device (Freeshu; Leizhou, Guangdong China). One disk was placed in each vial; 0.5 mL of solution was pipetted onto each floral foam disk. One tarnished plant bug was added per vial and each vial was closed with a cotton ball; a minimum of 18

replicates for each concentration per insecticide per location were used. Vials were kept at room temperature (approximately 21^{o}C) and mortality assessed after 24 hours. Adults were considered dead if they could not right themselves in five seconds or did not move when gently prodded.

Data were analyzed using Polo probit software (LeOra Software LLC; Berkshire, UK). Resistance ratios were calculated by dividing the LC50 of the field populations to the LC50 of the laboratory colony. This number represents the difference in resistance between the field populations and the susceptible laboratory colony. For this study, populations with a resistance ratio less than three were low, between three and 10 medium, and more than 10 high resistance (Dorman et al. 2020).

Results

Resistance to acephate was zero to low (0.00 – 2.6) in all regions sampled (Table 2.1). Resistance to bifenthrin was high in three of the six regions tested (Table 2.2): Monroe Co. (12.0), Macon Co. (13.4), and Limestone Co. (12.8). Only the Cherokee Co. population showed resistance to dicrotophos (Table 2.3), with a resistance ratio of 1.9. All regions had at least medium resistance to imidacloprid (3.2 – 5.9) except Cherokee Co., which showed high resistance (15.45) (Table 2.4). All regions tested had low to medium resistance to thiamethoxam (1.7 – 4.9) (Table 2.5). No region had high resistance to more than one insecticide tested. Populations had the most resistance to bifenthrin across the state of Alabama.

Discussion

These data add to TPB resistance data that has been made available over the last several decades. There has not been a recent comprehensive evaluation of resistance in Alabama TPB populations, however Dorman et al. (2020) collected and tested Alabama populations in the northeast, northwest, and central east for resistance to acephate, bifenthrin, and thiamethoxam in 2018 and 2019. There were similarities when compared to our results with acephate, but the two studies differed significantly concerning bifenthrin. Dorman et al. (2020) reported low to medium resistance to bifenthrin in the central east (0.4 – 3.8) whereas our results found a RR50 of 13.4 (high). The LC50 numbers in our tests were much higher than in Dorman et al. (2020) (as much as 4X). This could cause our RR50 values to be lower by comparison. High chi-squared values (ten or more) suggest that there is a large amount of variability in the data that could be rectified by a larger data set. However, the variability in these data is common for laboratory bioassays, which emphasizes the need to make thorough field observations for the best control recommendations.

Tarnished plant bug age has been documented as a significant factor when assessing mortality. Adults over 10 days old are reported to have significantly higher mortality than those 10 days or younger (Snodgrass 1996). While laboratory colony ages could be controlled, field populations were of unknown and likely variable age ranges. Therefore, the potential age differences may have skewed the results of field collected bioassays in either direction. If many older individuals were collected, that population would be significantly more susceptible to a given insecticide and the opposite may be true for younger individuals.

The prevalence of resistance to bifenthrin and, to a lesser extent, imidacloprid could be correlated with the intensity of their respective usage. Since its registration in cotton in 1978, bifenthrin has been used extensively in TPB and other cotton insect management programs. Resistance to bifenthrin and subsequent cross-resistance to other pyrethroids has been documented in the Mississippi Delta since the mid-1990's. Resistance to most classes of available insecticides has also been reported in the region (George et al. 2021). Furthermore, that resistance has been shown to increase in populations over the growing season (Dorman et al. 2020). These data suggest that continued monitoring of TPB insecticide resistance is important to determine recommended control measures as different regions of a state, as demonstrated here and in other trials, can have varying levels of resistance to respective insecticides. The disparate nature of these growing regions can help to decrease the rate of resistance formation due to a relative lack of gene flow when compared to large, dense regions of production. To inform best practices moving forward, understanding insecticide use rates for the different growing regions of Alabama would be beneficial. With these data, localized integrated resistance management plans could be implemented in these disparate areas.

Table 2.1 – Log-probit bioassays on adult tarnished plant bug to technical grade acephate. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance.

Location	*Chemical*	*LC 50*[a]	X^2	*N=*[b]	*CI*	*RR 50*[c]
Monroe Co., AL	acephate	0.818	26.83	112	-	-
Henry Co., AL	acephate	8.670	0.468	126	6.379 - 11.952	2.108
Dallas Co., AL	acephate	10.70	3.888	126	-	2.602
Macon Co., AL	acephate	7.893	0.468	126	-	1.920
Limestone Co., AL	acephate	4.825	4.259	116	2.405 – 10.360	1.173
Cherokee Co., AL	acephate	9.976	2.486	128	3.680 - 20.310	2.426
Laboratory	acephate	4.112	19.655	140	-	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR_{50} calculated using the LC_{50} values of a susceptible laboratory colony from Mississippi State University

Table 2.2 – Log-probit bioassays on adult tarnished plant bug to technical grade bifenthrin. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance.

Location	*Chemical*	*LC 50*[a]	X^2	*N*=[b]	*CI*	*RR 50*[c]
Monroe Co., AL	bifenthrin	2.648	4.109	112	1.089 - 6.446	11.982
Henry Co., AL	bifenthrin	1.740	43.558	126	-	7.873
Dallas Co., AL	bifenthrin	0.450	5.689	126	-	2.036
Macon Co., AL	bifenthrin	2.962	9.502	126	0.577 - 9.874	13.403
Limestone Co., AL	bifenthrin	2.821	5.764	126	0.629 – 17.993	12.765
Cherokee Co., AL	bifenthrin	0.128	4.640	126	0.001 - 0.764	-
Laboratory	bifenthrin	0.221	7.833	140	0.053 - 0.498	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR_{50} calculated using the LC_{50} values of a susceptible laboratory colony from Mississippi State University

Table 2.3 – Log-probit bioassays on adult tarnished plant bug to technical grade dicrotophos. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance.

Location	*Chemical*	*LC 50*[a]	X^2	*N=*[b]	*CI*	*RR 50*[c]
Monroe Co., AL	dicrotophos	0.629	20.737	112	-	-
Henry Co., AL	dicrotophos	1.508	0.792	126	0.847 - 2.170	-
Dallas Co., AL	dicrotophos	0.450	5.689	126	-	-
Macon Co., AL	dicrotophos	2.962	9.502	126	0.577 -1.13	-
Limestone Co., AL	dicrotophos	2.821	5.764	126	0.848 – 1.670	-
Cherokee Co., AL	dicrotophos	0.128	4.640	126	2.197 - 4.986	1.902
Laboratory	dicrotophos	0.221	7.833	140	1.235 - 2.452	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR_{50} calculated using the LC_{50} values of a susceptible laboratory colony from Mississippi State University

Table 2.4 – Log-probit bioassays on adult tarnished plant bug to technical grade imidacloprid. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance.

Location	*Chemical*	*LC 50*[a]	X^2	*N*=[b]	*CI*	*RR 50*[c]
Monroe Co., AL	imidacloprid	2.371	1.718	105	0.434 - 5.570	5.854
Henry Co., AL	imidacloprid	2.135	3.72	124	1.119 - 3.870	5.272
Dallas Co., AL	imidacloprid	2.030	1.49	105	0.604 - 4.200	5.012
Macon Co., AL	imidacloprid	1.290	6.50	105	0.153 - 3.652	3.185
Limestone Co., AL	imidacloprid	1.280	6.70	105	0.297 – 3.790	3.160
Cherokee Co., AL	imidacloprid	6.258	3.596	105	1.848 - 52.675	15.452
Laboratory	imidacloprid	0.221	7.833	126	1.235 - 2.452	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR_{50} calculated using the LC_{50} values of a susceptible laboratory colony from Mississippi State University

Table 2.5 – Log-probit bioassays on adult tarnished plant bug to technical grade thiamethoxam. LC50 values represent the concentration that causes death in 50 % of the individuals tested. RR50 values represent the LD50 of the field collection divided by the LC50 of the laboratory population. Dashes indicate no resistance.

Location	*Chemical*	*LC 50*[a]	*X^2*	*N=*[b]	*CI*	*RR 50*[c]
Monroe Co., AL	thiamethoxam	1.218	7.894	105	0.213 - 4.622	3.904
Henry Co., AL	thiamethoxam	0.803	3.550	105	0.334 - 1.638	2.574
Dallas Co., AL	thiamethoxam	0.544	2.560	105	0.163 - 0.970	1.744
Macon Co., AL	thiamethoxam	0.812	0.869	112	0.264 - 1.551	2.603
Limestone Co., AL	thiamethoxam	1.298	2.950	105	0.565 - 2.586	4.160
Cherokee Co., AL	thiamethoxam	1.525	1.837	105	0.163 - 4.396	4.888
Laboratory	thiamethoxam	0.312	4.520	126	0.982 - 1.666	-

[a] Units represented as ug/vial^{-1}
[b] Total number of tarnished plant bug adults
[c] RR_{50} calculated using the LC_{50} values of a susceptible laboratory colony from Mississippi State University

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Chapter 3: An Evaluation of Novaluron on Southern Green Stink Bug (Hemiptera: Pentatomidae) Nymphs and Sublethal Effects on Fecundity in Adults

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Abstract

Stink bug species have emerged as major insect pests of cotton in the mid-southern United States following the eradication of the boll weevil and the introduction of genetically modified *Bt* cotton for caterpillar pests. Considering the limited number of chemical classes available for insect control, further insights into other chemistries are necessary to inform management strategies with the overall goal of establishing and maintaining the most cost efficient and efficacious control programs for tarnished plant bug in Alabama. The insect growth regulator, novaluron, has shown control of tarnished plant bugs but little research has been done on its effect in stink bugs. The objective of our study is to evaluate the effects of novaluron, in a laboratory setting, on adult fecundity, nymphal mortality, and yield and damage in the field. We hypothesized that novaluron would have a direct effect on mortality in nymphs and could decrease fecundity in adult stink bugs. Although the effect on fecundity was counter to our hypothesis, this study shows effective control of nymphs in our model insect, the southern green stink bug (*Nezara viridula*). Future evaluation of proper timing of novaluron applications could make this a valuable tool for residual control of stink bugs in cotton.

Introduction

Stink bug species are a major insect pest of upland cotton, *Gossypium hirsutum* L., in the southeastern United States (Cook et al. 2022). The four economically important species that damage cotton in the region are the southern green stink bug (*Nezara viridula* L.), green stink bug (*Chinavia hilaris* (Say)), brown stink bug (*Euschistus servus* (Say)) and, more recently, brown marmorated stink bug (*Halyomorpha halys* (Stål)) (Greene et al. 2001; Reay-Jones 2009; Hebert and Toews 2011; Tillman 2013). In the 2022 growing season, stink bug spp. accounted for approximately $12.5 million in economic losses in Alabama cotton. Stink bugs infested 100% of the state's 426,458 acres planted, with nearly 88% percent of the acres treated an average of 1.5 times for stink bugs (Cook et al. 2022). Historically, stink bugs were not considered a major economic pest of cotton. The success of the Boll Weevil Eradication Program and the advent of *Bacillus thuringiensis* (*Bt*) genetically modified cotton cultivars in the mid-1990's resulted in a shift of pest complexes. These two advancements in pest management reduced the amount of broad-spectrum insecticide applications and this provided an opportunity for these highly polyphagous insects to emerge as major cotton pests (Greene et al. 2001).

Stink bugs damage cotton by puncturing the developing boll and feeding on seeds and nearby lint (Barbour et al. 1990). These punctures can stain lint, introduce boll rot pathogens, and cause complete abscission of the fruiting structure, reducing both quality of lint and total yield in cotton (Medrano et al. 2009). Bomireddy et al. (2007) found that the damage done to bolls is largely contingent on the maturity of the boll at feeding. Adults and nymphs that fed on bolls with less than 280 DD60's after flowering caused complete abscission of the boll, older

bolls (less than 500 DD60's) showed a significant reduction in seed cotton weight, lint yield, and lint quality (Bomireddy et al. 2007). Peak stink bug population densities normally coincide with peak bloom in cotton which occurs when all stages of developing bolls are present during the third, fourth, and fifth weeks of bloom (Greene et al. 2008; Bundy and McPherson 2000). While damage can be done on smaller or larger bolls, the southern green stink bug prefers to feed on bolls that are 2 to 2.5 cm in diameter (Huang and Toews 2012). Although there has been some evidence of feeding by first instar nymphs in a laboratory setting (Esquivel and Medrano 2014), economic damage in the field is by second through fifth instar nymphs and adults.

Chemical insecticides are the primary method of stink bug control in cotton. The insect growth regulator novaluron has insecticidal activity on tarnished plant bug *Lygus lineolaris* (Palisot de Beauvois) nymphs and sublethal effects on adult fecundity (Owen et al. 2011; Catchot et al. 2021). Novaluron has also shown effectiveness on economically important insect pests of humans, cattle, and crops (Elia-Amira et al. 2022; Lohmeyer et al. 2014). Novaluron is a benzophenyl urea insecticide that inhibits the formation of a new exoskeleton after molting. The objectives of this experiment are to identify effects of the formulated product Diamond (Adama, Ashdod, Israel), with the active ingredient novaluron, on possible sublethal effects on southern green stink bug (SGSB) adult egg lay and viability and direct mortality of stink bug nymphs in laboratory and field studies. This species was used as the model insect for this trial due to its availability and ease of rearing.

Materials and Methods

Southern green stink bug egg masses were obtained from the USDA-ARS facility in Tifton, Georgia and reared in a growth chamber at 27°C on a 16L:8D light cycle (Esquivel and Medrano 2014). Egg masses were placed in 2-quart plastic containers (PFS Sales Co., Raleigh, NC). The tops of these containers were modified by cutting an approximately 12 X 12 cm opening for ventilation. Containers were covered with a 30 X 30 cm piece of cotton cloth and the modified top was placed over the cloth (Figure 3.7). Egg masses were removed from containers after eclosion was completed. The SGSB nymphs were raised on a diet of fresh, prewashed green beans (*Phaseolus vulgaris*) and raw, blanched peanuts (*Arachis hypogaea*) along with a water source (Figure 3.6) (Huang and Toews, 2012; Panizzi and Slansky, 1991). Fresh diet and water were replaced every 48-72 hours.

Sublethal effects of Diamond on SGSB adults

To evaluate the sublethal effects of novaluron to SGSB adults, 5th instar nymphs were separated by sex and put into containers with no more than 30 nymphs per container. To differentiate between sexes, the last abdominal segment was inspected for the presence of claspers, which identifies males (Mitchell and Mau 1969). Upon molting, five male and five female SGSB adults were placed into "mating" buckets, designed as the rearing buckets above. Mating buckets were considered replications of each treatment (treated and untreated), with a total of 10 buckets (five replications of each treatment). Adults were either fed a diet of green beans dipped in a 0.17 kg ai/ha Diamond (novaluron) (Adama, Ashdod, Israel) solution or fresh green beans for the first 48 hours. Fresh green beans (i.e. not treated with novaluron) were then used for the remainder of the trial. An unbleached paper towel oviposition site was placed along

the sides of each mating bucket. These buckets were monitored daily for egg lay until all individuals died in each bucket. Mating buckets were kept in a growth chamber at 27 ^{o}C and 80% relative humidity with a 16L:8D light cycle. The egg masses were separated, counted, and allowed to incubate in a Petri dish. Egg hatch numbers were recorded upon emergence using a dissecting microscope (Motic SMZ-171, Motic Scientific, San Antonio, TX). These data were used to evaluate the effects of Diamond (novaluron) on the number of eggs laid and hatch rate.

Effects of Diamond on nymphal mortality

To assess direct mortality to nymphs, five newly molted (within 48 hours) individuals of each nymphal instar (second – fifth instars) were placed in a 100 mm X 15 mm Petri dish (VWR Scientific; Radnor, PA). Petri dishes were held in a growth chamber at 25 ^{o}C and 80 % relative humidity with a 16L:8D light cycle. The treatments consisted of a group fed green beans dipped in 0.087 kg ai/ha of Diamond for 96 hours and an untreated control group fed fresh green beans for 96 hours. Each treatment was replicated five times. Mortality was assessed at 24, 48, 72, and 96 hours after application. Insects were considered moribund/dead if they could not right themselves from a supine position in five seconds.

Effects of Diamond on SGSB damage and lint yield

A field trial was conducted at Prattville Agricultural Research Unit (PARU) in Prattville, AL in 2022 and at PARU and the Wiregrass Research and Extension Center (WGREC) in Headland, AL during the 2023 growing season. This trial was designed as a randomized complete block with four replications. Plots were eight rows spaced 0.91 m apart and 7.6 m long. Cotton, DP1646B2XF, was planted on 9 May 2022 at PARU and on 17 May 2023 at PARU and WGREC. Treatments consisted of one application of Diamond (novaluron) applied (Mud Master, Bowman Manufacturing, Newport, AR) at rates of 0.042, 0.063, 0.087, and 0.17 kg ai/ha and were initiated at the third week of bloom. This timing generally coincides with the initial peak migration of adult stink bug species into cotton in Alabama and pre-counts of susceptible bolls showed internal stink bug damage. To evaluate initial and residual effects of novaluron on stink bug injury, 15 (2.5 cm diameter) bolls were examined for internal boll damage from the center two rows of each plot at 7, 14 and 21 days after application (DAA). A boll was considered damaged if it showed symptoms of pinprick marks inside the carpel wall, warts inside the carpel wall or stained lint (Bundy et al. 2000). Because novaluron also has direct mortality on tarnished plant bug nymphs and residual control could confound yield data, cotton plots were monitored using a drop cloth sampling 3 m row per plot in 2022. However, threshold for tarnished plant bugs was not reached in any treatment that year. In 2023, all plots were over sprayed with sulfoxaflor (Transform WG, Corteva Agriscience, Indianapolis, IN, USA) 0.11 kg ai/ha at the 3rd and 5th weeks of bloom to account for tarnished plant bug populations and damage. This chemical was chosen due to its efficacy on plant bugs and no effect on stink bugs. Yield data was collected by harvesting the center two rows of each plot.

Data Analysis

Laboratory data for SGSB adult fecundity and mortality of SGSB nymphs were analyzed using a normal generalized linear mixed model of analysis of variance PROC GLIMMIX of SAS (Version 9.4, SAS Institute, Cary, NC). Egg viability was calculated by dividing the number of eggs hatched by the number of eggs laid. Replication was treated as a random effect for both tests. Nymphal mortality data were sorted by treatment, insect stage, and hours. Treatment (Diamond) was considered the fixed effect and replication (mating bucket or Petri dish) was considered random.

Field data were analyzed using PROC GLIMMIX (SAS Version 9.4) to evaluate the effects of Diamond rates on internal boll damage and yield. In the initial model, location was considered a fixed effect, however no significant differences were observed for damage or yield ($P > 0.05$), thus location was removed from the model. Replication was considered the random effect.

For all analyses, means were estimated using LSMEANS and separated based on Fisher's protected least significant differences (LSD) ($\alpha = 0.05$). The Kenward-Roger method (Kenward and Roger 2009) was used to estimate degrees of freedom.

Results

Sublethal effects of Diamond on SGSB adults

There were no significant differences between the treated and control groups with respect to: number of egg masses laid (F= 1.0; df = 1, 3; P= 0.3910), number of eggs laid (F= 5.59; df = 1, 3; P= 0.0989), number of eggs hatched (F= 8.19; df = 1, 3; P= 0.0645), and overall hatch rate (F= 1.27; df = 1, 3; P= 0.3424). Adult SGSB laid an average of 1.50 ± 0.29 eggs masses in the non-treated group, while those fed novaluron treated green beans laid an average of 1.75 ± 0.29 egg masses (Figure 3.1). For the number of eggs laid, the non-treated group laid an average of 74.50 ± 11.8 eggs, while the novaluron treated group laid an average of 104.80 ± 14.3 eggs (Figure 3.2). The number of eggs that hatched from those egg masses reflect the disparity in total eggs laid but were also not significantly different from each other. In the non-treated group, there was an average hatch of 67.50 ± 12.1, while the Diamond treated group had an average of 97.25 ± 14.2 eggs hatch. Finally, when rates were compared, no differences were observed. An average of 89.7 % ± 3.0 eggs hatched from the non-treated group, while 92.7 % ± 4.0 hatched from the novaluron treated group (Figure 3.3).

Effects of Diamond on nymphal mortality

Data were analyzed after the final evaluation period (96 h) for each instar since mortality occurs when nymphs molt to the next instar. Novaluron significantly impacted mortality for second instar nymphs (F= 15.01; df= 1, 34; P= 0.0005). The novaluron-treated group had a mortality rate of 32.0 % ± 8.0, whereas the non-treated group had no mortality at 96 h. Novaluron significantly impacted survival of third instar nymphs (F= 9.44; df= 1, 34; P=

0.0042), where 27.0 % ± 7.0 of novaluron fed nymphs died and 7.0 % ± 2.0 died in the non-treated group. Mortality approached significance for fourth instar nymphs (F= 3.74; df= 1, 34; P= 0.0616). The novaluron-treated group had 15.0% ± 5.0 mortality and the non-treated group had 5.0 % ± 2.0 mortality. There was significant impact for fifth instar nymphs (F= 4.77; df= 1, 34; P= 0.0359), where 8.0 % ± 3.0 of the novaluron-treated group died and 1.0 % ± 1.0 of the non-treated group died (Figure 3.4). When analyzed across all instars by treatment, the novaluron-treated group showed significantly higher mortality (21.0 % ± 3.0) than the non-treated group 3.0 % ± 1.0.

An analysis was done comparing "small" (second and third) and "large" (fourth and fifth) instar nymphs and we found a significant interaction of treatment by size (F=15.9; df= 3, 152, P<0.0001). A significantly higher mortality was observed for small nymphs fed novaluron-treated green beans (29.5 % ± 5.0) than large nymphs fed novaluron-treated green beans (11.5 % ± 3.0) than either small or large nymphs in the non-treated groups.

Effects of Diamond on SGSB damage and lint yield

All treatments averaged a damage rating above the action threshold (=10%) and were not significantly different from each other (F=1.07; df= 4, 112; P= 0.3733). Damage was highest in the untreated check (26.4 % ± 3.3 damaged bolls) and lowest in the 0.63 and 0.84 kg ai/ha treatments (20.1 % ± 2.7 and 20.1 % ± 1.8, respectively) (Figure 3.6).

Novaluron did not significantly impact yield (F=1.57; df= 4, 32; P= 0.2065) regardless of rate used (Figure 3.5). The highest numerical yield was in treatments with the highest rate (1.64 kg ai/ha) of novaluron (1,693.4 ± 37.7 kg/ha), followed by the second highest rate (0.84 kg ai/ha) of novaluron (1,522.6 ± 48.0 kg/ha). The third highest yield was found in the non-treated check (1,503.1 ± 190.3 kg/ha), however, this treatment had the highest variability among plots (approximately 3X the standard error of the treated plots). The lowest yields were found in cotton treated with novaluron at 0.63 and 0.42 kg ai/ha (1433.0 ± 60.0 and 1,368.7 ±62.7 kg/ha, respectively) (Figure 3.5).

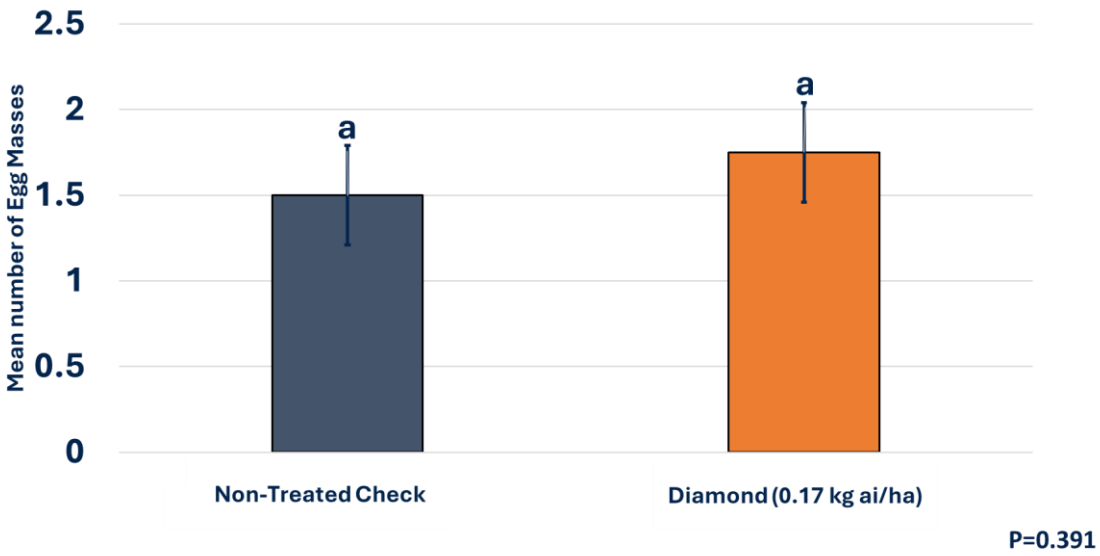

Figure 3.1 – Mean number of egg masses laid by treatment. Letters that are different are statistically significant. Mean number of southern green stink bug egg masses laid by adults fed green beans dipped in water (UTC) or Diamond (0.17 kg ai/ha). Error bars represent 95 % confidence intervals. Common letters above bars indicate treatments are not different (Fisher's protected LSD, α=0.05)

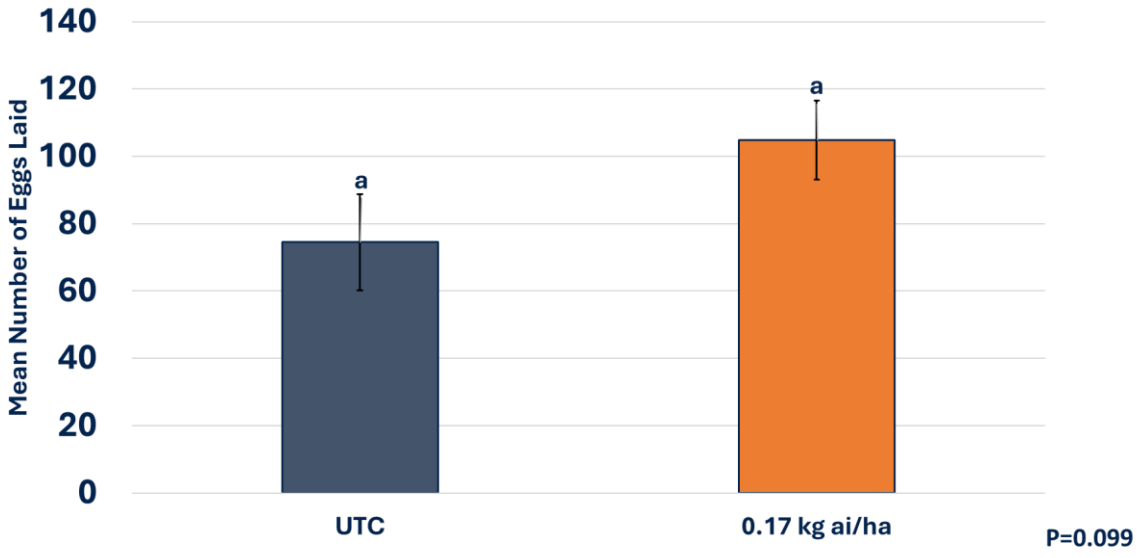

Figure 3.2 – Mean number of eggs laid by treatment. Letters that are different are statistically significant. Mean number of southern green stink bug eggs laid by adults fed green beans dipped in water (UTC) or Diamond (0.17 kg ai/ha). Error bars represent 95 % confidence intervals. Common letters above bars indicate treatments are not different (Fisher's protected LSD, α=0.05)

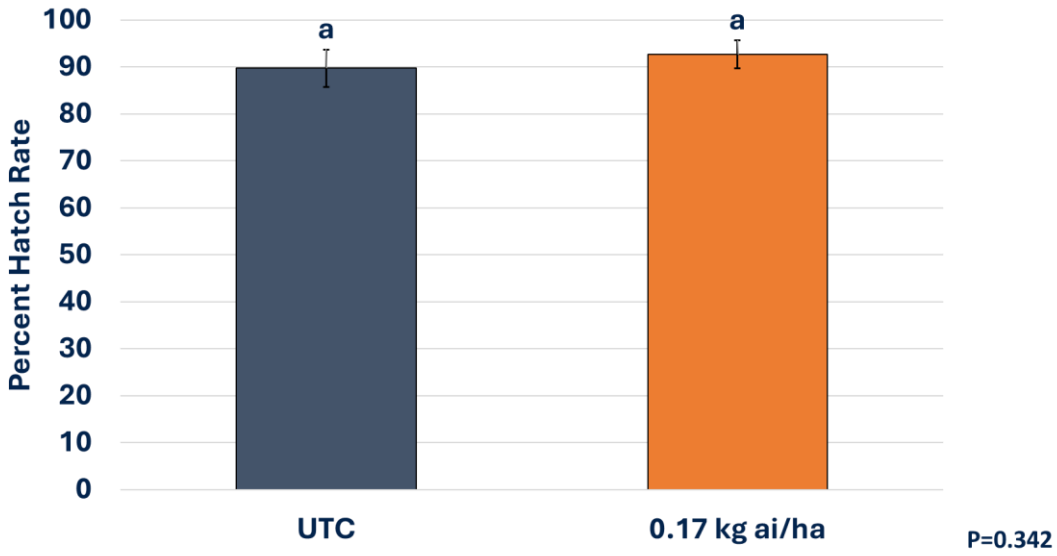

Figure 3.3 – Mean percent hatch rate by treatment. Letters that are different are statistically significant. Mean number of southern green stink bug egg masses laid by adults fed green beans dipped in water (UTC) or Diamond (0.17 kg ai/ha). Error bars represent 95 % confidence intervals. Common letters above bars indicate treatments are not different (Fisher's protected LSD, α=0.05)

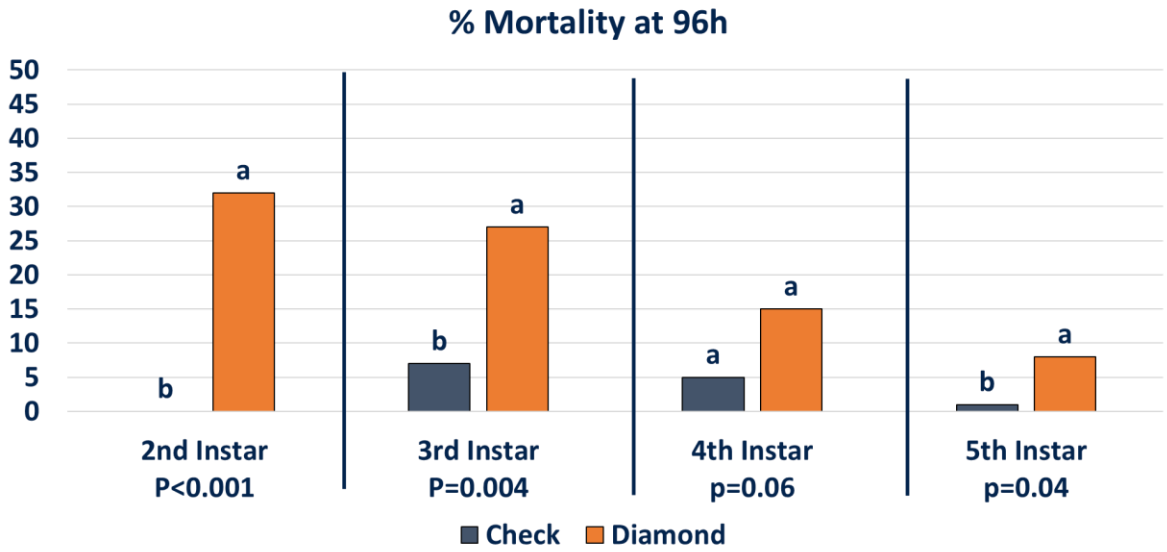

Figure 3.4 – Effects of Diamond on nymphal mortality by instar. Letters that are different are statistically significant (Fisher's protected LSD, α=0.05). Mean percent mortality of nymphs fed fresh green beans (UTC) or Diamond (0.087 kg ai/ha) for second, third, fourth, and fifth instars.

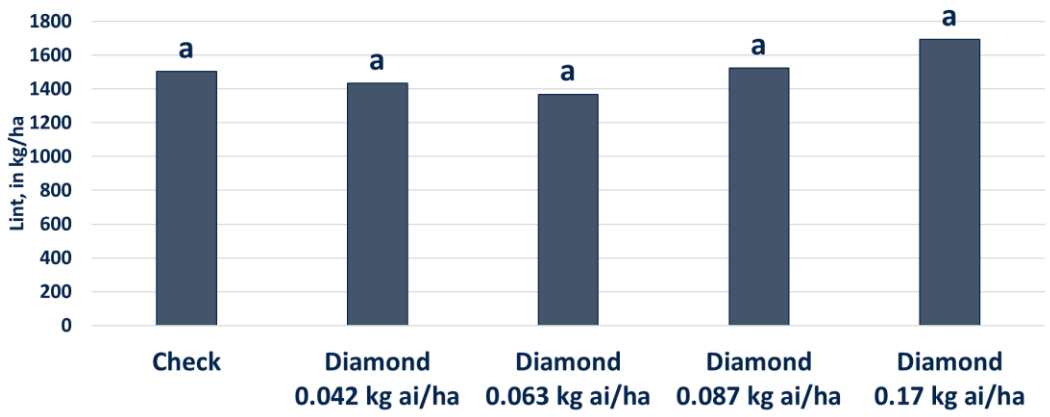

Figure 3.5 – Mean yield of DP1646B2XF when treated with 0.042, 0.063, 0.087, and 0.17 kg of novaluron per hectare. Yield was taken from the center two rows of each 8 row plot. This field trial was a RCBD with 4 replications. Letters that are different are statistically significant. These data represent 3 site-years.

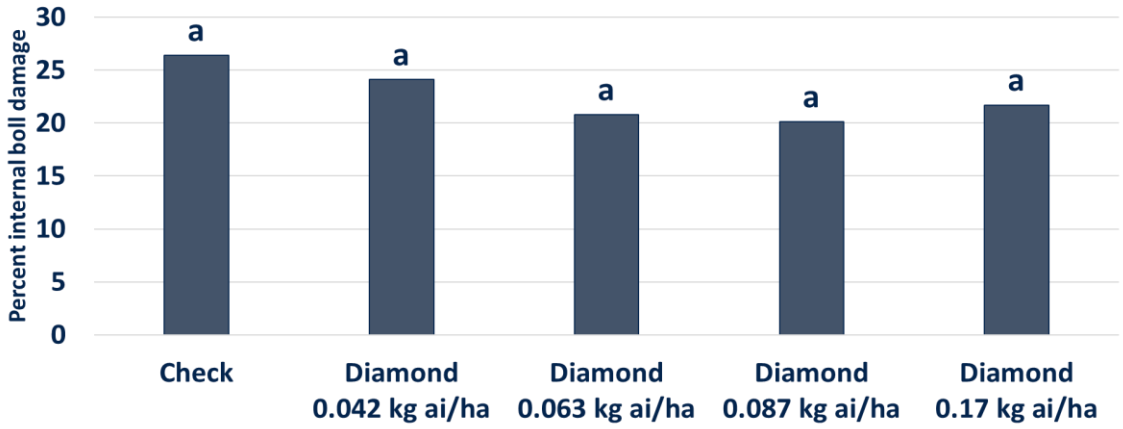

Figure 3.6 - Mean internal boll damage of DP1646B2XF when treated with 0.042, 0.063, 0.087, and 0.17 kg of novaluron per hectare. Boll damage was taken at 7, 14, and 21 days after application. 45 bolls were taken from the center two rows of each 8 row plot. This field trial was a RCBD with 4 replications. Letters that are different are statistically significant. These data represent 3 site-years.

Discussion

Diamond had the opposite effect on fecundity than hypothesized, although these results were not significant. Southern green stink bug adults fed green beans dipped in Diamond had higher fecundity than those fed green beans dipped in water only. These results are similar to those reported by Kamminga et al. (2012), who found that brown marmorated stink bug (BMSB) adults fed with green beans dipped in novaluron (Rimon 0.83EC at 363.2 g ai/ha) and diflubenzuron (Dimilin 2L at 280.2 g ai/ha) had similar numbers of egg masses, numbers of eggs and hatch rates compared to BMSB fed green beans dipped in water only. This finding is also not unprecedented in the literature and is known as pesticide hormoligosis (Hardin et al. 1995; Guedes et al. 2016: Wu et al. 2020; Yang et al. 2023). While all mechanisms of this phenomenon are not fully understood, female fecundity has been shown to increase in several systems and across multiple chemistries. For example, Abdallah (1968) found that sublethal doses of DDT, parathion, and dieldrin increased fecundity in Colorado potato beetle. Azzam et al. (2009) found that deltamethrin and triazophos increased fecundity in brown leafhopper, a pest of rice. Chintalapati et al. (2015) found that the neonicotinoid insecticides thiamethoxam and imidacloprid caused higher fecundity in rice leaffolder. Wu et al. (2020) found that a sublethal dose of a variety of traditional insecticides induced increased fertility in the brown leafhopper in rice. Similarly, Yang et al. (2023) found that exposure of brown leafhoppers to emamectin benzoate in rice caused female fecundity to increase.

In our laboratory study, Diamond significantly impacted SGSB nymphal mortality for second, third and fifth instars, but not fourth instars. It should be noted that for fifth instars, although significant, only approximately 8 % of the nymphs died, whereas approximately 15 % (not significant) died in the fourth instar group. In our bioassay, we evaluated impacts up to 96 h

after treatment and found that approximately 27 % of third instars died by this time. These results are consistent with the findings of Kamminga et al. (2012) when they performed a bean dip assay on third instar nymphs of BMSB. A previous study by López et al. (2008) evaluated the effects of novaluron on SGSB using two nozzle sizes and three rates of Diamond in a spray chamber with artificially infested cotton plants at similar rates to our study. Lopez et al. (2008) also found that second instar nymph mortality was significantly increased by Diamond. Our data did deviate from theirs in that we found significant mortality in third and fifth instar nymphs, and they found no significant difference in the third instars and did not evaluate fifth instars. The differences could be explained by the fact that we used laboratory reared SGSB whereas they used field collected SGSB; also, we had a more controlled environment that ensured all nymphs received a similar dose of the product. We both found that Diamond did not effectively kill fourth instar SGSB nymphs.

The increase in mortality, however, was not reflected in an increase in yield in the field trial. This is likely because fourth and fifth instars and adult stink bugs do the most damage to cotton bolls when compared to the lower instars. A third instar nymph is estimated to do only about half as much damage as a fourth or fifth instar nymph (Khan 2004). When we compare the effectiveness of Diamond in younger instars to older instars in our study, we can see the steep decline in mortality associated with that nymphal development. It is also possible that our damage data could have been better supported by evaluating the test at 28-35 days to account for the time it takes the eggs to hatch and develop throughout their life cycle. It is possible that those extended evaluations could better reflect residual control of stink bugs in the field. Additionally, in our lab study, Diamond killed approximately 30 % of second – third instars, meaning approximately 70 % of the nymphs survived to the more damaging stage. It is possible that even if timed properly, Diamond would leave too many nymphs behind to reduce damage

below threshold levels. There was no significant increase in cotton yield between the higher novaluron application and untreated control, although there was a numerical increase. This could be an issue of incorrect timing of application. Ideally, Diamond would be applied 4-5 days after eggs are observed on the underside of the leaves. In our study, Diamond was applied at the third week of bloom, when the peak migration of adults is expected to occur.

Another limitation of this study was that in the laboratory only SGSB were tested. There are other species of stink bugs able to cause damage in the field. Even though SGSB has been observed as the most common stink bug species in Alabama cotton (Unpublished data, R. Smith), no species identification sampling was successful in this study to ensure that was the case in our fields. Internal boll damage is used to determine control measures in cotton due to an uneven distribution of stink bug densities across the field and the difficulty of visible counts (Reay-Jones et al. 2010). We believe more research in the field should be done to determine how novaluron can be used to aid in managing stink bugs in southeastern cotton, especially as it relates to our findings on nymphal mortality.

Figure 3.6 – A rearing bucket containing green beans, peanuts, and a water source.

Figure 3.7 – The outside of the rearing buckets with a hole cut out of the lid and the interior covered with cloth. Unbleached paper towels are allowed to hang on the inside walls as an oviposition substrate.

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