

**EFFICACY AND NON-LETHAL EFFECTS OF CLOTHIANIDIN FOR THE
MANAGEMENT OF STINK BUGS (HEMIPTERA: PENTATOMIDAE) IN COTTON**

by

BRIAN ANDERSON LITTLE

(Under the Direction of Michael D. Toews)

ABSTRACT

Experiments were conducted to assess the efficacy of selected insecticides for managing stink bugs in cotton. Bioassays on treated leaves demonstrated that bifenthrin provided the fastest time to knockdown and the minimum amount of time spent feeding following exposure. In comparison, dicrotophos was slower acting than bifenthrin but significantly faster than clothianidin. Direct application bioassays showed that all stink bug species were highly susceptible to dicrotophos and individuals never recovered. Results from cage studies showed that there was no repellency or residual activity remaining 7 days after treatment. Results from field studies indicated that stink bug damage was highly variable and was generally less when treated with dicrotophos, bifenthrin, or a reduced rate tankmix of clothianidin and bifenthrin. These results strongly suggest that dicrotophos applications are necessary for management of *E. servus*, but any of the other products tested provide acceptable control of remaining species.

INDEX WORDS: Stink bugs, neonicotinoid, chemical control, efficacy, insect behavior, cotton

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DEDICATION

To my God, parents: Rev. Mark E. and Belinda Little; sisters: Kimberly and Ashley; grandparents: Margie, Virginia, and John Little; Uncle Robert; cousins: Michael, Kristen and Lisa; and lastly my friends.

In memory of: Gene Culp, Jeffery Culp, Sharon Culp, Ruben A. Tucker

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CHAPTER 1

EFFICACY AND NON-LETHAL EFFECTS OF CLOTHIANIDIN FOR THE MANAGEMENT OF STINK BUGS (HEMIPTERA: PENTATOMIDAE) IN COTTON: AN INTRODUCTION AND LITERATURE REVIEW

Introduction

This thesis is aimed at understanding the efficacy and toxicology of clothianidin, a neonicotinoid insecticide. Products within the neonicotinoid insecticide class (IRAC group 4A) generally exhibit similar efficacy to organophosphate and carbamates insecticides, but are less toxic to non-target organisms including vertebrates. Research is needed to better understand how these products can replace older insecticide classes for managing emerging insect pests. Trials were conducted on stink bugs (Hemiptera: Pentatomidae) in cotton, *Gossypium hirsutum* L. (Malvaceae). Efficacy of clothianidin was compared with the following insecticides; dicotophos, bifenthrin as well as a reduced rate tankmix of clothianidin and bifenthrin (Table 1.1).

The first objective of this study, which is described in Chapter 2, was to measure the time to knockdown, incidence of feeding, and potential for recovery following insecticide exposure with insecticides from different insecticide classes. This study included a treated leaf bioassay and a direct application bioassay conducted under time-lapse videography. The second objective of this study, which is described in Chapter 3, was to investigate potential repellency of stink bugs in cages placed over treated and untreated cotton rows. The third objective, which is also described in Chapter 3, was to document efficacy through estimated boll damage, stink bug

captures, physical fiber properties and yield following treatments in commercial cotton fields. The final chapter is the summary of all research findings and suggestions for integrated pest management programs for stink bugs in cotton production.

Literature Review

Stink Bugs in Cotton

Stink bugs have been reported as a pest in cotton since the early 20th century (Morrill 1910). More than a century later, management of these insects is a growing issue within cotton production in the southeast and other parts of the cotton belt. They are also a growing issue in many other major crops grown in the southern US (Olson et al. 2012). Over the last few years management for stink bugs has increased in cotton, soybeans [*Glycine max* (L.) Merr.] and rice crops (Greene and Capps 2005). There are two main factors that contributed to stink bugs becoming a major pest in cotton. During the active boll weevil, *Anthonomus grandis grandis* Boheman, infestation period and the subsequent time period during eradication, broad spectrum insecticides were used weekly as standard practice in cotton production. Once the boll weevil was eradicated from Georgia, those insecticide applications decreased by at least 50%. Five years after boll weevil eradication ended in Georgia, transgenic Bt-cotton was introduced to control lepidopteran larvae, which further decreased insecticide use. The decrease in insecticide use in cotton due to the eradication of the boll weevil and new cotton plant cultivars released stink bugs, a previous occasional pest, within this production system (Blinka et al. 2010). Key stink bug pests found in cotton include the southern green stink bug, *Nezara vidula* (L), green stink bug, *Chinavia hilaris* (Say) and the brown stink bug, *Euschistus servus* (Say). Stink bugs have piercing mouthparts, with which they pierce through the carpal wall and into the developing seeds located inside the immature cotton boll. Stink bug feeding damage to developing cotton

bolls can cause lint discoloration, decrease lint quality and overall yield. These cotton pests caused approximately 26 million dollars in cotton losses in Georgia alone (Williams 2014). This study mainly focuses on *N. viridula* and the *E. servus*, two of the most common species of stink bugs found in cotton within the southeastern United States (Sullivan et al. 1996).

Cotton Production within the Southeast

Cotton is a very important agricultural crop in the southeast due to the many commodities it helps produce. Cotton lint is used to make textiles and the seeds are used produce cottonseed oil as well as livestock feed. The most important element in the growth of cotton is the availability of water within the soil. However, during a period of drought the fruits or bolls are less sensitive than the leaves to this condition (VanIersel and Oosterhuis 1996). The cotton production system has been under attack by many pests throughout its history, from the boll weevil to many lepidopteron pests. Due to the variety of pest within this system, a good understanding of the pest species and their biology is necessary for insect pest management.

Major Cotton Pest

According to the 2014 Cotton Insect Losses publication by M. Williams, the most important pest that contribute to cotton losses included: the boll weevil, bollworm, *Heliothis zea* (Boddie); tobacco budworm, *Heliothis virescens* (Fabricius); pink bollworm, *Pectinophora gossypiella* (Saunders); cotton fleahopper, *Pseudatomoscelis seriatus* (Reuter); tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois); cotton leaf perforator, *Bucculatrix thurberiella* (Busck); spider mites, *Tetranychus* spp.; thrips, *Thrips* spp. and *Frankliniella* spp.; beet armyworm, *Spodoptera exigua* (Hubner); fall armyworm, *Spodoptera frugiperda* (J.E. Smith); European corn borer, *Ostrinia nubilalis* (Hübner); stink bugs including *C. hilare*, *E. servus*, and *N. viridula*; grasshoppers; saltmarsh caterpillar *Estigmene acrea* (Drury); cotton aphid, *Aphis*

gossypii (Glover); banded winged whitefly, *Trialeurodes abutilonea* (Haldeman); silverleaf whitefly, *Bemisia argentifolii* Bellows & Perring; sweetpotato whitefly B biotype, *Bemisia tabaci* (Gennadius); loopers such as the cabbage looper, *Trichoplusia ni*, (Hübner) and soybean looper, *Chrysodeixis includens*, (Walker); southern armyworm, *Spodoptera eridania* (Stoll); cutworms, *Agrotis* spp. and clouded plant bugs, *Neurocolpus nubilus* (Say).

Stink Bug Biology

Stink bugs undergo a hemimetabolous metamorphosis or what is sometimes called incomplete metamorphosis. After hatching from eggs, nymphs go through five instars and then after the final molt the adult emerges (Gullan and Cranston 2010). Most insects found within Hemiptera are phytophagous or plant-feeding insects, however a few are predacious. Stink bugs have piercing-sucking mouthparts that they use to pierce through the outer cuticle and into the internal plant tissues, typically seeds, where they ingest the nutrients. The act of feeding can mechanically transmit plant pathogens inside the boll, affecting both yield and lint quality (Medrano et al. 2007). Stink bugs overwinter as adults and become active in the spring when cued by photoperiod and rise in the temperatures. In *E. servus*, the first generation of eggs are typically deposited in the field in late April and continue to late September, nymphs emerge in early May, and adults will start to appear around mid-June (Rolston and Kendrick 1961). Stink bugs immigrate to cotton as the plants produce flowers until formation of bolls (Bundy and McPherson 2000). *N. viridula* has approximately 4 to 5 generations per year (Todd 1989), whereas, *E. servus* has approximately 2 generations per year (McPherson and McPherson 2000).

Boll Damage and Economic Impact

These plant feeding insects are an economic issue in cotton because of the impact they have on both cotton yield and fiber quality. From 2010 to 2013, stink bugs in Georgia alone

caused estimated losses of 46,817 bales in 2010; 34,135 bales in 2011; 45,229 bales in 2012; and 63,235 bales in 2013 (Williams 2011, 2012, 2013, 2014). *N. viridula* is known to transmit a bacterium, *Pantoea agglomerans*, while feeding on bolls (Medrano et al. 2007, Medrano et al. 2009). The impact of this pathogen depends on both the age of the boll and the amount of time that has passed since inoculation (Medrano et al. 2009). If a boll is damaged early and becomes infected it is often aborted by the plant and thus can cause a decrease in seedcotton yield. *E. servus* adults typically select bolls from 7-27 days past anthesis (Siebert et al. 2005). External feeding damage is also reflective of internal damage and a decrease in lint quality (Blinka et al. 2010). Older treatment thresholds for stink bugs in South Carolina and Georgia were set at one individual per 2 m of row of cotton or at a level of 20% internal damage to medium-sized bolls (Greene et al. 2001). Currently, Cooperative Extension in the southeastern states worked together to produce a dynamic treatment threshold that suggests treatment at different levels of boll damage based on week of bloom. The threshold is lowest (10-15% injury) during weeks 3 to 5 of the bloom cycle and higher earlier and later in the bloom cycle.

Stink Bug Sampling and Boll Damage Assessment

Stink bug populations within cotton fields can be estimated by using sweep nests or beat cloths. These two methods are more efficient than other direct sampling methods used to sample hemipterans in cotton (Musser et al. 2007). Another way to document populations without insect sampling is to look at internal boll injury. However, this method can be more time consuming as shown by a 2010 study that documented one 20-boll sample required 569 s to complete versus 48 and 135 s for beat cloth and sweep net (Reay-Jones et al. 2010). Drop cloth sampling is conducted by placing a cloth beneath the plant canopy and then the plants are shaken or beaten to allow the insects to fall on the cloth. Sweep net sampling is conducted by swinging the net

through the top canopy of a single row of cotton. A typical sample is comprised of 10-20 sweeps before counting the insects that were captured. This type of sampling tends to have a bias for capturing adults rather than nymphs, whereas drop cloth sampling tends to have a bias for capturing nymphs (Reay-Jones et al. 2010). Internal boll damage is assessed by collecting medium sized bolls (2.3-2.8 cm diameter); the bolls are then opened and checked for internal feeding symptoms such as lint discoloration, carpel wall penetrations and the formation of callous or warts on the internal carpel wall. Due to different types of bias inherent with each sampling type, multiple sampling techniques are typically used to assess pest populations within the field (Reay-Jones et al. 2010).

Farmscape Ecology

The surrounding cropping system has an impact on both population dynamics and colonization preferences within a field (Olson et al. 2011). Development of stink bugs as a pest in cotton may be attributed to abundant alternative hosts adjacent to cotton fields. Research has shown that corn (*Zea mays* L.), peanut (*Arachos hypogaea* L.) and soybean [*Glycine max* (L.)] are all good host plants for stink bugs (Olson et al. 2011). *N. viridula* prefer cotton over peanut, however both *E. servus* and *N. viridula* prefer soybeans over cotton (Bundy and McPherson 2000, Olson et al. 2011). Research suggests that cotton fields near peanut fields produces a predictable movement of late instars from peanut to cotton, resulting in boll damage (Tillman et al. 2009, Toews and Shurley 2009). Cotton planted next to soybeans may also result in boll damage. However cotton planted adjacent to corn shows no significant difference in boll damage from bolls collected on the edge and bolls collected 18.7 m within the cotton field. Seedcotton yields can be decreased up to 5.3 m in cotton planted adjacent to peanuts and up to 18.7 m in

cotton planted adjacent to soybeans (Toews and Shurley 2009). Studies show that woodlands adjacent to fields are not a major source of stink bug colonizing populations (Olson et al. 2012).

Stink Bug Management Strategies

Stink bug management in cotton requires the use of broad-spectrum insecticides such as organophosphates and pyrethroids. However, other classes of insecticides, such as neonicotinoids, have shown some activity on stink bugs. Chemical rotation and avoiding sole reliance on a single compound are important components to a resistance management program. *E. servus* is at least tolerant, if not resistant, to pyrethroids in the field.

Biological Control

The spined soldier bug, *Podisus maculiventris* (Say), will feed on stink bug nymphs and adults (Jones 1918, McPherson 1980, McPherson et al. 1982). A study by P.G. Tillman and B.G. Mullinix, Jr. (2004) showed that commonly used insecticides used in managing stink bug populations are highly toxic to *P. maculiventris*. Similarly, *Trichopoda pennipes* (F.) (Diptera: Tachinidae), is an endoparasitoid of *N. viridula* adults and nymphs. *T. pennipes* is highly susceptible to the chemistries commonly used to manage stink bugs in cotton (Tillman 2006).

Biotechnology

Genetically modified cotton was introduced in 1996 to control tobacco budworms, and pink bollworms. These insect pests had developed a resistance to synthetic pyrethroids and required alternative methods of control. *Bacillus thuringiensis* (Bt) cotton contains genes which encode for a group of endotoxins known as the Cry group. The initial toxin developed in Bt cotton was known as Cry 1Ac. Research continued and led to the development of the next generation of Bt cotton which contained a second gene which codes for Cry 2Ab. The toxins are dissolved in the GI tract of the insect and bind to the midgut and allow fluid to pass through the

membrane leading to desiccation. Bt cotton has been extremely effective in controlling lepidopteran pest in cotton. However, Bt cotton is ineffective against stink bugs, and plant bugs, which require other methods of control.

Chemical Control

Organophosphates. Organophosphates interfere with the nervous system by acting on a highly conserved enzyme, acetylcholinesterase; therefore, organophosphate insecticides are toxic to both insects and vertebrates. The organophosphate acephate has been shown to be more toxic than dicotophos on *E. servus* (Kamminga et al. 2009). A study by Hopkins et al. (2009) testing insecticide susceptibility of stink bugs in cotton in Texas found that dicotophos was most effective against all species of stink bugs. Dicotophos has exhibited both good residual activity and oral toxicity against *E. servus* (Tillman and Mullinix 2004). Dicotophos mixed with methyl parathion, another organophosphate (trade name Methyl 4E), was 95-100% effective in controlling populations of *C. hilaris*, *E. servus* and *N. viridula* (Greene and Capps 2004). Dicotophos at rates of both 0.33 and 0.50 lb [AI]/A provided 96-100% mortality in both *C. hilaris* and *E. servus* (Greene and Capps 2002).

Pyrethroids. Synthetic pyrethroids are typically used for bollworm control in cotton but can also provide effective control of stink bug populations, especially when mixed with methyl parathion (Sullivan et al. 1996, Greene et al. 2001). Pyrethroid insecticides are known to provide excellent control against *C. hilaris* nymphs and adults, but poor control of *E. servus* populations (Greene and Capps 2002). Bifenthrin is the most effective pyrethroid on both *N. viridula* and *E. servus* nymphs as well as adults (Greene and Capps 2002, Snodgrass et al. 2005, Hopkins et al. 2009). The use of the pyrethroid λ -cyhalothrin (trade name Karate® Insecticide with Zeon

Technology) has been shown to control *C. hilare* nymphs more so than *E. servus* adults (Kamminga et al. 2009).

Neonicotinoids. Neonicotinoid insecticides are classified as Group 4A by the Insecticide Resistance Action Committee (IRAC) (Jeschke 2008). Neonicotinoids have been the fastest-growing class of insecticides since the introduction of pyrethroids (Nauen and Bretschneider 2002). This class of insecticides acts as an agonists of the post-synaptic nicotinic acetylcholine receptors in insects. Neonicotinoids are typically used as a seed treatment; however, some of the compounds can be applied to the plants directly. Foliar broadcast applications of neonicotinoid insecticides for stink bugs could provide growers with an integrated pest management alternative to manage stink bug populations (Jeschke 2008, Kamminga et al. 2009). Recent studies suggest that neonicotinoid insecticides can perform comparably with most organophosphates (Kamminga et al. 2009). A study in 2006 located in North Carolina concluded that the neonicotinoid insecticide dinotefuran provided greater mortality than acetamiprid for the management of *C. hilaris* and *E. servus* (Kamminga et al. 2009). After neonicotinoids were introduced, they began to replace pyrethroids, chlorinated hydrocarbons, organophosphates, and carbamates for management of pests in a variety of cropping systems (Denholm et al. 2002, Nauen and Denholm 2005).

Techniques for Testing Insecticide Efficacy

There are several standard techniques used to access the efficacy of an insecticide on a certain pest. Common techniques for stink bugs include: oral toxicity, direct application, residual toxicity, cage trials and field efficacy studies.

Oral Toxicity

Oral toxicity to insecticides is typically assessed by treating food and then allowing insects to feed. A study in 2004 used this technique to document oral toxicity of selected insecticides against *E. servus* as well as the impact of those compounds on the spined soldier bug, *Podisus maculiventris* (Say), a beneficial stink bug (Tillman and Mullinix 2004).

Direct Toxicity

In topical application bioassays, the diluted insecticide is applied directly to the insect as a means to test efficacy. However this method only tests the efficacy of the active ingredient. In this technique a specific amount of dilute active ingredient (0.16-1.0 μ l) is applied to the center of the sternite (ventral abdominal segments) of each insect, this simulates the theoretical efficacy within the field (Greene and Capps 2004, 2005, Takeuchi and Endo 2012). Mortality is typically scored at a defined time period following treatment.

Residual Toxicity

Residual toxicity bioassays document the residual efficacy of insecticides. Glass vial bioassays were first used to estimate insecticide resistance in adult plant bugs, *Lygus lineolaris* (Palisot de Beauvois) (Snodgrass 1996). One method is to dissolve the diluted active ingredient in acetone and apply it to the inside of a glass vial (Snodgrass 1996, Owen et al. 2011). The insect is then placed into the glass vial and is exposed to insecticide residues via tarsal contact with the vial. Other studies have applied treatments to the top and bottom of a plastic Petri dish allowed the residues to dry and then place insects into the Petri dish (Tillman and Mullinix 2004, Tillman 2006). Behavior as well as time to mortality may be monitored for a specific duration.

Systemic bioassays are a technique often used when using active ingredients which have systemic properties to document how well these compounds work once taken up by the plant.

This technique applies a small amount of diluted insecticide to living plant material and allowing the compounds to dry for a period of time before adding insects.

Cage and Field Trials

Field efficacy trials are conducted within commercial production fields to evaluate how well a product works on a large scale. Insect populations are monitored and insecticide applications are made once the trial area exceeds economic threshold. The crop will be harvested and yield values are taken to aid in the evaluation of the products. Insecticide efficacy trials in the field can also be conducted within insect cages. This technique has been used in many studies to document the efficacy of selected insecticides (Greene and Capps 2005, Hopkins et al. 2009). Field cages are commonly used when there is potential for feral insect populations to confound observed results.

Insect Behavioral Studies

Video tracking is a technique that has been used in many behavioral studies. Early studies on locomotor behavior in rodents aided the development of better behavioral study techniques (Vorhees et al. 1992). Understanding insect behavior is the key to understanding dispersal as well as development of integrated pest management programs (Romero et al. 2010). Advanced video tracking systems such as EthoVision® provides both object detection as well as data analysis (Bengtsson et al. 2004). Video tracking studies have been conducted on stink bugs within growth chambers. Studies include the movement and feeding preference of stink bugs caged on cotton plants (Huang and Toews 2012). In 2011, Cooper and Spurgeon conducted a laboratory studying using time-lapse videography on *Lygus hesperus*, to document feeding behavior differences between genders and reproductive states.

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Table 1.1 Selected insecticides evaluated for stink bug efficacy

Active Ingredient	Trade name	Rate ml per ha (oz per acre)	Manufacturer	IRAC class (insecticide group)	Mode of Action
Dicrotophos	Bidrin® 8	387 (5.3)	Amvac Chemical Corp.	1B (organophosphates)	acetylcholinesterase inhibitor
Bifenthrin	Discipline™ 2EC	373 (5.1)	Amvac Chemical Corp.	3A (pyrethroids)	sodium channel modulator
Clothianidin	Belay® + NIS ^a	292 (4.0)	Valent U.S.A Corp.	4A (neonicotinoids)	nicotinic acetylcholine receptor antagonist
Sulfoxaflor	Transform® WG	146 (2.0)	Dow AgroSciences	4C (sulfoxaflor)	nicotinic acetylcholine receptor agonist
Clothianidin + bifenthrin	Belay® + Discipline™ 2EC + NIS ^a	219 (3.0) 292 (4.0)	.	-	-

^anonionic surfactant at 2.5 ml per 1000 ml of finished spray solution

CHAPTER 2

A VIDEO BEHAVIORAL ANALYSIS: TOXICITY ASSOCIATED WITH SELECTED
INSECTICIDES AGAINST *NEZARA VIRIDULA* (L.), *EUSCHISTUS SERVUS* (SAY) AND
HALYOMORPHA HALYS (HEMIPTERA:PENTATOMIDAE)

Little, B.A., P.M. Roberts, D.G. Riley, and M.D. Toews. To be submitted to *Journal of
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Abstract

Laboratory bioassays were conducted in upright growth chambers equipped with digital video cameras to evaluate the efficacy of insecticides including bifenthrin, clothianidin, dicotophos, and a reduced rate tankmix of bifenthrin and clothianidin for management of *Nezara viridula* (L.), *Euschistus servus* (Say) and *Halyomorpha halys* in cotton. Bioassays on treated leaves demonstrated that bifenthrin provided the fastest time to knockdown and the minimum amount of time spent feeding following exposure. In comparison, dicotophos was slower acting than bifenthrin but significantly faster than clothianidin. *E. servus* adults on clothianidin treated leaves required nearly threefold longer than bifenthrin to reach knockdown; further, more than half of the *E. servus* test subjects recovered following clothianidin exposure. Direct application bioassays showed that all three stink bug species were highly susceptible to dicotophos and individuals never recovered. In comparison, greater than 10% recovery was observed in bifenthrin and clothianidin treated individuals. There were no significant differences in time to knockdown or time spend feeding among insecticides or the reduced rate tankmix of bifenthrin and clothianidin for *N. viridula* adults. These results strongly suggest that dicotophos applications are highly effective for the control of *E. servus*, but any of the other products provide effective control of remaining species. Data from these experiments is valuable for integrated pest management programs, as well as providing baseline data for resistance management of pentatomids in cotton.

KEY WORDS: Stink bug, neonicotinoid, chemical control, efficacy, insect behavior, cotton

Introduction

Stink bugs (Hemiptera: Pentatomidae) are an economically important group of pests affecting cotton (*Gossypium hirsutum* L.) production in the southeastern U.S. These insects are also a growing issue in other principle cash crops grown in the southern US (Olson et al. 2012). Stink bugs were first reported as cotton production pests in the early 1900s (Morrill 1910). In the past two decades, stink bug presence has increased in cotton, soybean [*Glycine max* (L.) Merr.] and rice (*Oryza sativa* L) (Greene and Capps 2005). Key stink bugs found in cotton in the Southeastern U.S. include *Chinavia hilaris* (Say), green stink bug, *Euschistus servus* (Say), brown stink bug, and *Nezara viridula* (L), southern green stink bug.

Phytophagous stink bugs are seed feeders and preferentially feed on seeds in developing cotton bolls (Bundy and McPherson 2000). Cotton lint, the harvestable part of the plant, is part of the seed. In *E. servus*, the first generation of eggs is typically deposited in late April resulting in nymphs from May to July. Nymphs emerge typically in early May, and the adults will appear around mid-June (Rolston and Kendrick 1961) with the second generation emerging in late September and October (Herbert and Toews 2011). Conversely, *N. viridula* starts reproducing about the same time but has three to five overlapping generations per year depending on latitude (Todd 1989).

The arrival of stink bugs as a serious economic cotton pest is thought to be related to general decreases in insecticide usage for cotton production. Prior to *Anthonomus grandis grandis* Boheman (boll weevil) eradication, weekly insecticide applications were required to manage this pest. The second factor leading to increased stink bug incidence was the introduction of transgenic *Bacillus thuringiensis* (Bt) cotton in 1996 to manage lepidopteran pests. Confluence of these two events produced new production environments that enable

growers to produce a crop with less than three insecticide applications per year. However, greatly reduced broad spectrum insecticide applications have fostered stink bug populations, especially in the southeastern US.

Currently, stink bug management in cotton requires application of foliar-applied, broad-spectrum insecticides such as organophosphates and pyrethroids. The susceptibility of stink bugs to these insecticides differs among species. Newer classes of insecticides, such as neonicotinoids, have some activity on stink bugs and are purported to be softer on beneficial insects than broad spectrum materials (Kilpatrick et al. 2005). Scouts and cotton specialists report that *E. servus* is tolerant of many pyrethroids, which mandates the use of organophosphates (Greene and Capps 2002). While *N. viridula* populations are susceptible to pyrethroids (Roberts et al. 2001, Hopkins et al. 2009), the organophosphate insecticide dicrotophos is known to be very effective in managing *A. hilaris*, *N. viridula*, and *E. servus* in cotton production (Greene and Herzog 1999a, Greene and Capps 2002, Hopkins et al. 2009).

In 2013, stink bugs infested approximately 485,623 ha (1.2 M acres) of cotton in Georgia, with 432,204 ha (1,068 M acres) treated at a total cost of \$25 per ha (\$18 per acre) with three total applications per ha (Williams 2014). Georgia Cooperative Extension recommends the use of a dynamic threshold for making stink bug treatment decisions (Bachelier et al. 2009). The threshold is based on the percentage of developing cotton bolls with evidence of stink bug feeding. Treatment is more aggressive in the middle of the bloom cycle when comparatively more harvestable bolls are susceptible to stink bug feeding. Phytophagous stink bugs feed on developing seeds in the bolls, which directly leads to loss of lint yield as the lint is part of the seed (Roberts et al. 2005). *E. servus* adults typically select bolls from 7-27 days past anthesis or plant flowering (Siebert et al. 2005).

Sampling for stink bug damage can be performed by quantifying internal boll damage or external boll feeding lesions (Toews and Shurley 2009). External feeding damage is reflective of internal damage and a decrease in lint quality (Blinka et al. 2010), but is not as sensitive as assessment of internal feeding damage. Seedcotton yields can be reduced by feeding damage caused by stink bug adults and late instar nymphs. Data show that comparatively more feeding damage is inflicted by *N. viridula* fifth instars compared to younger instars (Greene et al. 1999b, Willrich et al. 2004b). Stink bug feeding can have deleterious impacts on fiber quality and fiber quantity. Fiber length, micronaire, and fiber strength are significantly shorter in stink bug damaged cotton bolls (Bommireddy et al. 2007). Cotton plants under attack will abort larger bolls that are heavily damaged and very small undamaged bolls thereby decreasing lint yield (Huang and Toews, 2012). A study by Willrich et al. (2004a) demonstrated that boll-rotting pathogens (*Diplodia* spp. and *Fusarium* spp.) could be isolated from bolls caged with *N. viridula*. Further, this species is known to transmit a boll rot pathogen, *Pantoea agglomerans*, Sc-1-R strain, which can colonize the whole boll (Medrano et al. 2007, Medrano et al. 2009, Medrano et al. 2011). Finally, stink bug feeding leaves a wound channel that makes the boll susceptible to colonization by additional boll-rotting pathogens (fungal or bacterial) (Kiyomoto and Ashworth 1974, Roncadori 1974).

Two general techniques were used in this study to determine the toxicity of insecticides to stink bug adults: 1) exposure of the insect to dried/absorbed residues of the insecticides applied to cotton leaves, 2) topical application of the insecticide on the dorsal side of the insect. These two techniques were observed under time lapse videography and provided valuable information on insect behavior, feeding and time to knockdown. Time lapse videography is an important tool for studying insect biology, behavior and feeding. Cooper and Spurgeon (2011)

demonstrated the use of a video-based assay to monitor movement, stylet probing, feeding location, and oviposition by *Lygus hesperus* Knight. Video studies using stink bugs on individual cotton plants have aided in understanding movement, as well as insect feeding preferences on specific boll sizes (Huang and Toews, 2012). Managing stink bug populations with minimal disruptive insecticide applications, avoiding potential insecticide resistance and avoidance of secondary pest outbreaks have necessitated alternatives to widespread use of organophosphates. The objective of this research was to evaluate and compare the efficacy of bifenthrin, clothianidin, dicotophos, and a reduced rate tankmix of bifenthrin and clothianidin for management of *Nezara viridula*, *Euschistus servus* and *Halyomorpha halys* in cotton.

Materials and Methods

Insects

E. servus (colony established in 2009) and *N. viridula* (colony established in 2007) were maintained in a lab colony year round using the published methods (Harris and Todd, 1981) and (Huang and Toews 2011). Colonies were maintained in upright growth chambers (model I-36 LLVL, Percival Scientific, Perry, IA) running at 65% RH, 25° C during the day and 21° C at night with a 14:10 (L:D) photoperiod. Feral adults were introduced each spring to maintain genetic diversity within the lab colony. Adults were reared in 37.9 liter aquaria fitted with screen lids and the bottom was lined with paper cut to the dimensions of the aquaria bottom. Adult stink bugs were provisioned three times a week with fresh insecticide free green beans, *Phaseolus vulgaris* (L.), or okra, *Abelmoschus esculentus* (Moench), depending on seasonal availability. Additionally, they were provisioned with a few grams of organic shelled sunflower seeds and soybean pods. A 9-cm plastic Petri dish bottom was filled with absorbent cotton (U.S. Cotton™, Gastonia NC) and provisioned with free water. For *E. servus*, the aquaria sides were

lined with grade 50 cheese cloth (Uline®, Pleasant Prairie, WI) for use as an oviposition substrate. Aquaria containing *N. viridula* were simply lined with plain paper towels. Eggs were removed every other day to prevent cannibalism and transferred to a 9 cm ventilated Petri dish (Fisher Scientific, Pittsburgh, PA) lined with filter paper (cat. no. 09-795C, Fisher Scientific, Pittsburgh, PA) and a small okra pod or a green bean. Individuals were transferred to 0.95 liter plastic containers (part no. JSS32-120PP, Olcott Plastics, St. Chas, IL) after reaching the third instar growth stage.

H. halys (colony established in 2014) was reared following methods outlined by Medal et. al. (2012). Rearing methods were similar to those described above for *E. servus*, except that both immatures and adults were additionally supplemented with a small organically grown carrot. Once the nymphs reached the third instar growth stage, they were transferred to 0.95 liter plastic containers lined with strips of Kimwipes® (Kimberly-Clark Corporation, Roswell GA) to provide refugia.

Plant cultivation

Three cotton seeds ('DP 0912 B2RF') were sown 3 cm deep in 11.36 liter plastic pots filled with Commercial Metro Mix 300 growing medium (Sun Gro Horticulture, Bellevue, WA). Prior to planting, the entire bag (0.079 m³) of growing medium was mixed with 68 g of Osmocote 14-14-14 and 40.8 g of Micromax 90505 (The Scotts Co. LLC, Marsville, OH) to provide seedlings with nutrients to grow. Once seedlings were approximately 7.62 cm tall, the most vigorous plant was supported using a 1 m tall bamboo rod, while the remaining plants were discarded. Plants were watered daily and fertilized twice monthly using 6 g of 14-14-14 and 7.5 g of Micromax 90505. Once the plants started squaring, individual leaves that exceeded 9 cm across were selected for use in trials. Cotton plants received no pesticide applications in the

greenhouse other than the base fungicide [metalaxyl, pyraclostrobin, trifloxystrobin, myclobutanil, ipconazole, fluxapyroxad and chlorpyrifos (for storage insects)] provided in a commercial seed treatment.

Experimental Conditions

All experiments were conducted in experimental arenas placed in an upright growth chamber (model I-36 LLVL) maintaining conditions at $25 \pm 0.5^{\circ}\text{C}$, 65% RH and a 14:10 (L:D) photoperiod. The growth chamber was provisioned with 6 high-resolution miniature color video cameras (model HCCM474M, Honeywell, Morristown, NJ) equipped with vari-focal lenses (model YV5x2.7R4B-SA2L 2.7-13.5mm F1.3 A/I, Fujinon Corp., Tokyo, Japan). The cameras were mounted to the chamber racks using flex mounts (model 817-13, PanaVise Products Inc., Reno, NV) such that the optical end of the lens was approximately 14 cm above the chamber shelf (Fig. 2.1). Cameras were attached to an external digital video recorder (model EDR920 Powerplex, EverFocus Electronics Corp., Taipei, Taiwan) using coaxial cable; the video recorder was configured to enable view of date and timestamp on each frame to allow tabulation of time elapsed for various behaviors. LED infrared illuminators (model IR-200, Speco Technologies®, Amityville, NY) were placed below each experimental arena to enable viewing of the stink bugs through the cameras when the growth chamber lights were turned off (Fig. 2.1). A display monitor was connected to the DVR to allow for proper positioning and focusing of the cameras on the arenas.

Treated Leaf Bioassays

These bioassays were conducted to investigate insect feeding behavior and time to knockdown following insecticide exposure to dry insecticide residues on cotton leaves. Treatments included a negative control (water), bifenthrin, clothianidin, dicotophos, and a

reduced rate tankmix of bifenthrin and clothianidin. All treatments that included clothianidin also included A non-ionic surfactant (Penetrator® Plus, BASF, Research Triangle Park, NC) at a rate of 2.5 ml/liter (32 oz/100 gal.) finished spray solution. The appropriate amount of insecticide (Table 2.1) was diluted in water to mimic a grower spraying a finished spray solution at 93.5 liters per ha (10 gallons to the acre). For each individual replicate, a suitable sized cotton leaf attached to the plant in the greenhouse was evenly sprayed with 500µl of diluted insecticide using a dual action airbrush (model 150TM, Badger, Franklin Park, IL) applied at 34.4 kPa (5 PSI). The airbrush was cleared of pesticide residues by spraying 3 ml acetone into a metal trash can after each treatment. Since clothianidin and to a lesser degree dicotophos are systemic, leaves were left on the plants in the greenhouse for a period of 4 h following insecticide application to permit the insecticide to dry and be absorbed by the plant.

Prior to excising treated leaves in the greenhouse, experimental arenas were prepared in the lab using 100 x 20 mm plastic Petri dishes (Fisher Scientific, Pittsburgh, PA) containing an article of clean food (fresh okra or bean pod) and a <14 d old stink bug adult. Treated leaves were removed from the plant in the greenhouse and immediately transported to the lab where they were positioned with the treated side up and the untreated side placed in contact with the inverted lid of the Petri dish. Clean food and the bug were then gently placed on the treated leaf surface before pushing the inverted Petri dish bottom into the lid. The entire experimental arena was immediately positioned on the growth chamber rack directly underneath a video camera. Less than one minute elapsed between assembling the experimental arenas and initiation of video recording and all treatments were run simultaneously.

Video recording (Fig. 2.2) lasted 24 hours from the initiation of the trial. The bugs were then moved from the treated leaves into a clean Petri dish lined with a piece of filter paper and

provisioned with a fresh green bean or okra pod. The bugs were examined for recovery at 24 h and 48 h post insecticide exposure. Behaviors tabulated from the video recording included elapsed time moving (included both walking and running), time feeding and time until knockdown. The latter category was defined as when the bug could no longer make coordinated movements and flipped ventral side up (Kamminga et al. 2009). Conversely, insects had to right themselves and be able to walk to be classified as recovered. Following each experiment, insects were frozen for 48 h and discarded.

Direct Application Bioassay

Trials were conducted similar to those described above, except that treatments consisted of 250µl of the diluted insecticide delivered directly to the dorsal side of each individual bug using the airbrush. In addition to trials with *E. servus* and *N. viridula*, trials were also conducted with *H. halys*. Further, sulfoxaflor was added to the insecticide treatment list. Treatment of each bug was accomplished by placing an individual bug in a small box lined with removable paper under the fume hood. The bug was sprayed directly with the airbrush and then immediately transferred using fine forceps to an inverted Petri dish lined with filter paper and placed beneath a camera in the growth chamber. After 24 h in the growth chamber, the Petri dishes were placed on a lab bench and observed at 24 and 48 h post exposure to document recovery.

Statistical Analysis

Statistical comparisons were conducted separately by species using PROC GLIMMIX using SAS 9.3 software (SAS Institute Inc. 2012, Cary, NC). No data transformations were performed on the response variables and differences among treatments followed the LSMEANS separation at the $P \leq 0.05$ level of significance. Regardless of experiment, treatments were arranged in a completely randomized design; there were 10 replicates per treatment combination

in the leaf bioassay and 20 replicates per treatment combination in the direct application bioassay. Primary response variables in the leaf bioassays were duration of insect movement, amount of time spent feeding (on okra pod or green bean) and time to knockdown. There was no statistical comparison of recovery at 24 and 48 hours because recovery was limited to a single compound. The response variables in the direct application bioassays were elapsed time until knockdown and recovery at 24 and 48 hours; the lack of variation in mean recovery responses among compounds made them unsuitable for statistical analyses. Data plots were created using SigmaPlot 11.0.1 (Systat Software Inc. 2008 San Jose, CA).

Results

Approximately 960 h of video footage was recorded for the five treatments and two stink bug species included in treated leaf bioassays. In the direct application bioassays, approximately 1,333 h of video footage was recorded for the six insecticides and three stink bug species. Although water was included as a negative control in each experiment, those data were not included in the statistical analyses and are not shown because there was no knockdown attributed to treatment with water and the bugs fed intermittently throughout the assays. Incorporation of those results would greatly inflate the experimental variability leading to increased Type II errors.

Treated Leaf Bioassay

Results generally show that there were few differences among treatments for *N. viridula*, but there were obvious differences among insecticide treatments for *E. servus*. There were no differences among insecticides in the minutes required to reach knockdown for *N. viridula* adults exposed on cotton leaves ($F=2.03$; $df=3,10$; $P=0.173$) (Fig. 2.3). Similarly, there were no significant differences in the minutes of feeding prior to knockdown among the selected

treatments: ($F=0.92$; $df=3,10$; $P=0.465$) (Fig. 2.4). While no bugs recovered from insecticide poisoning from dicotophos or bifenthrin, several bugs (14.3%) recovered 48 hours after clothianidin exposure (Table 2.2).

There were differences among insecticides when assessing the amount of time to knockdown for *E. servus* ($F=5.98$; $df=3,17$; $P<0.001$). Dicotophos and bifenthrin treatments required less than half of the time to reach knockdown compared with clothianidin (Fig. 2.5). The reduced rate combination treatment reduced knockdown time by 1/3 compared with clothianidin alone. Similarly, there were large differences in the number of minutes spent feeding prior to knockdown for *E. servus* ($F=14.04$; $df=3,17$; $P<0.001$). Bifenthrin treated leaves resulted in the quickest knockdown (Fig. 2.6). While no bugs recovered from exposure to bifenthrin or dicotophos, more than half of the bugs recovered 48 hours after being transferred to a clean dish following exposure to clothianidin (Table 2.2).

Direct Application Bioassay

Regardless of stink bug species being investigated, there were always differences among insecticide treatments in the direct application bioassays and no individuals recovered following exposure to dicotophos. *N. viridula* adults treated with dicotophos were knocked down the fastest, whereas the clothianidin treated adults took five times longer to reach knockdown; meanwhile, sulfoxaflo was very slow acting compared to the remaining treatments ($F=65.97$; $df=4,84$; $P<0.001$). Dicotophos, bifenthrin, and the reduced rate combination treatment resulted in a similar number of minutes to reach knockdown (Fig. 2.7). There was no recovery from insecticide exposure except for treatment with sulfoxaflo.

E. servus adults treated with dicotophos were numerically fastest to knockdown; however, there were no significant differences among bifenthrin, dicotophos and the reduced

rate combination treatment ($F=3.97$; $df=3,28$; $P=0.018$). Clothianidin treatments required over six times longer, at 127.75 min, compared to dicotophos, 19.98 min, to reach knockdown (Fig. 2.8). While no bugs recovered when exposed with dicotophos, more than 10% of *E. servus* adults recovered following direct treatment with bifenthrin and clothianidin.

H. halys adults treated with dicotophos were knocked down in 13.74 min, whereas the clothianidin treated adults took almost 6 times longer at 78.57 min ($F=17.53$; $df=4,90$; $P<0.001$). Bifenthrin and the reduced rate combination (clothianidin + bifenthrin) treatments were not significantly different in minutes to knockdown: (Fig. 2.9). Recovery of *H. halys* following direct exposure was similar to that observed with *E. servus*.

Discussion

This study adapted the use of digital video cameras to document the efficacy of insecticides for management of stink bugs in cotton. Analyses of video footage allowed the ability to precisely measure time to knockdown and incidence of feeding following insecticide exposure.

After comparing the selected insecticides in the treated leaf bioassay, it was evident that bifenthrin was effective in providing the least amount of time spent feeding and the fastest time to knockdown in *E. servus* adults. This is important because less feeding in the field could directly reduce opportunities for pathogens to be introduced and thus protecting yield. Bifenthrin has been shown in other studies to provide effective control of *N. viridula*, whereas *E. servus* is less susceptible (Hopkins et al. 2009). Bifenthrin is considered the most effective pyrethroid for all life stages of *E. servus* (Snodgrass et al. 2005). *N. viridula* adults placed on cotton leaves treated with clothianidin + bifenthrin resulted in the numerically fastest time to knockdown; however, it was not significantly different from dicotophos or bifenthrin treatments alone. There

were no significant differences in the time spent feeding in any of the treatments with *N. viridula*. Direct application bioassays with *H. halys* showed that this species is highly susceptible to bifenthrin or dicotophos.

Although the different exposure methods were not designed to be statistically compared, it is interesting to examine crude differences among compounds. As expected, exposure to dicotophos residues on treated leaves resulted in slower time to knockdown compared to direct application bioassays with both *N. viridula* and *E. servus*. Similarly, direct application of the reduced rate tankmix of clothianidin and bifenthrin resulted in a faster time to knockdown compared to exposure of *E. servus* on treated leaves. However, it required approximately the same amount of time (140 to 150 min) to reach knock with clothianidin treatments regardless of insect species or method of exposure.

The use of broad-spectrum insecticides, such as organophosphates and pyrethroids, is disruptive to natural enemies and can lead to outbreaks of other secondary pest species (i.e. spider mites, whiteflies, aphids). Studies designed to quantify the impacts of selected neonicotinoids compared with dicotophos on nontarget arthropods in cotton show a 75% reduction in predators following treatment with dicotophos, 55-60% in the neonicotinoid thiamethoxam, and only 30% in neonicotinoid treatments including acetamiprid and imidacloprid treatments (Kilpatrick et al. 2005). Another study showed that the pyrethroid cyfluthrin was highly toxic to *Trichopoda pennipes* (F.), a beneficial insect that is an endoparasitoid of *N. viridula* (Tillman 2006). Similarly, the predacious spined soldier bug, *Podisus maculiventris* (Say) is highly susceptible to the pyrethroid cyfluthin and the carbamate oxamyl (Tillman and Mullinix 2004).

Direct applications of sulfoxaflor required significantly longer time to knockdown in both *N. viridula* and *E. servus* compared to all other treatments. Stink bugs treated with sulfoxaflor were knocked down eventually, but more than one fourth of the *N. viridula* test population recovered. These data strongly suggest that sulfoxaflor is a poor insecticide choice for control of stink bugs.

N. viridula and *H. halys* were highly susceptible to nearly all the products tested except sulfoxaflor. These results are consistent with previous studies that showed *E. servus* are highly susceptible to dicotophos (Tillman and Mullinix 2004). Therefore, the authors conclude that dicotophos applications are required for effective *E. servus* management in cotton. Variability was evident in the susceptibility to insecticides among the species, thus illustrating the importance of correctly determining species identification and composition within the field. In fact, correct species identification should drive insecticide selection in the field.

Stink bug recovery following exposure to neonicotinoids is known from the literature. Here, the authors observed greater than 10% recovery of *N. viridula* following exposure on clothianidin treated leaves. This documented recovery was consistent with previous studies showing that *N. viridula* recovered after initial knockdown in clothianidin treatments (Sugimura et al. 2007, Takeuchi and Endo 2012). Based on stink bug knockdown and recovery in treated leaf bioassays with clothianidin, it is evident that this xylem-mobile compound is translocated at both lethal and sublethal concentrations within the leaf tissue. This observation is consistent with the study by Stamm et al. (2011), which documented a similar occurrence when studying the susceptibility of the western chinch bug, *Blissus occiduus* Barber, exposed to different neonicotinoids in treated leaf bioassays. *N. viridula* adults will probe and feed on the vascular

tissues when caged on cotton leaves (personal observation), which undoubtedly could increase exposure to the toxicant.

Clothianidin by itself was generally slower acting than dicotophos or bifenthrin. However, the reduced rate tankmix of clothianidin and bifenthrin generally provided statistically similar results to dicotophos or bifenthrin alone. Conversely, bifenthrin alone was not slower than the reduced rate tankmix. Cullen et al. (2007) documented that there were no statistically significant advantages to a reduced rate tankmix of a neonicotinoid and pyrethroid versus the pyrethroid alone for managing consperse stink bug, *Euschistus conspersus* (Uhler), in tomatoes (*Lycopersicon esculentum* Miller). A study by Hopkins et al. (2009) also showed that there were no significant differences between a reduced rate tankmix of a neonicotinoid (imidacloprid) and pyrethroid (cyfluthrin) versus a pyrethroid alone on *E. servus* caged on cotton 72 hr after exposure. Provided that by reducing the rate of the pyrethroid from 373 ml/ha to 292 ml/ha it potentially may preserve some additional natural enemies, the reduced rate tankmix of clothianidin and bifenthrin may be a good strategy for management of *N. viridula* and *H. halys*, but not *E. servus*.

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Table 2.1. Selected insecticides evaluated for stink bug efficacy

Active Ingredient	Trade name	Rate ml per ha (oz per acre)	Manufacturer	IRAC class (insecticide group)	Mode of Action
Dicrotophos	Bidrin® 8	387 (5.3)	Amvac Chemical Corp.	1B (organophosphates)	acetylcholinesterase inhibitor
Bifenthrin	Discipline™ 2EC	373 (5.1)	Amvac Chemical Corp.	3A (pyrethroids)	sodium channel modulator
Clothianidin	Belay® + NIS ^a	292 (4.0)	Valent U.S.A Corp.	4A (neonicotinoids)	nicotinic acetylcholine receptor antagonist
Sulfoxaflor	Transform® WG	146 (2.0)	Dow AgroSciences	4C (sulfoxaflor)	nicotinic acetylcholine receptor agonist
Clothianidin + bifenthrin	Belay® + Discipline™ 2EC + NIS ^a	219 (3.0) 292 (4.0)	.	-	-

^anonionic surfactant at 2.5 ml per 1000 ml of finished spray solution

Table 2.2. Percent insect recovery from selected insecticides evaluated for stink bug efficacy in treated leaf and direct application bioassays

Treatment	<i>N. viridula</i>	<i>E. servus</i>	<i>H. halys</i>
Treated Leaf Bioassay: Percent Recovery Post 24 hrs			
Bifenthrin	0	0	-
Clothianidin	0	62.5	-
Dicrotophos	0	0	-
Clothianidin + bifenthrin	0	0	-
Treated Leaf Bioassay: Percent Recovery Post 48 hrs			
Bifenthrin	0	0	-
Clothianidin	14.28	62.5	-
Dicrotophos	0	0	-
Clothianidin + bifenthrin	0	0	-
Direct Application Bioassay: Percent Recovery Post 24 hrs			
Bifenthrin	0	12.5	0
Clothianidin	0	12.5	5
Dicrotophos	0	0	0
Clothianidin + bifenthrin	0	0	0
Sulfoxaflor	27.27	-	6.25

Treatment	<i>N. viridula</i>	<i>E. servus</i>	<i>H. halys</i>
Direct Application Bioassay: Percent Recovery Post 48 hrs			
Bifenthrin	0	12.5	0
Clothianidin	0	12.5	5
Dicrotophos	0	0	0
Clothianidin + bifenthrin	0	0	0
Sulfoxaflor	27.27	-	6.25

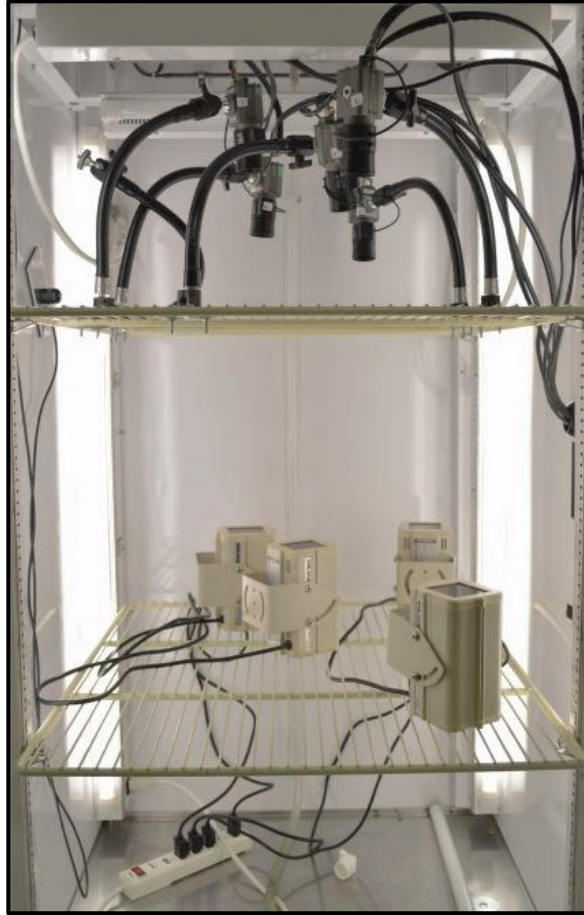


Fig. 2.1. Upright growth chamber equipped with digital video cameras mounted on top of growth chamber rack. Note LED infrared illuminators positioned below the cameras for viewing subject when the chamber lights shut off.



Fig. 2.2. Treated leaf bioassay with experimental arenas positioned below digital video cameras.

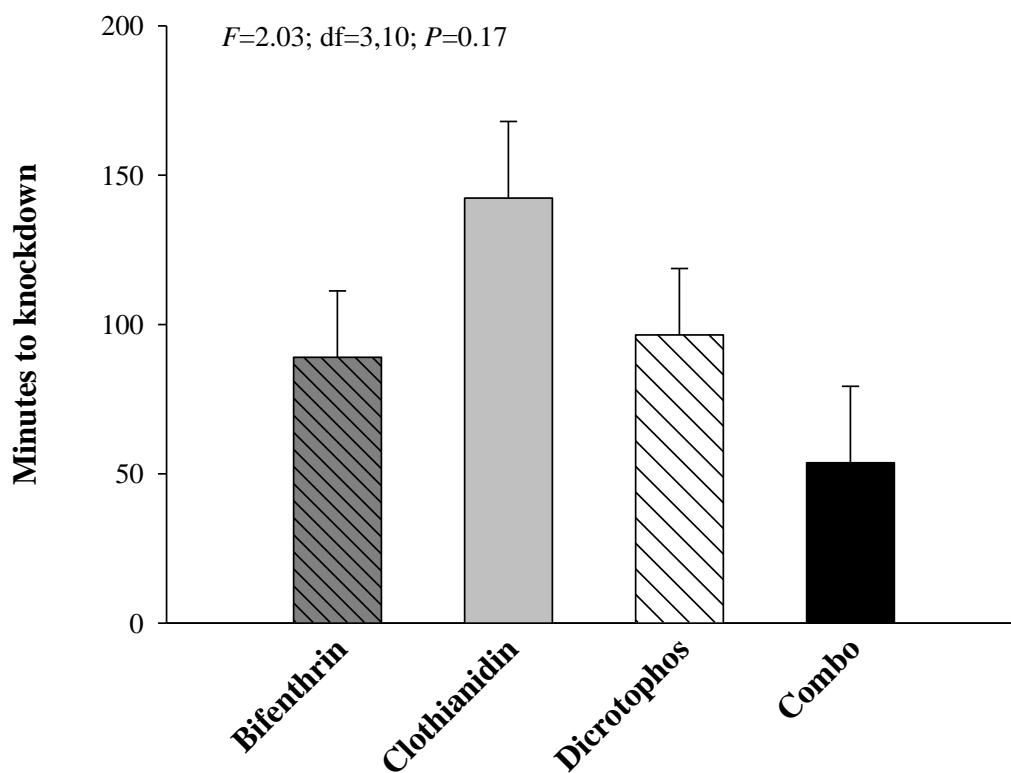


Fig. 2.3. Mean \pm SD minutes required to reach knockdown in treated leaf bioassays with *N. viridula* adults.

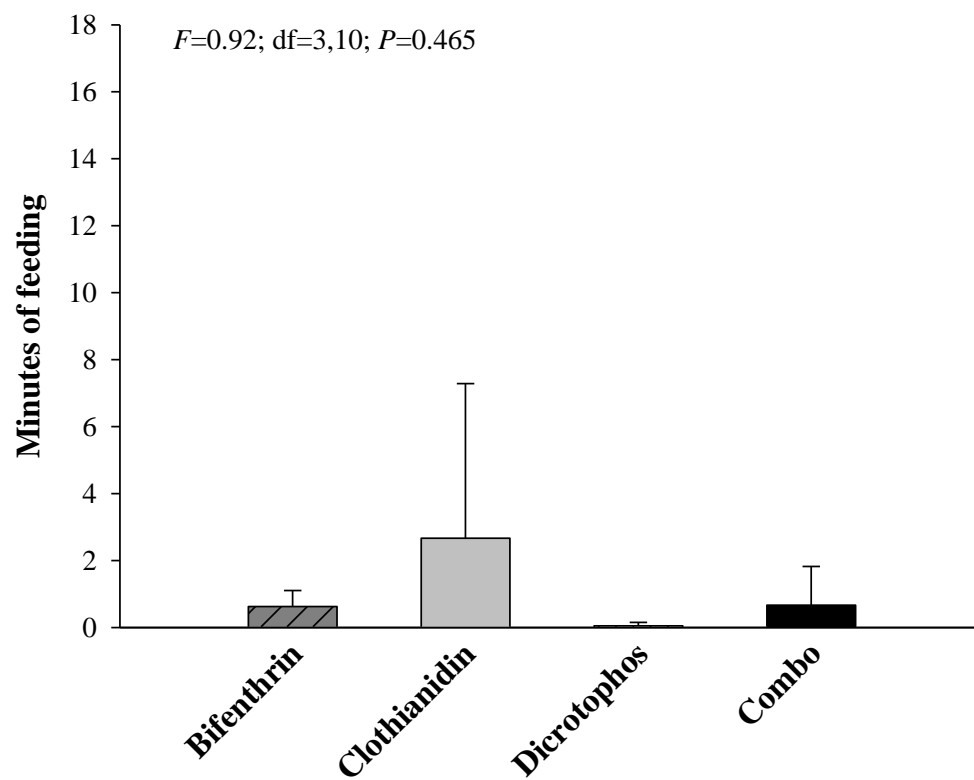


Fig. 2.4. Mean \pm SD minutes spent feeding on a small bean or okra pod in treated leaf bioassays with *N. viridula* adults.

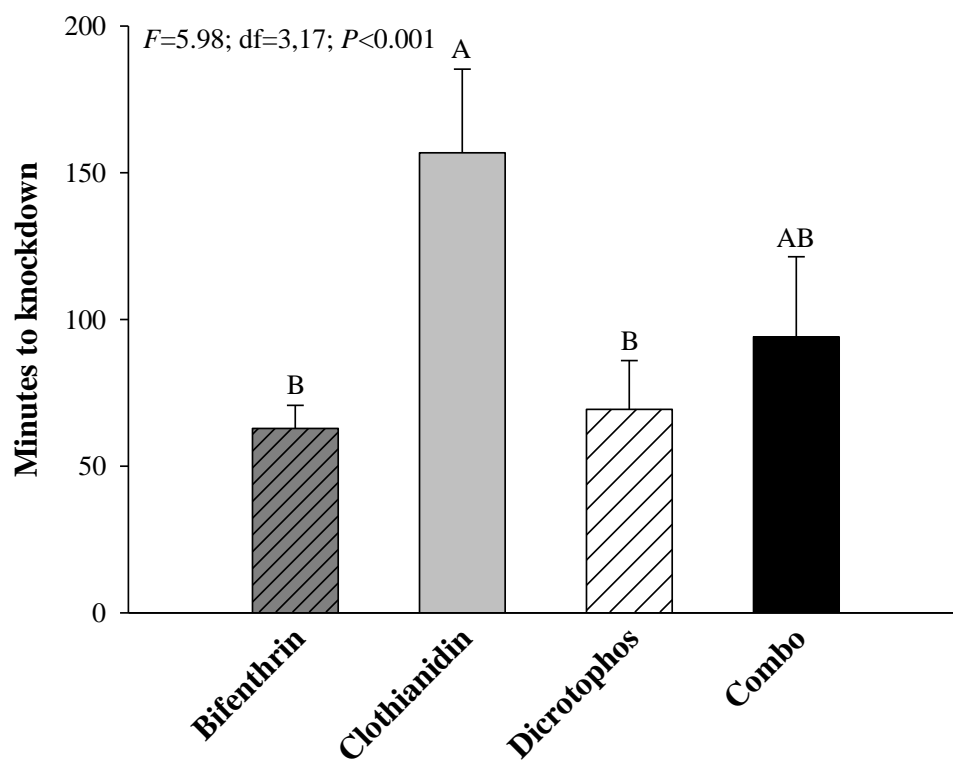


Fig. 2.5. Mean \pm SD minutes required to reach knockdown in treated leaf bioassays with *E. servus* adults. Means followed by the same letter are not significantly different ($P<0.05$; LSMEANS).

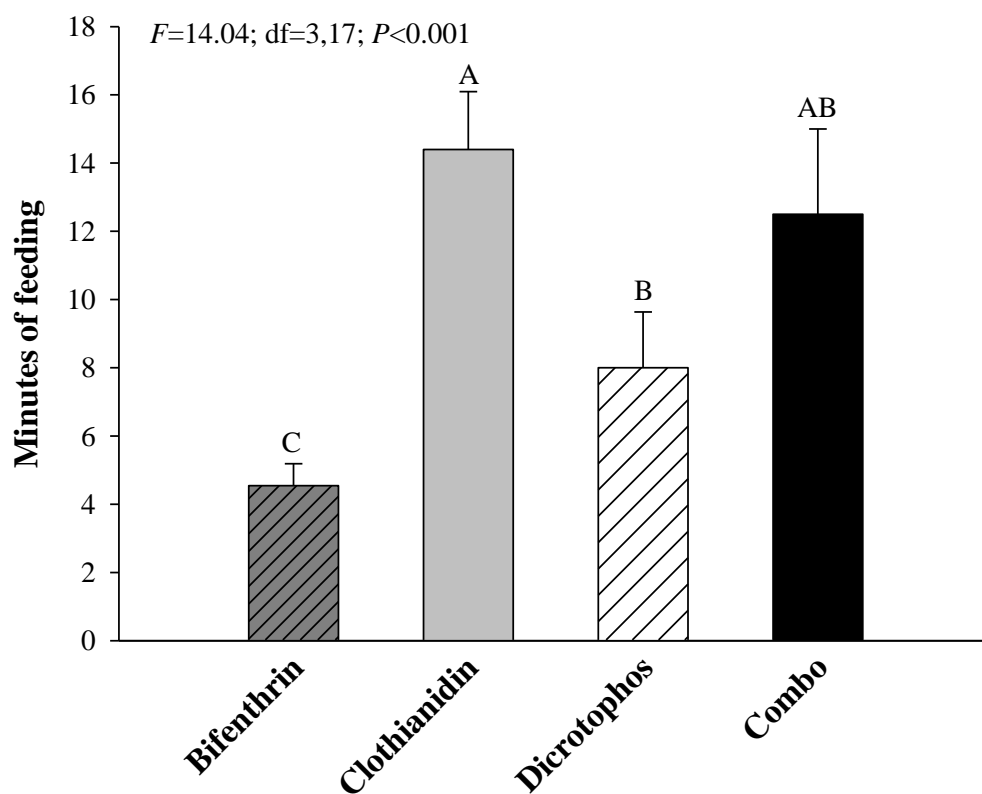


Fig. 2.6. Mean \pm SD minutes spent feeding on a small bean or okra pod in treated leaf bioassays with *E. servus* adults. Means followed by the same letter are not significantly different ($P<0.05$; LSMEANS).

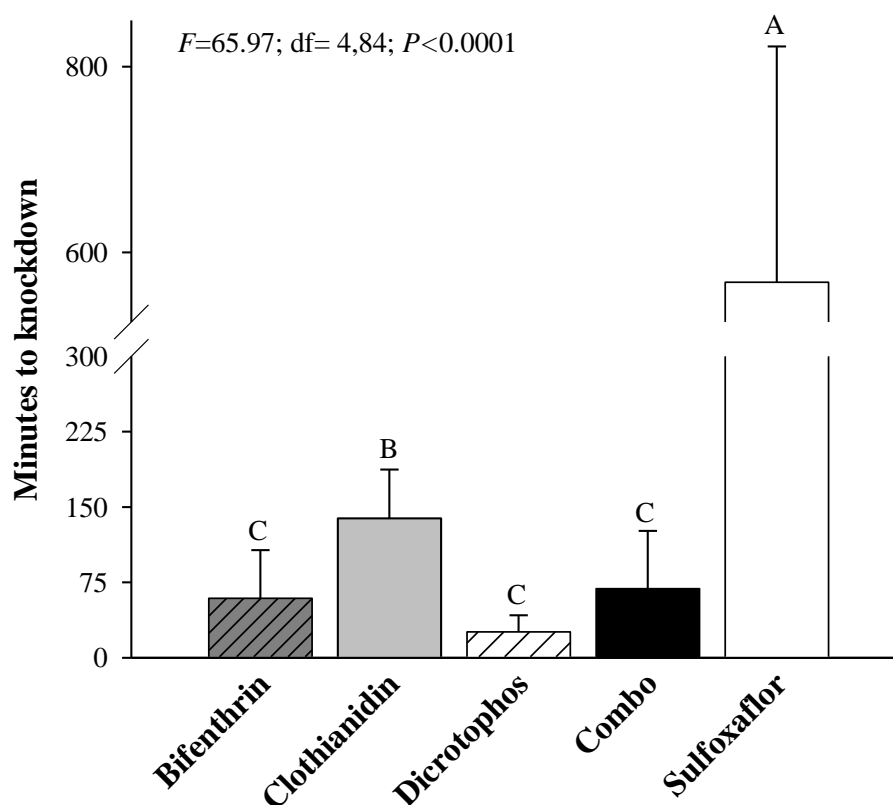


Fig. 2.7. Mean \pm SD minutes required to reach knockdown in direct application bioassays with *N. viridula* adults. Means followed by the same letter are not significantly different ($P<0.05$; LSMEANS).

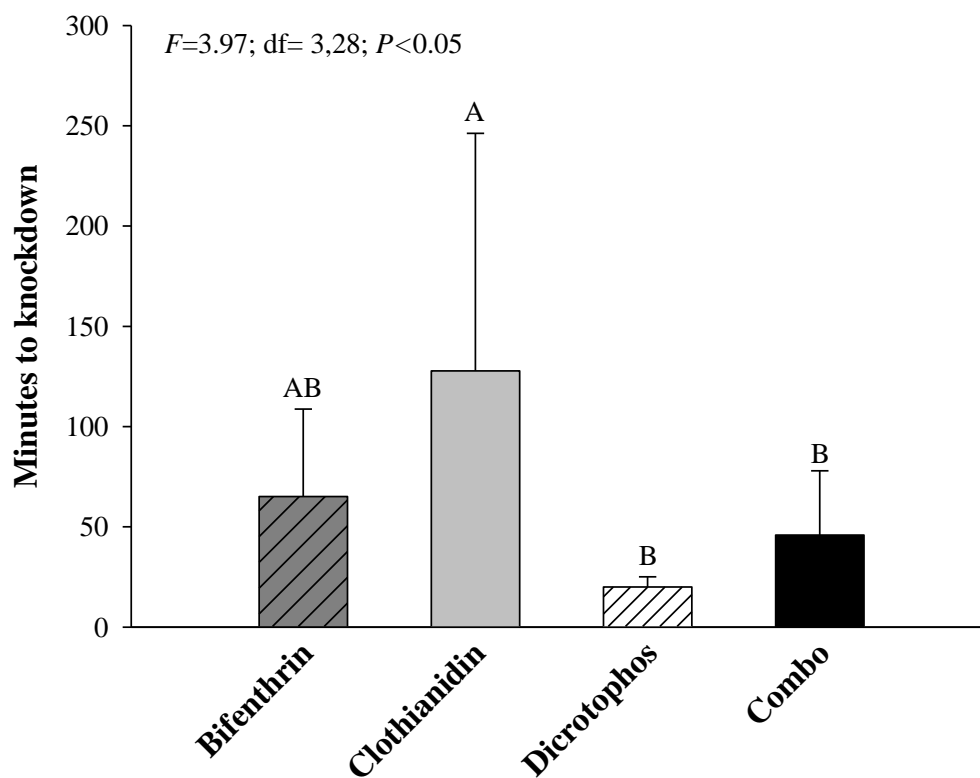


Fig. 2.8. Mean \pm SD minutes required to reach knockdown in direct application bioassays with *E. servus* adults. Means followed by the same letter are not significantly different ($P<0.05$; LSMEANS).

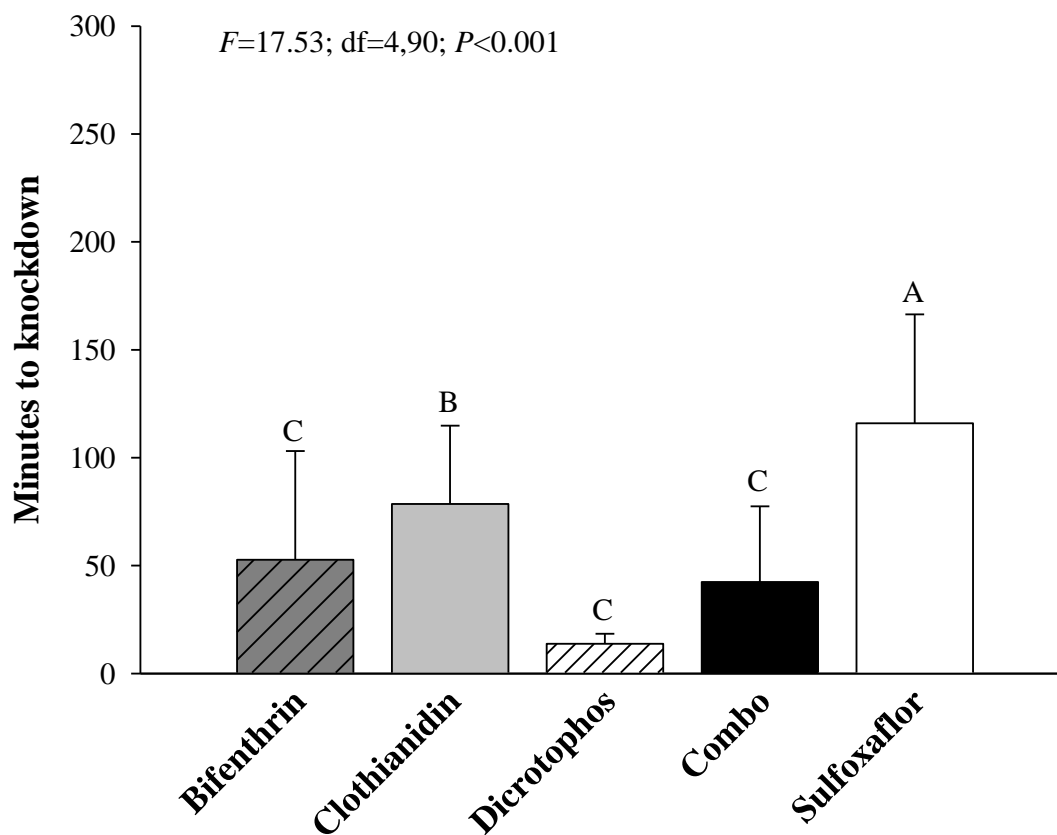


Fig. 2.9. Mean \pm SD minutes required to reach knockdown in direct application bioassays with *H. halys* adults. Means followed by the same letter are not significantly different ($P<0.05$; LSMEANS).

CHAPTER 3

EFFICACY OF SELECTED INSECTICIDES FOR THE MANAGEMENT OF STINK
BUGS (HEMIPTERA: PENTATOMIDAE) IN GEORGIA COTTON FARMSCAPES

Little, B.A., P.M. Roberts, D.G. Riley, and M.D. Toews. To be submitted to *Journal of Economic Entomology*.

Abstract

Experiments were conducted to evaluate the efficacy of clothianidin, dicotophos, bifenthrin, and a reduced rate combination of clothianidin and bifenthrin against the southern green stink bug, *Nezara viridula* (L.), and the brown stink bug, *Euschistus servus* (Say) in cotton, *Gossypium hirsutum* (L.). Efficacy of these selected insecticides was evaluated in field cage studies and on commercial farms during 2013 and 2014. Results from cage studies showed boll damage from stink bug feeding increased with boll diameter between 1.6 and 2.8 cm in diameter. Bolls that were less than 1.6 cm in diameter received little feeding damage while damage was more inconsistent on bolls greater than 2.8 cm in diameter. Within the 1.6 to 2.3 cm boll class, there were no differences among insecticides 7 days after treatment in the total number of bolls exhibiting stink bug feeding symptoms in *N. viridula* adults. However, there were differences among treatments in the total number of bolls exhibiting stink bug feeding symptoms in *E. servus* adults. Results from commercial field studies showed that *E. servus* was the primary stink bug observed and boll injury from stink bugs ranged from 20 to nearly 100%. There were few statistical differences among treatments, but the water treated (positive control) plots always endured more boll injury than insecticide treated plots one week after treatment. While not 100% consistent, treatments including dicotophos, bifenthrin, or a reduced rate tankmix of clothianidin + bifenthrin generally reduced boll injury compared to water or clothianidin alone.

KEY WORDS: cotton, stink bugs, physical fiber properties, feeding preferences, integrated pest management

Introduction

Stink bugs (Hemiptera: Pentatomidae) are a pest in row crop and vegetable production in the southeastern United States. Stink bugs are a known agricultural pest of corn, beans, and cotton. Since the early 20th century, stink bugs have been reported as a pest of cotton (Morrill 1910). The three most common stink bug species that attack cotton in Georgia include the brown stink bug, *Euschistus servus*; southern green stink bug, *Nezara viridula*; and green stink bug, *Chinavia hilaris* (Sullivan et al. 1996). The need for stink bug management in cotton has greatly increased in the south (Greene and Capps 2005). The development of stink bugs as cotton pests in the southeastern US is thought to be attributed to fewer applications of broad-spectrum insecticides as a function of the successful boll weevil, *Anthonomous grandis grandis* (Boheman), eradication program and widespread adoption of transgenic *Bacillus thuringiensis* cotton.

Broad-spectrum insecticides, primarily organophosphates (IRAC group 1B) and pyrethroids (IRAC group 3A), are used to provide effective control of stink bugs. However, these chemistries are disruptive to natural enemies, may flare secondary pest outbreaks (cotton aphids, whiteflies or spider mites) and are highly toxic to vertebrates including humans. Newer classes of insecticides, such as neonicotinoids (IRAC group 4A), may be a potential alternative for managing stink bugs. Neonicotinoid insecticides have a low affinity for vertebrate nicotinic receptors but do have a high affinity to insect nicotinic receptors, which makes them a potential replacement for other classes of insecticides (Tomizawa and Casida 2005). Research is necessary to understand the susceptibility of stink bugs to these newer classes of insecticides and foster the development of better management programs.

Stink bugs begin to infest cotton during the early boom stage and continue to increase while all stages of developing bolls are present. Stink bugs continue to infest cotton until the last boll is formed (Bundy and McPherson 2000). Phytophagous stink bugs have piercing-sucking mouthparts that are inserted into plant tissues, typically seeds, to extract the nutrients within. Feeding injuries caused by adult *N. viridula* feeding is characterized by a water-soaked ring on the interior carpel wall with a small brown necrotic spot at the center (Medrano et al. 2011). *N. viridula* feeding on bolls can significantly affect the physical fiber properties of length, micronaire, strength, uniformity, and discoloration (Bommireddy et al. 2007). *E. servus* feeding on developing bolls can cause a reduction in seedcotton yields (Willrich et al. 2004b). Plant pathogens introduced during feeding can cause lint staining, discoloration or even lead to the plant aborting the infected boll. *N. viridula* can transmit a pathogen, *Pantoea agglomerans* strain Sc 1-R, while feeding on unopened cotton bolls (Medrano et al. 2007, Medrano et al. 2009). *E. servus* is known to target bolls that are approximately 2-27-d-old or 165.2 to 672 heat units beyond anthesis (flowering) (Siebert et al. 2005).

According to the National Cotton Council of America, Georgia cotton growers harvested 542,278 ha (1.34 M acres) of the 554,419 ha (1.37 M acres) planted and produced 2.32 M bales of cotton during the 2013 crop year. Stink bugs accounted for approximately 486,230 ha (1.2 M acres) infested and 63,235 bales lost in Georgia during the 2013 crop season (Williams 2014). Georgia Cooperative Extension recommends that growers utilize a dynamic threshold decision aid for making insecticide applications. The dynamic threshold suggests treatment at 20% internal boll damage during the second week of bloom, but decreases to 10-15% internal boll damage during the third through

fifth weeks of bloom, followed by 20% in the sixth and 30% in the seventh week of bloom.

Stink bug populations are currently managed in the southeastern US with broad-spectrum insecticides, such as organophosphates and pyrethroids. Organophosphates are known to provide excellent control of stink bugs in cotton; both *E. servus* and *N. viridula* adults are equally susceptible to the organophosphate dicrotophos (Snodgrass et al. 2005). Organophosphates are acetylcholinesterase inhibitors that are toxic to insects and vertebrates. Due to high vertebrate toxicity, US EPA has removed some registrations while restricting use patterns of others. Prior to widespread use of transgenic Bt cotton, synthetic pyrethroids were routinely applied for bollworm management, which could be mixed with methyl parathion if stink bugs exceeded thresholds (Sullivan et al. 1996). However, methyl parathion is no longer commercial available, thus other methods of effective stink bug control are necessary. Bifenthrin, a common pyrethroid, is known to be equally toxic to both *E. servus* and *N. viridula* adults (Emfinger et al. 2001, Willrich et al. 2003). However, *E. servus* populations have developed a tolerance to many available pyrethroids (Snodgrass et al. 2005). With the development of newer chemistries, such as neonicotinoids, there is a possible alternative to the use of organophosphates and pyrethroids for managing stink bugs. Neonicotinoids are growing in popularity and sales worldwide (Matsuda et al. 2001). After the discovery of nitenpyram, research led to the replacement of the nitromethylene group with a nitroimino group and the development of clothianidin (Uneme 2010). Clothianidin is currently available as a foliar applied material for management of stink bugs in cotton. Neonicotinoid insecticides target insect nicotinic acetylcholine receptors (Matsuda et al. 2001, Ihara et al. 2006). Thiamethoxam and

acetamiprid are two neonicotinoids that are known to be toxic to *E. servus* adults (Kamminga et al. 2008). In soybean field trials, neonicotinoids perform comparable to both organophosphates and pyrethroids for managing stink bug populations (Kamminga et al. 2008).

Objectives of this study were to 1) investigate evidence of repellency following insecticide treatment in field cages, and 2) assess insecticide efficacy through estimated boll damage, yield, and lint quality following treatment in commercial fields.

Materials and Methods

Insects

The *N. viridula* lab colony was established from feral adults captured from Tift County, GA, in 2007. The *E. servus* lab colony was established from feral adults captured in corn from Tift County, GA, in 2009. Each spring, approximately 50 feral adults were introduced into each colony to maintain the genetic diversity in the established lab colonies. *E. servus* and *N. viridula* adults were reared in 37.9 liter aquaria with metal screen lids. Stink bugs were provisioned with fresh green beans, *Phaseolus vulgaris* (L.) or okra, *Abelmoschus esculentus* (Moench), as well as a dry mixture of organic sunflower and soybean seeds. A 9 cm Petri dish of PADCO (non-sterile) absorbent cotton (U.S. Cotton™, Gastonia NC), with water was added to supply the insects with free water. For oviposition, *E. servus* adults were provisioned with grade 50 cheese cloth (Uline®, Pleasant Prairie, WI), while *N. viridula* adults were provisioned with white single fold hand towels (no, SB18408, Tork® Universal, SCA AFH Professional Hygiene, Philadelphia, PA). The oviposition substrates were affixed via tape to the sides of the glass aquariums.

Eggs were removed daily to prevent cannibalism and then placed in 9 cm ventilated Petri dishes (Fisher Scientific, Pittsburgh, PA) lined with 9 cm dia. filter paper (cat. no. 09-795c, Fisher Scientific, Pittsburgh, PA) and a small okra pod or green bean pods to maintain humidity. Once the nymphs reached third instar, they were transferred to 0.95 liter plastic containers (part no. JSS32-120PP, Olcott Plastics, St. Chas, IL) where they remained there until they molted into adults. All insect life stages (eggs, nymphs, and adults) were held in upright growth chambers (model I-36 LLVL, Percival Scientific, Perry, IA) maintaining 65% RH, 25° C during the day and 21° C at night with a 14:10 (L:D) photoperiod.

Caged Field Studies

Caged field bioassays were conducted to document any repellency caused by insecticide application. Research was conducted during the summers of 2013 and 2014 at the University of Georgia Coastal Plain Experiment Station at Tifton, GA. Plots were planted every three weeks with cotton cultivar ‘DP 1252 B2RF’ beginning in late May. Agronomic practices conformed to general practices recommended by Georgia Cooperative Extension, except that no foliar insecticides were applied. Experiments were initiated during the fourth week of bloom when there were all possible sizes of bolls present. Insecticide applications were made using a two-row backpack sprayer equipped with hollow cone nozzles (Conejet TXVS 6, TeeJet Technologies, Wheaton, IL) at 276 kPa (40 PSI). Two liter plastic bottles were prepared with each insecticide which simulated field rates applied at a volume of 93.5 liters per ha (10 gallons/acre. Three liters of clean water were passed through the sprayer to rinse out residual insecticide between treatments.

Treatments included the following: dicotophos (Birdin 8, Amvac Chemical Co., Los Angeles, CA) at 0.388 liter/ha (5.3 oz /acre), bifenthrin (Discipline 2EC, Amvac chemical Co.) at 0.373 liter/ha (5.1 oz/acre), clothianidin (Belay®, Valent U.S.A. Corp., Walnut Creek, CA) at 0.293 liter/ha (4 oz/acre) and a reduced rate tankmix of clothianidin at 0.219 liter/ha (3 oz/acre) plus bifenthrin at 0.293 liter/ha (4 oz/acre). All applications that included clothianidin were prepared with a non-ionic surfactant (Penetrator® Plus, BASF, Research Triangle Park, NC) at a rate of 2.5 ml/liter (32 oz/100 gal.) finished spray solution as suggested on the Belay® label.

Experimental plots were 5.57 m long with 3.05 m alleys. Treatments were administered by first completely covering an adjacent cotton row with a plastic drop cloth (Husky®, Poly-America, Grand Prairie, TX.) followed by spraying two rows adjacent to the covered row with one of the five insecticide mixtures. The drop cloth was immediately removed and then twenty four hours post application, 183 cm x 183 cm x 183 cm field cages (no. 1406A, BioQuip®, Rancho Dominguez, CA) equipped with Lumite® screen (18 x 14 mesh size) and supported by steel cage frames (no. 1406S, BioQuip®, Rancho Dominguez, CA) were erected over one row of treated cotton and the adjacent untreated row of cotton (Fig.3.1). To alleviate potential environmental condition bias attributed to row cardinal direction position in the field cage, position of the field cages was staggered so that the treated row alternated with each application (ex. south row untreated and north row treated in one rep followed by the opposite in the next rep). Seven days after treatment (DAT) the cages were infested with 30 (>14 day old) unsexed adult stink bugs of a given species. Stink bugs were released from the center of the cages to allow the bugs to disperse equally. Stink bugs were allowed to feed within cages for 96

h, at which time they were chemically terminated using a high rate of the organophosphate acephate (Orthene® 97, Valent USA Corp., Walnut Creek, CA) at 1.172 liter/ha (16 oz/acre) tankmixed with bifenthrin at 0.440 liter/ha (6oz/acre).

Twenty four hours after termination, all cotton bolls from within the cage were manually harvested, pooled by row within cage and measured using a veneer caliper. For the first replicate only, every single boll was dissected and examined for symptoms of stink bug feeding (Toews et al. 2009); in subsequent replicates only bolls with a diameter between 1.6 and 2.3 cm were dissected and examined for symptoms of stink bug feeding. Although Extension guidelines suggest sampling bolls in the 2.3 to 2.8 cm range, the authors further restricted bolls to the 1.6 to 2.3 cm range in this study since the exposure period was only 3 days long as opposed to a 7 day scouting interval that commercial scouts follow. Following this study, a follow up study was conducted using the same methods except that the stink bugs (*N. viridula* only) were introduced 24 h after treatment.

Statistical Analyses

The experiment was organized as a two way factorial arrangement of treatments in a randomized block design. Treatments included insecticide with 4 levels and treated or untreated row with two levels; there were a total of 5 replicates each with *N. viridula* and *E. servus*. Response variables included: total number of bolls available for feeding, percent damaged bolls, and ratio of damaged bolls from the treated rows to damaged bolls from untreated rows. The latter response variable is a test for evidence of repellency. If the ratio is less than 1 it would indicate repellency from the treated plants whereas a ratio greater than 1 would indicate a preference for feeding on treated plants.

All data were analyzed by analysis of variance (ANOVA) (PROC GLIMMIX, SAS Enterprise Guide version 4.2); treatments were modeled as fixed effects while replicates were modeled as random effects. Treatment means were separated using LSMEANS test at $P \leq 0.05$ level of significance.

Field Studies

Field trials were conducted during the summers of 2013 and 2014 in irrigated commercial cotton fields to document efficacy of the selected insecticides. Trial locations were selected where a cotton field was located directly adjacent to a peanut field with the rows of cotton and peanuts running parallel. Cotton was planted in early May and managed by the grower (no foliar insecticides were applied by the grower) until bloom. The 2013-A experimental location was located near Tifton, GA (31° N 30° 10.992" 83° W 35° 4.503") and planted with 'DP 1050 B2RF.' A total of 16 plots were marked into four rows on 101.6 cm centers and 10.66 m in length with 3.04 m alleys. The 2013-B experimental location was located near Ty Ty, GA (31° N 27° 5.934" 83° W 37° 43.556"), planted with cotton cultivar 'DP1252 B2RF'. A total of 20 plots, 12.19 m in length with 3.04 m alleys, were marked into four rows with a 101.6 cm row spacing. In 2014 trials were in the same location as the 2013 study and each location had a total of 30 plots. Tifton (2014-C), was planted with cotton cultivar 'DP1252 B2RF' and Ty Ty (2014-D). Insecticide treatments followed compounds and rates shown in the caged experiment, except that an untreated control (water) was added and space limitations in at the 2013-A site prevented use of dicotophos in that trial. Treatments were arranged in a randomized block design with 4 replicates at the 2013-A and 2013-B location and 5 replicates at the 2014-C and 2014-D locations.

Experimental plots were scouted weekly starting in the second week of bloom and included an assessment of stink bug presence, plant growth stage and internal boll damage. Stink bug populations in each plot were estimated using 25 single row sweeps with a 38.1 cm sweep net. Plant growth stage was determined by the average number of nodes above white flower from three plants within each plot. Internal boll damage was determined by collecting 20 soft bolls (2.3 and 2.8 cm diameter) from two rows in each plot. Bolls were dissected and assessed for internal damage due to stink bug feeding. A boll was deemed damaged if any warts, calluses, lint discoloration or boll rot pathogens were evident. Percent internal boll damage from each plot was calculated for each field location and treatments were administered when the whole study area exceeded the extension recommended threshold. Insecticide applications were made using a Lee Spider high-clearance sprayer (LeeAgra, Inc., Lubbock, TX) equipped with a four row multi-spray boom outfitted with Conejet® TXVS 6 hollow cone nozzles calibrated to deliver 93.5 liter/ha (10 gallons/acre) at 276 kPa (40psi). In Trial 2013-A treatments were applied on 8 Aug, 15 Aug, 23 Aug, and 4 Sep. Trial 2013-B treatments were applied on 12 Aug and 16 Aug. Trial 2014-C treatments were applied on 2 Aug and 8 Aug whereas treatments were applied on 2 Aug and 12 Aug in trial 2014-D.

At the end of the growing season, plots were chemically defoliated by the grower and a hedge trimmer was used to remove the plants in the alleys. The center two rows from each plot were picked using a two row mechanized spindle cotton picker. Trials were harvested as follows: 2013-A on 19 Nov, 2013-B on 16 Oct and 2014-C and D on 20 Oct. Seedcotton was collected from plots into individual bags and seedcotton weights were taken at the time of harvest to determine yield. Seedcotton samples were ginned at

The University of Georgia's MicroGin at Tifton, GA and then subsamples (~450 g) of lint from each plot were classed at the USDA Classing office at Macon, GA to assess lint fiber quality. Lint yields by treatment were extrapolated to kg lint per hectare based on 40% average gin turnout.

The experiment was organized as a randomized complete block design. Response variables include counts of each stink bug species, percent boll injury by week, nodes above white flower (NAWF), end of season aggregate boll injury, seedcotton yield, and physical fiber properties (lint quality parameters). The data were analyzed by date using mixed model analysis (PROC GLIMIX, SAS Enterprise Guide version 4.2) and treatment means were separated using the LSMEANS at $P \leq 0.05$ level of significance.

Results

Caged Field Studies

During this two-year study, a total of 4552 cotton bolls were collected, measured and evaluated for feeding symptoms while 1950 stink bugs (1200 *N. viridula* and 750 *E. servus* adults, were used in experimental cage studies. After completion of the first replicate, the proportion of damaged bolls by boll diameter across insecticide treatments was graphed for *N. viridula* (Fig. 3.2A) and *E. servus* (Fig.3.2B). Regardless of species, boll damage increased in a significant linear curve between 1.6 and 2.8 cm in diameter. Bolls that were less than 1.6 cm in diameter received little feeding damage while damage was more inconsistent on bolls greater than 2.8 cm in diameter. Bolls 3.18 cm in diameter are no longer susceptible to stink bug feeding (Bachelier, 2009a; Green and Herzog, 2001; Emfinger et al., 2014). Across all treatments, there were no differences in the mean number of bolls available in the 1.6 to 2.3 cm boll class in either *N. viridula* (1 or 7

DAT), or *E. servus* (7 DAT) (Fig. 3.3). Similarly, there were no differences in the mean number of bolls (1.6 to 2.3 cm dia) exhibiting stink bug feeding symptoms for either 7 DAT or 1 DAT across all treatments for *N. viridula*. However, there were differences in the mean number of bolls exhibiting stink bug feeding symptoms across treatments in *E. servus*, 7 DAT (Fig. 3.4). To account for minor differences in the available bolls across treatments, the data was also analyzed as a proportion of available bolls in each field cage. This statistic was marginally not significant in the case of *N. viridula*, 1 DAT or 7 DAT but was significantly different in *E. servus* (Fig. 3.5). Finally, the potential for repellency by previously treated bolls was examined 7 DAT for both species and 1 DAT in *N. viridula*. Although these data were highly variable, there was no significant bias detected among treatments for either *N. viridula* or *E. servus* (Fig. 3.6). In comparing the DAT across treatments there was a significant interaction between the total percent of bolls damaged and the DAT ($F=3.53$; $df=4,18$; $P<0.05$) in *N. viridula*. Clothianidin 1 DAT (20.31%), and bifenthrin 1 DAT (22.79%) had significantly lower percent boll damage when compared to dicotophos at 7 DAT (37.83%), clothianidin 7 DAT (41.96%), and clothianidin + bifenthrin 7 DAT (40.87%) (Fig. 3.7).

Field Study

In trial 2013-A, species composition consisted of 56% *E. servus*, 34% *E. quadrator*, 10% *N. viridula* and 10% green *C. hilaris*. There were no differences in stink bug captures on the dates sampled among treatments (Fig. 3.8). Further, there were no differences in percent boll injury among treatments on the first, fourth and sixth sampling dates: 26 July 2013 ($F=0.23$; $df=3,7$; $P=0.86$), 15 August 2013 ($F=0.01$; $df=3,12$; $P=0.99$) and 30 August 2013 ($F=0.69$; $df=3,9$; $P=0.57$). There were differences in percent

boll injury among treatments in the remaining sampling dates (Fig. 3.9): 1 August 2013 ($F=3.01$; $df=3,12$; $P<0.05$), 8 August 2013 ($F=2.81$; $df=3,12$; $P<0.05$), 22 August 2013 ($F=4.32$; $df=3,12$; $P<0.05$), and 11 September 2013 ($F=2.66$; $df=3,12$; $P<0.05$). There were differences in seedcotton yields ($F=6.98$; $df=3,11$; $P<0.05$) among treatments as lint yield per ha in the water treatment was significantly lower than all other treatments. Fiber quality measurements including staple length, micronaire values, uniformity, and discoloration were not different among treatments (Table 3.1). However, there were detectable differences in fiber strength among treatments ($F=4.2$; $df=3,12$; $P<0.01$), which suggested that treatments suffering greater stink bug damage yielded less fiber strength.

In trial 2013-B, species composition consisted of 74% *E. servus*, 20% *E. quadrator*, and 6% *N. viridula*. There were differences in stink bugs captured on the first and second sampling dates among treatments (Fig. 3.10), but the percent boll injury among treatments by sampling dates was not different (Fig. 3.11). There were no differences among physical fiber properties (Table 3.1) and no differences in mean seedcotton weights ($F=0.32$; $df=4,14$; $P=0.86$) among treatments.

In trial 2014-C, species composition consisted of 54.2% *E. servus*, 37.5% *E. quadrator*, and 8.3% green *C. hilaris*. There were no differences in stink bugs captured on sampling dates among treatments (Fig. 3.12) and no differences in percent boll injury among treatments on sampling dates (Fig. 3.13). There were no differences in any of the physical fiber properties (Table 3.2). There were no differences in mean seedcotton weights ($F=2.02$; $df=4,19$; $P=0.13$) among treatments.

In trial 2014-D, species composition consisted of 55.3% *E. servus*, 42.3% *E. quadrator*, and 2.4% *N. viridula*. There were differences in sink bugs captured on the 3rd date sampled among treatments (Fig. 3.14). There were no significant differences in percent boll injury among treatments on any of the dates samples (Fig. 3.15); 1 Aug (F=0.32; df=4,20; *P*=0.85), 7 Aug (F=2.06; df=4,20; *P*=0.12), 14 Aug (F=1.59; df=4,20; *P*=0.21), 19 Aug (F=1.82; df=4,20; *P*=0.16). There were no differences in any of the physical fiber properties (Table 3.2). There were no differences in mean seedcotton weights (F=0.82; df=4,18; *P*=0.52) among treatments (Fig. 3.16).

Discussion

The combination of cage and field studies provided valuable data on the overall efficacy of each insecticide against *E. servus* and *N. viridula* adults. For *N. viridula*, there were no significant differences among treatments in total number of bolls or percentage of bolls exhibiting stink bug feeding symptoms 7 DAT. In addition to concluding that repellency is not an issue one week after application, one could reasonably also conclude that there is no residual activity remaining one week after application. Results with *E. servus* were similar, but there was evidence that bifenthrin treated plots experienced less stink bug feeding than bifenthrin + clothianidin treated plots one week after application.

Plots of stink bug damage by boll size confirm earlier studies that bolls with a size between 1.6 and 2.8 cm in diameter are most appropriate for estimation of feeding damage by *N. viridula* and *E. servus*. Bolls approximately 2.4 cm in diameter size are most at risk for stink bug feeding (Greene and Herzog, 1999; Green et al., 2001, 2009). A recent report on cotton boll size preference by *Halyomorpha halys* Stål showed a strong preference for feeding on very large (>3.2 cm) bolls (Kamminga et al. 2014). If *H.*

halys were to become established in cotton growing areas and scouts had to sample larger bolls, these data here suggest that sampling these very large bolls may be effective for *N. viridula*, but would underestimate damage by *E. servus*. Ironically, *E. servus* was the most common species in all four field trials.

Although there were no statistical differences detected in the preference for treated vs. untreated bolls at 1 and 7 DAT in *N. viridula* cage trials, the trend was for more feeding at 7 DAT compared with 1 DAT in the clothianidin treated plots. This was unusual because in all other treatments there were few indications of differences between intervals.

Previous experiments have shown that the first four rows of cotton planted adjacent to peanut fields are highly susceptible to stink bug infestation (Tillman et al., 2009; Toews and Shurley 2009; Olson et al., 2011). This observed phenomenon makes a cotton field adjacent to peanuts an ideal location for efficacy studies. However, it should be expected that there will be more immigration into those areas than would be expected across the entire field. For that reason, growers could expect longer stink bug suppression from any insecticide treatment than was observed in these experiments.

A key issue with the 2013 field studies was the extremely high levels of boll damage that preceded insecticide application. Due to extremely wet weather conditions, insecticides could not be applied in the 2013-A field until the mean boll damage exceeded 80%. Similarly, access to the 2013-B field was not gained until the fourth week of bloom when the percent internal boll damage ranged from 50 to 60%. Extension recommendations suggest application when the threshold exceeds 15 to 20%. These data strongly suggest that growers who are not able to spray before damage exceeds the

threshold should not expect that any of the insecticides tested will bring the damage below threshold. Similar to boll injury, in the 2013 field studies, insecticide application for the management of stink bugs did not improve physical fiber properties or cotton yield. This is congruent to past studies by Bommireddy et. al. (2007), which found no differences in yield and fiber quality among treatments to control stink bugs. The authors suggest that the extremely high levels of damage may have masked any fiber quality differences.

Cotton lint fiber quality analysis showed that in trials 2013-B, 2014-C and 2014-D all of the fiber quality parameters were not significantly different among treatments. Trial 2013-A fiber quality analysis showed that all quality parameters were not significantly different among treatments except for fiber strength. The lack of differences in fiber quality among treatments could possibly be explained by the fact that the plots were mechanically harvested as opposed to hand harvested. Therefore, bolls that were hardlocked or not fully open due to boll rot pathogens would not be picked by the harvester. Similarly, a study in 2006 showed that there were no differences in yield or physical fiber properties in plots treated with insecticides verses untreated plots (Bauer et al. 2006). However, water treatments in trial 2013-A resulted in significantly lower seedcotton weights and lint yields per ha than all other treatments indicating that excessive injury in one particularly poor treatment could reduce yield.

These data suggest different insecticides are required for different species of stink bugs. Previous field trials in both cotton and soybeans have shown that organophosphate insecticides, such as acephate, dicrotophos, and methyl parathion, provide effective control of both *N. viridula* and *E. servus* (Fitzpatrick et al, 2001; Greene and Capps,

2004; Hopkins et al., 2009; Willrich et al., 2003). Pyrethroids are effective in controlling *N. viridula*, however the susceptibility of *E. servus* varies (Hopkins et al., 2009; Snodgrass et al., 2005 Willrich et al., 2003). Overall, the reduced rate tankmix of clothianidin + bifenthrin performed just as well as bifenthrin alone. This is supported by other research that found that a neonicotinoid mixed with a pyrethroid performed up to the standards of the pyrethroid alone (Cullen et al., 2007; Hopkins et al. 2009). Takeuchi and Endo (2012) and Sugimura et al. (2007), have documented *N. viridula* recovering from initial knockdown in treatments with clothianidin.

These data confirm the importance of treatment at recommended thresholds. If pest populations well exceed the treatment thresholds than it is extremely difficult to bring stink bug populations at or below thresholds using any of the classes of insecticides used in this study. Both commercial field studies and cage studies confirm that none of the selected insecticides have any residual activity 7 DAT. In commercial studies the data showed that there was residual activity in most of the selected insecticides 5 DAT.

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Table 3.1. Effects of selected insecticides evaluated for stink bug efficacy on cotton physical fiber properties from 2013 field studies

Treatment	Length	Micronaire	Strength	Uniformity	Discoloration
2013-A Commercial Field Study: Mean Physical Fiber Properties \pm SEM					
bifenthrin	36.25 \pm 0.47	3.90 \pm 0.20	31.17 \pm 0.47 AB	80.77 \pm 0.54	8.87 \pm 0.02
clothianidin	36.50 \pm 0.50	3.47 \pm 0.18	30.45 \pm 0.61B	81.62 \pm 0.36	8.97 \pm 0.04
clothianidin + bifenthrin	37.25 \pm 0.25	4.05 \pm 0.30	32.77 \pm 0.51 A	81.55 \pm 0.69	8.90 \pm 0.14
water	36.50 \pm 0.28	3.55 \pm 0.32	30.42 \pm 0.54 B	81.50 \pm 0.59	8.90 \pm 0.14
2013-B Commercial Field Study: Mean Physical Fiber Properties \pm SEM					
dicotophos	36.75 \pm 0.47	4.37 \pm 0.07	29.65 \pm 0.646	82.95 \pm 0.45	9.00 \pm 0.07
bifenthrin	37.25 \pm 0.75	4.22 \pm 0.02	31.05 \pm 0.78	83.70 \pm 0.20	9.12 \pm 0.14
clothianidin	36.50 \pm 0.28	4.30 \pm 0.07	29.60 \pm 1.08	83.32 \pm 0.40	9.00 \pm 0.20
clothianidin + bifenthrin	37.50 \pm 0.50	4.35 \pm 0.06	30.47 \pm 0.24	83.07 \pm 0.39	9.07 \pm 0.07
water	37.50 \pm 0.28	4.32 \pm 0.13	31.30 \pm 0.47	83.12 \pm 0.31	8.42 \pm 0.62

Physical fiber quality parameters from each commercial farm study location. Means followed by common letters within columns are not significantly different ($P < 0.05$; LSMEANS).

Table 3.2. Effects of selected insecticides evaluated for stink bug efficacy on cotton physical fiber properties from 2014 field studies

Treatment	Length	Micronaire	Strength	Uniformity	Discoloration
2014-C Commercial Field Study: Mean Physical Fiber Properties \pm SEM					
bifenthrin	34.80 \pm	4.90 \pm 0.08	30.28 \pm	81.00 \pm 0.28	8.04 \pm 0.24
	0.37		0.71		
clothianidin	34.40 \pm	4.86 \pm 0.08	30.54 \pm	81.76 \pm 0.24	7.82 \pm 0.26
	0.24		0.55		
dicrotophos	34.40 \pm	4.86 \pm 0.09	28.92 \pm	81.16 \pm 0.42	7.76 \pm 0.17
	0.24		0.70		
clothianidin +	34.20 \pm	4.88 \pm 0.06	29.14 \pm	81.02 \pm 0.36	7.98 \pm 0.18
bifenthrin	0.48		0.67		
water	34.40 \pm	4.86 \pm 0.08	29.06 \pm	81.14 \pm 0.71	8.14 \pm 0.19
	0.50		0.50		
2014-D Commercial Field Study: Mean Physical Fiber Properties \pm SEM					
bifenthrin	35.80 \pm	4.94 \pm 0.04	28.78 \pm	82.68 \pm	7.60 \pm 0.11
	0.47		0.32	0.19	
clothianidin	36.20 \pm	4.96 \pm 0.02	28.28 \pm	82.46 \pm 0.20	7.70 \pm 0.03
	0.20		0.27		
dicrotophos	36.00 \pm	5.05 \pm 0.08	28.62 \pm	82.37 \pm 0.25	7.65 \pm 0.09
	0.00		0.64		
clothianidin +	36.00 \pm	5.00 \pm 0.03	28.44 \pm	82.68 \pm 0.40	7.60 \pm 0.05
bifenthrin	0.32		0.32		

water	$36.20 \pm$	4.98 ± 0.03	$28.60 \pm$	82.40 ± 0.08	7.76 ± 0.13
	0.37		0.28		

Physical fiber quality parameters from each commercial farm study location.



Fig. 3.1. Cage study testing the effects of selected insecticides on stink bug feeding preferences. Cages were placed over a treated and an untreated row of cotton.

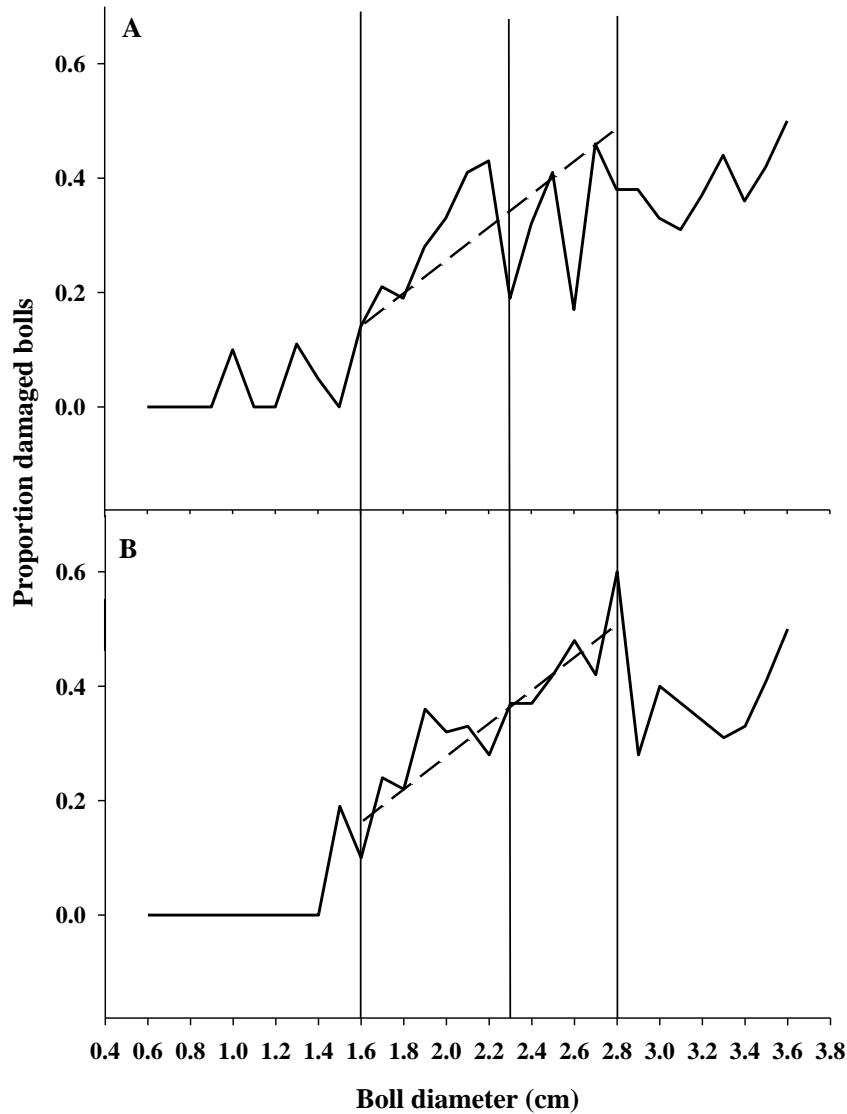


Fig. 3.2. Incidence of stink bug feeding by boll size after the first replicate in 7-DAT cage study in both *N. viridula* (A) and *E. servus* (B). Vertical lines delineate the different boll diameter classes. Bolls smaller than 1.6 cm in diameter were too small for feeding, 1.6 to 2.3 cm range were the most likely damaged during this experiment, 2.3 to 2.8 cm compose the field scouting range, and bolls larger than 2.8 cm were too large for consistent feeding pressure.

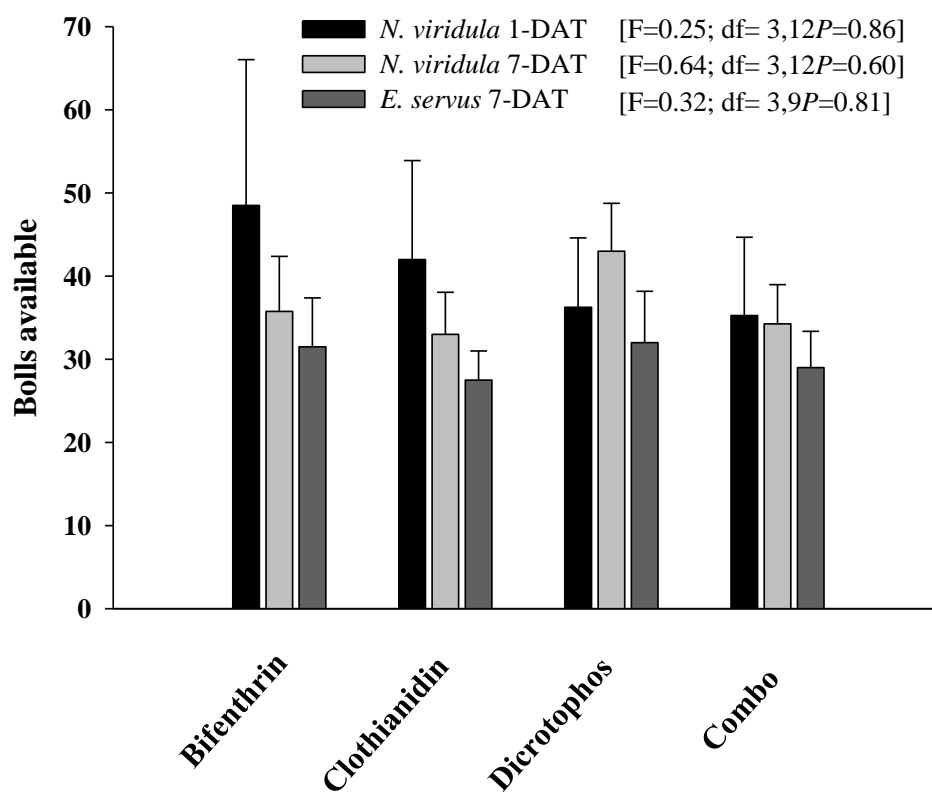


Fig. 3.3. Mean \pm SEM number of bolls available in the 1.6 to 2.3 cm diameter class by treatments in cage studies with *N. viridula* and *E. servus*.

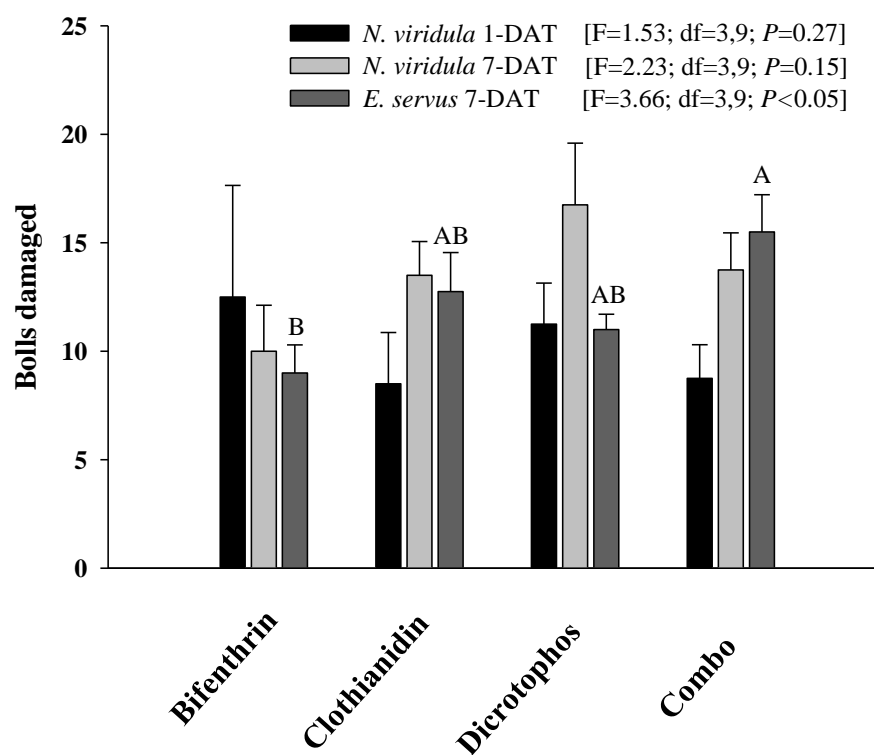


Fig. 3.4. Mean \pm SEM number of bolls damaged in the 1.6 to 2.3 cm diameter class by treatments in cage studies with *N. viridula* and *E. servus*.

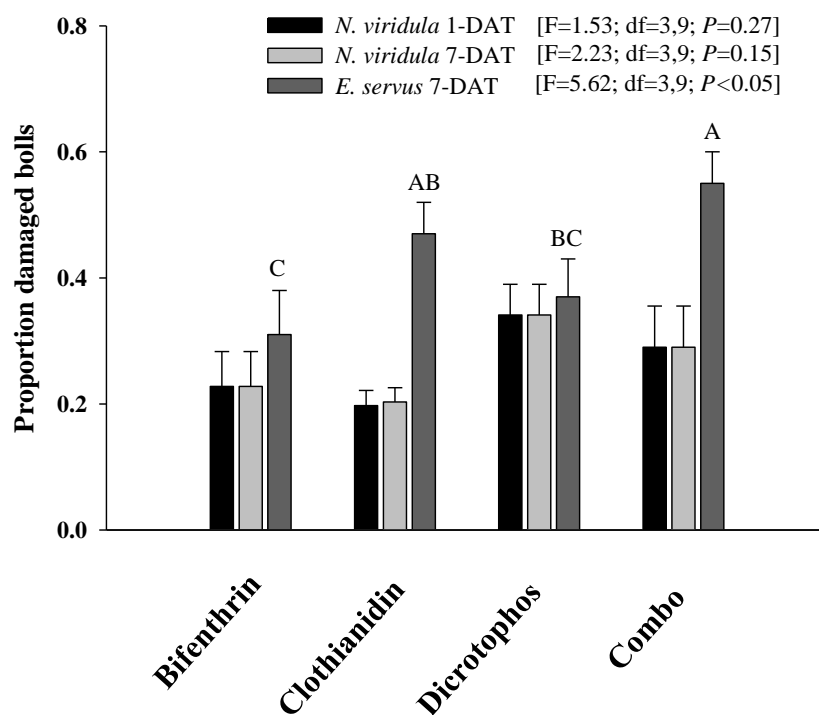


Fig. 3.5. Mean \pm SEM proportion of damaged bolls, as a function of total available bolls in the 1.6 to 2.3 cm diameter class, by treatment in both *N. viridula* and *E. servus* cage studies. Different letters above bars indicates significant differences among treatments and proportion of damaged bolls as a function of available bolls (LSMEANS, $P<0.05$).

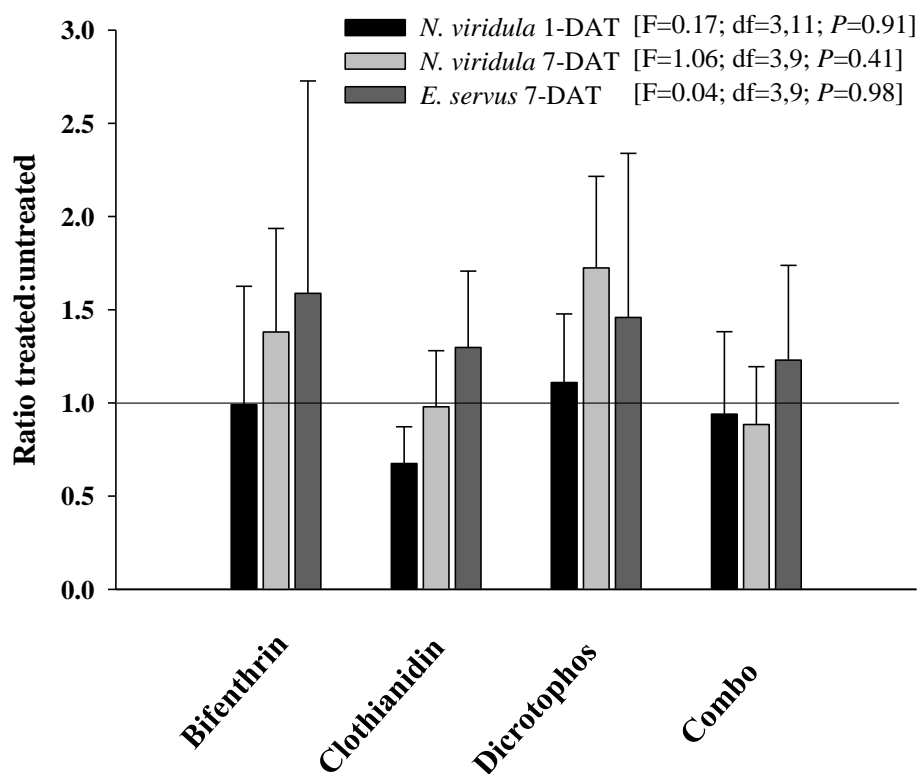


Fig. 3.6. *N. viridula* 7-DAT cage study: Ratio of damaged bolls from treated rows to damaged bolls from untreated rows. Bars greater than 1.0 suggest a feeding preference for treated bolls. Bars less than 1.0 suggest a feeding preference for untreated bolls.

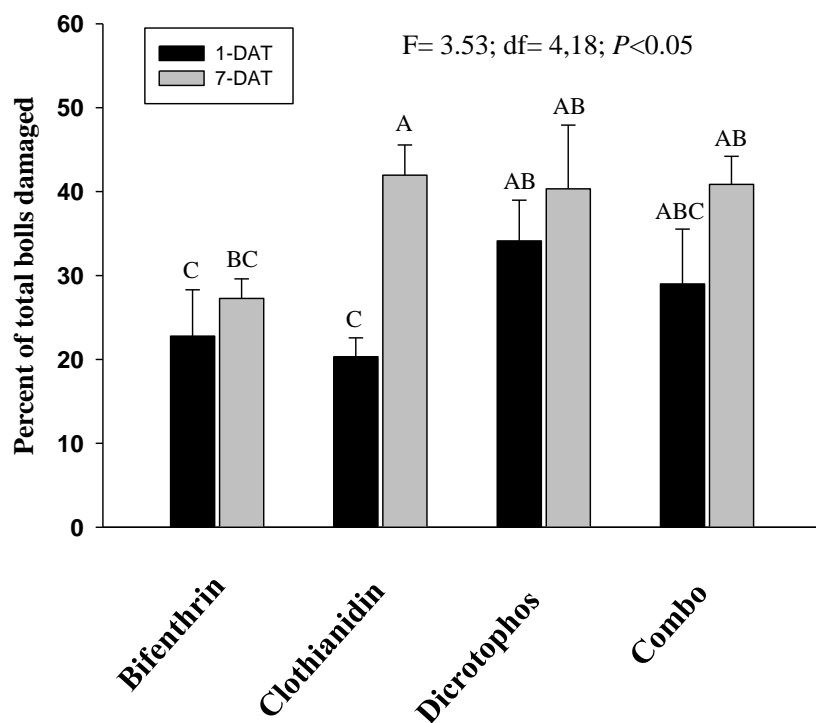


Fig. 3.7. *N. viridula* 1-DAT vs 7-DAT cage study: Percent of total bolls damaged bolls from treated and untreated rows. Different letters indicates significant differences among treatments and percent of total bolls damaged (LSMEANS, $P<0.05$).

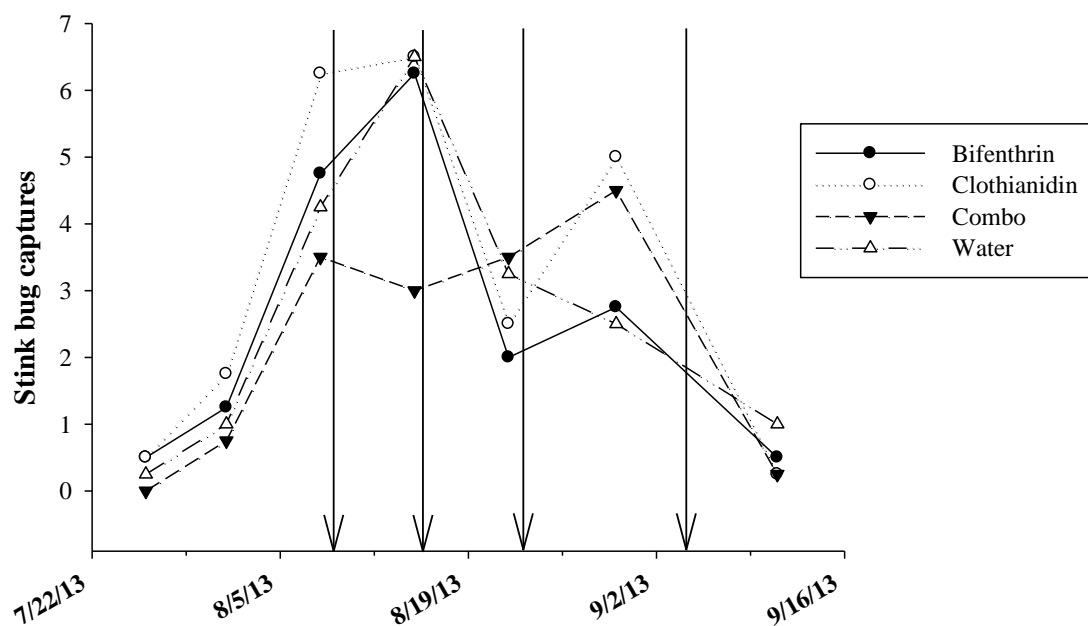


Fig. 3.8. Mean number of stink bugs per 25 sweeps captured in the 2013-A commercial field trial. Treatment application is indicated using arrows. Different letters indicates significant differences among treatments and number of stink bug captured (LSMEANS, $P < 0.05$).

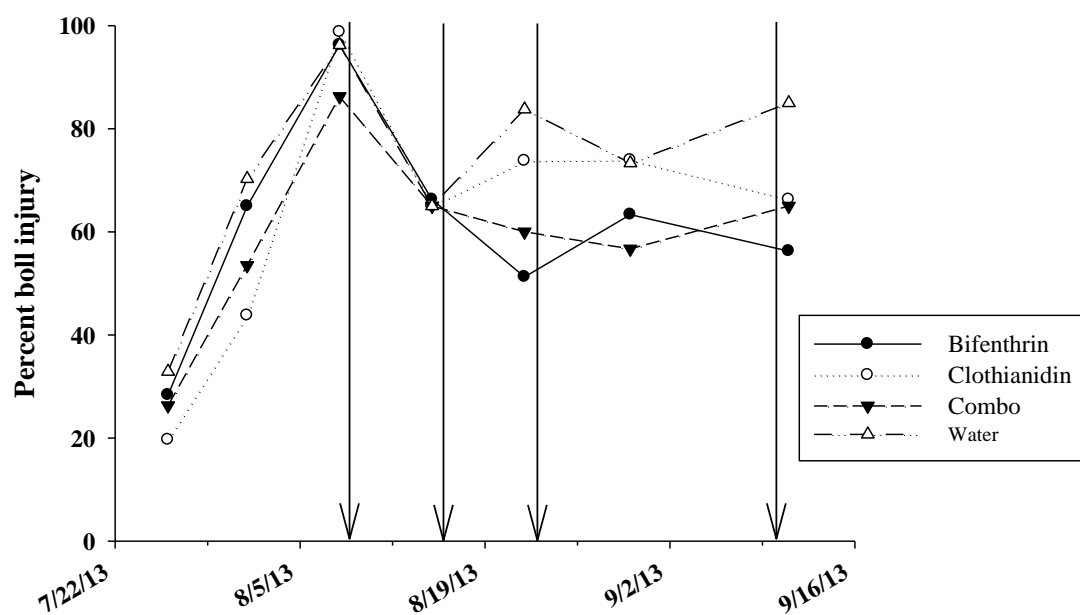


Fig. 3.9. Percent internal boll damage at the 2013-A commercial field location. Arrows indicate application of insecticides. Different letters indicates significant differences between treatments and percent internal boll (LSMEANS, $P < 0.05$).

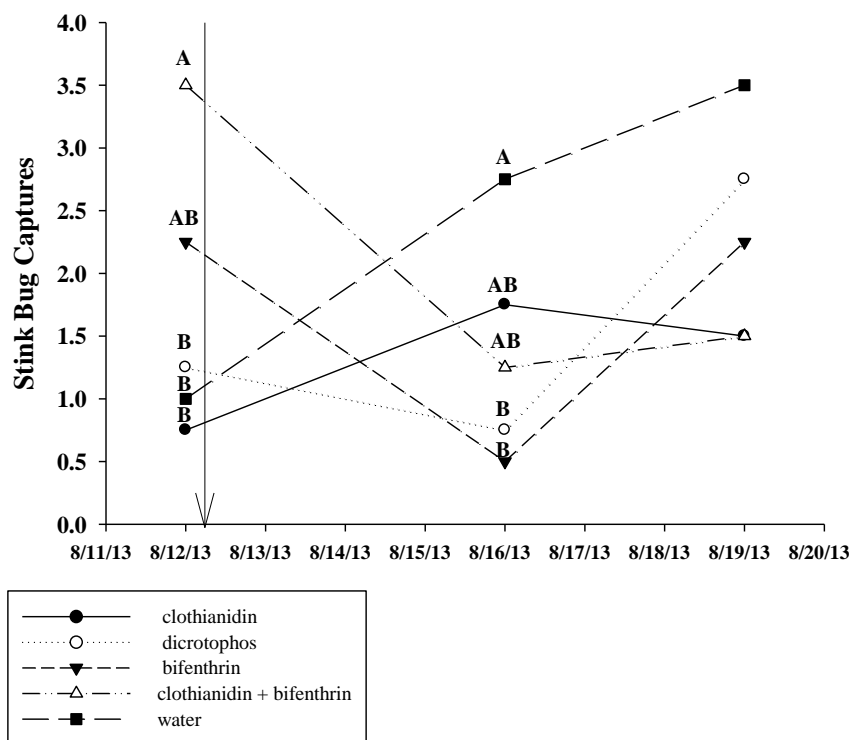


Fig. 3.10. Mean number of stink bugs per 25 sweeps captured in the 2013-B commercial field trial. Treatment application is indicated using the arrow. Different letters indicates significant differences among treatments and number of stink bug captured (LSMEANS, $P < 0.05$).

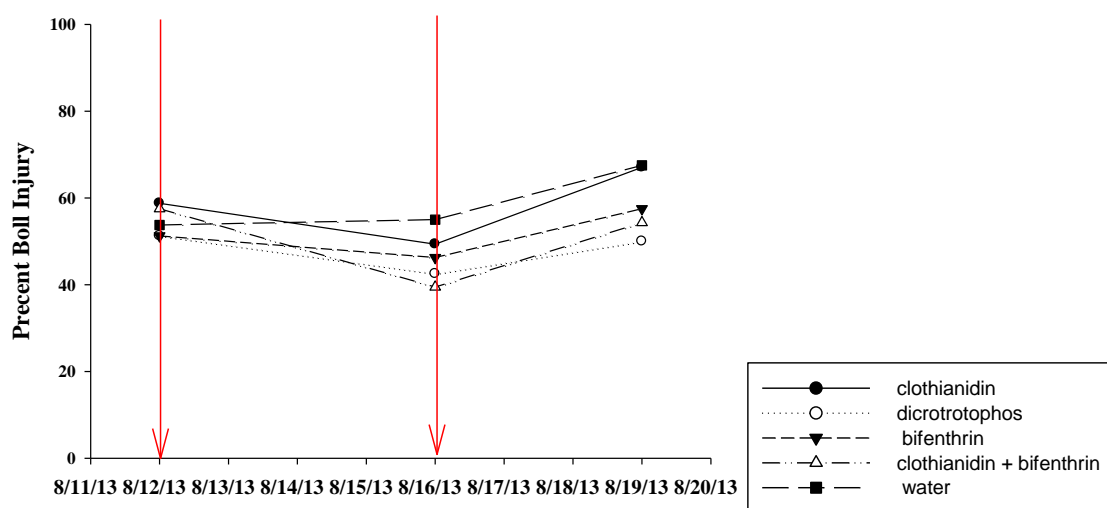


Fig. 3.11. Percent internal boll damage for the 2013-B commercial field location.

Arrows indicate application of insecticides. Different letters indicates significant differences between treatments and percent internal boll (LSMEANS, $P < 0.05$).

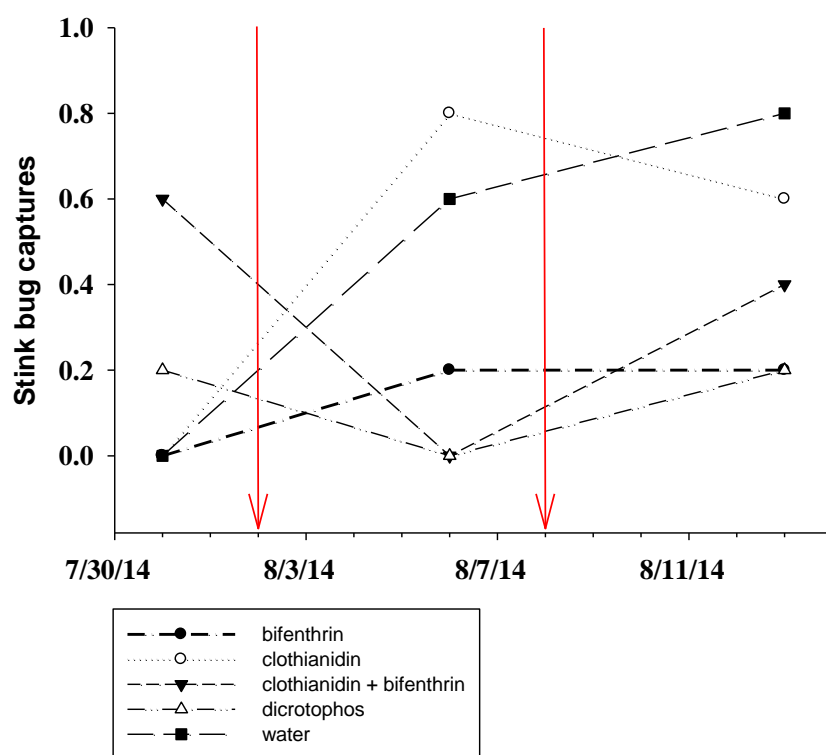


Fig. 3.12. Mean number of stink bugs per 25 sweeps captured in the 2014-C commercial field trial. Treatment application is indicated using arrows.

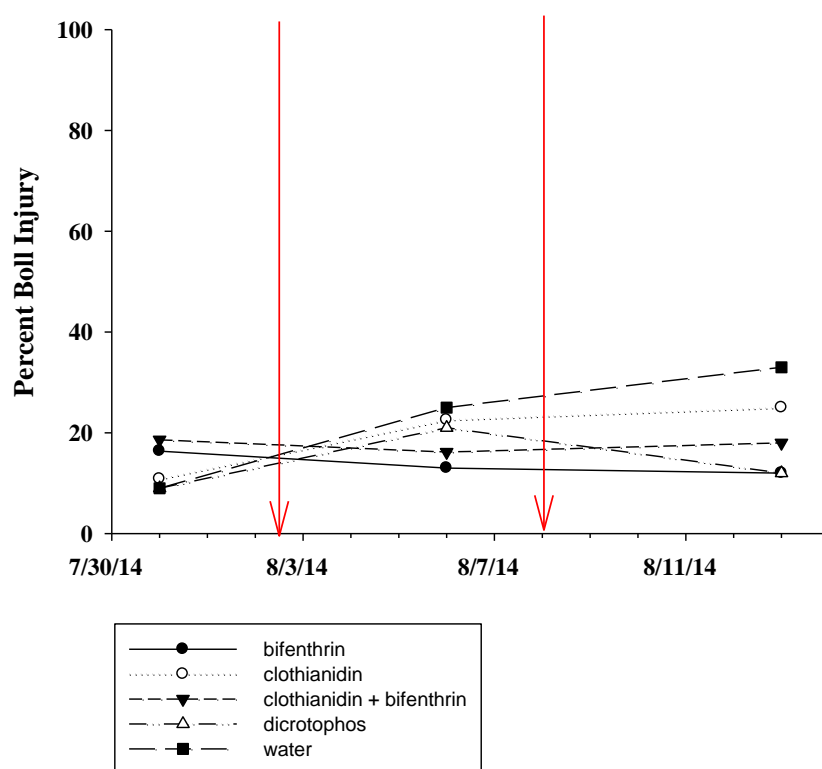


Fig. 3.13. Percent internal boll damage at the 2014-C commercial field location. Arrows indicate application of insecticides.

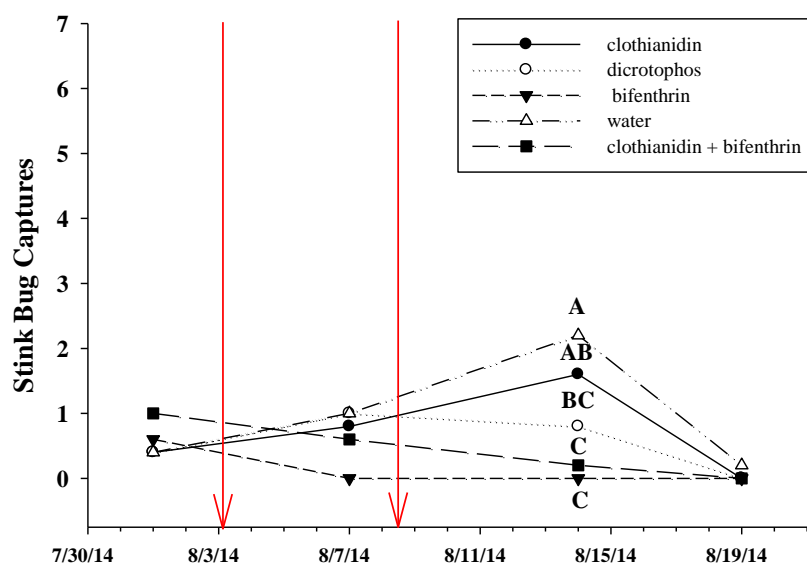


Fig. 3.14. Mean number of stink bugs per 25 sweeps captured in 2014-D commercial field trial. Treatment application is indicated using arrows. Different letters indicates significant differences between treatments and stink bug captures (LSMEANS, $P < 0.05$).

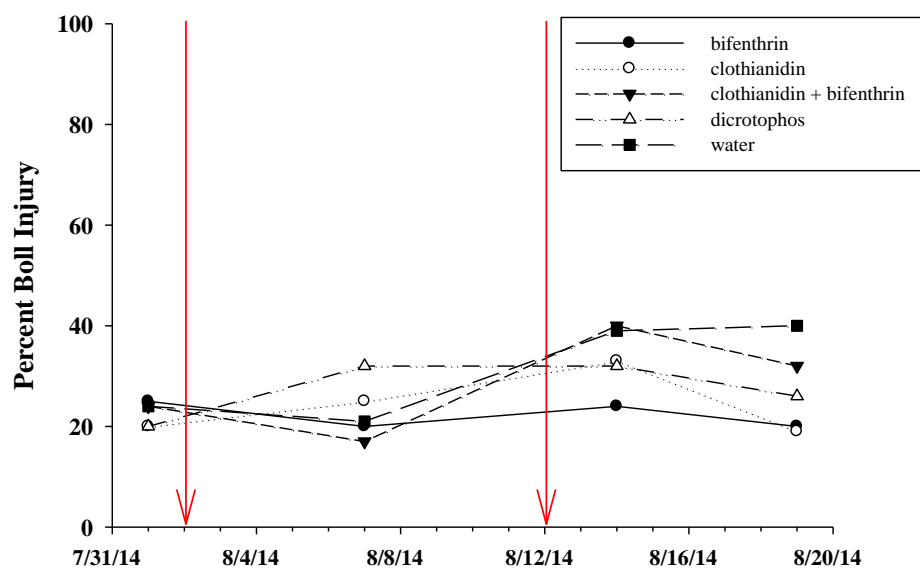


Fig. 3.15. Percent internal boll damage for the 2014-D commercial field location.

Arrows indicate application of insecticides.

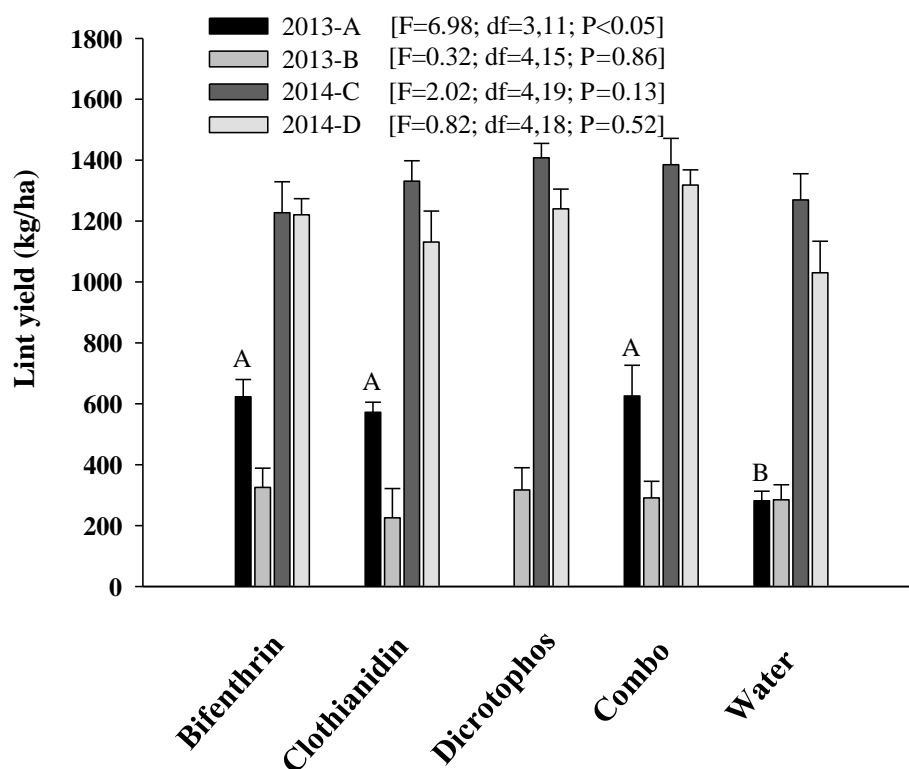


Fig. 3.16. Extrapolated mean lint yield (kg per hectare) \pm SEM by treatment in commercial field trials. Different letters indicates significant differences among treatments and number of stink bug captured (LSMEANS, $P < 0.05$).

CHAPTER 4

CONCLUSIONS

The primary investigator used laboratory bioassays, detail video analyses, field cage experiments, and trials in commercial fields to document differences in the efficacy of selected insecticides for managing stink bugs in cotton. Insecticides in interest included the neonicotinoid clothianidin, the organophosphate dicotophos, the pyrethroid bifenthrin, and a reduced rate tankmix of bifenthrin and clothianidin.

Stink bugs were assayed in the lab through treated leaf bioassays and direct application bioassays. Results demonstrated that there were differences in susceptibility among insecticide treatments by stink bug species. Video analyses of treated individuals showed that bifenthrin provided the fastest time to knockdown and the minimum amount of time spent feeding following exposure. In comparison, dicotophos was slower acting than bifenthrin, but significantly faster than clothianidin. *E. servus* adults on clothianidin treated leaves required nearly threefold longer, compared to bifenthrin, to reach knockdown; further, more than half of the test subjects recovered suggesting that clothianidin is a poor insecticide for managing this species. Direct application bioassays showed that all three stink bug species were highly susceptible to dicotophos. Although the test bugs were knocked down, >10% recovery was observed in bifenthrin and clothianidin treated individuals. These results strongly indicate that majority populations of *E. servus* in cotton fields should be managed with dicotophos.

Results from field and cage studies provided a lens whereby the laboratory results could be applied in the field. Cage studies showed that there were no differences among treatments 7 days after treatment (DAT) in the total number of bolls exhibiting stink bug feeding symptoms in *N. viridula* adults. However, there when the same assay was conducted using *E. servus* adults, there was a detectable increase in damaged bolls previously treated with a reduced rate tankmix of bifenthrin and clothianidin compared to bolls previously treated with bifenthrin. A comparison of repellency by *N. viridula* from clothianidin treated bolls between 1 DAT and 7 DAT suggested that the bugs avoided the recently treated bolls. There was no statistical evidence of this repellency in the remaining treatments including bifenthrin, dicotophos, or the reduced rate tankmix of bifenthrin + clothianidin.

Field studies demonstrated that most physical fiber properties were not affected by treatments. Possible reasons for lack of differences among treatments include: possible bias against acquisition of damaged lint by the mechanized harvester, cotton cultivar, biotic and abiotic factors, and extreme stink bug pressure across treatments due to study design. Results from commercial field studies showed that seedcotton yields were not significantly different at either location in either 2013 or 2014. These results seem to confirm the observation from the field cages that there was no residual activity in any of the selected compounds at 7 DAT.

In conclusion, this research showed that proper stink bug species identification is a prerequisite to treatment, due to the variability in susceptibility, including recovery, to different insecticide chemistries. Because boll damage that greatly exceeded the treatment threshold was difficult to reduce, these trials indicate the importance of

initiating treatment at or very close to the recommended treatment threshold. Once damage greatly exceeded the treatment threshold and populations become established, it was nearly impossible to bring the level of damage below the threshold with repeated applications of any insecticide. Finally, these data strongly support dicotophos use patterns for effective management of *E. servus*. When populations consist of *N. viridula* or *H. halys*, bifenthrin or the reduced rate tankmix of clothianidin and bifenthrin is a reasonable management strategy. Although non-target organisms were not monitored in this study, a reduced rate tankmix of clothianidin and bifenthrin may allow growers to preserve additional natural enemies beyond what would survive dicotophos or stronger rates of bifenthrin alone.