Determining residual control and systemic activity of commonly used insecticides in soybean and cotton

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Determining residual control and systemic activity of commonly used insecticides in soybean and cotton

By

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

Mississippi State, Mississippi

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Chemical control is a critical component of integrated pest management in cotton and soybean production. Residual efficacy of foliar insecticides can be highly variable and difficult to quantify due to several factors. The purpose of this research was to determine residual control and concentrations in flowering structures of commonly used insecticides. This research focused on the concentrations of active ingredient within the plant as well as efficacy over time. Previous research suggested chlorantraniliprole had a long residual and was highly lethal on corn earworm up to 28 days after treatment (DAT) in soybean; however, the results found in cotton were inconclusive. From this research, concentrations of chlorantraniliprole were found in flowering structures of both soybean and cotton up to 14 DAT. Bioassays conducted from chemical concentrations suggest reduced rates of chlorantraniliprole, similar to what was found in the flowering structures, provided mortality of corn earworm up to 64%.
DEDICATION

I dedicate this research to my loving family. Foremost, I am eternally grateful for my parents, Dale and Kim, for always being a constant throughout my life and teaching me the value of hard-work and determination. Next, my sister Kristina, and her children, Olivia and Sawyer. Thank you for being my support system in trying times. Lastly, I would like to dedication this research to my brother Ty. You were always a phone call away and willing to help at a minute’s notice. Thank you for being a big influence in my life.
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Of course, I have to offer a special thank you to Mr. Boise Stokes, who was always willing to help no matter the time and provide a good laugh when times were stressful. If there is a harder working person in this world, I have yet to find them. I would also like to thank Mrs. Beverly Catchot and Ms. Katie Huff for providing me with supplies, support, and knowledge for my research. Additionally, I would like to thank the Mississippi Soybean Promotion Board for funding this research.

I am forever appreciative of my fellow graduate students- Sara Leininger, Cade Francis, Seth Permenter, Michael Huoni, Mary Jane Lytle, Judge Fortenberry, Brett Farmer, TJ Douglas, Ryan Mann, and Tyler Towles. The colleagues and friends I have met in my time at this university will always be something I am grateful for, and I am certain we will all remain friends in the future. Lastly, thank you to all the intermittent workers for the hard work and endless hours in the field.
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CHAPTER I
INTRODUCTION

Soybean Production

Soybean, *Glycine max*, L., is an important row crop planted in several countries around the world. The United States, Brazil, Argentina, and China rank among the top producing countries totaling 299.72 million metric tons in 2018 (Soymeal 2018). The crop is prized for its protein content for livestock feed as well as its high-quality oil and is the dominant oilseed crop accounting for approximately 90% of oilseed production in the United States (USDA Economic Research Service 2021). In 2019, an estimated 30 million hectares were harvested in the U.S., and Mississippi accounted for 650,000 of those hectares. According to the Mississippi Department of Agriculture (2019), soybean ranked third in value in Mississippi behind poultry and forestry.

Growth Stages and Maturity Groups of Soybean

Soybean, a short-day annual plant that is sensitive to photoperiod, initiates flowering with a certain critical value that is specific to maturity groups (Purcell et al. 2014). Soybean is divided into maturity groups that range from 00 to VIII. These maturity groups are based on the amount of daylight needed to initiate the flowering process (Hartwig 1973). Plants can possess either a determinate or indeterminate cultivars. For the determinate growth habit, all vegetative growth is ceased at the initiation of flowering (Kogan and Turnipseed 1980). In contrast, indeterminate
soybean cultivars continue vegetative growth after flower initiation (Kogan and Turnipseed 1980).

Soybean, whether determinate or indeterminate, progresses through a set of ordered growth stages described by Fehr and Caviness (1977). Soybean undergoes vegetative (V) growth stages as well as reproductive (R) growth stages. These stages are based on the appearances of leaves (V), flowers (R), and pods (R). Soybean seeds should be planted when soil temperatures are between 27.7 and 29.4°C. A soybean plant is determined to be at vegetative emergence (VE) when cotyledons emerge from the soil (Purcell et al. 2014). Following emergence, soybean plants advance through a series of vegetative stages and reproductive stages until harvest maturity is reached (Table 1.1). The final growth stage, R8, is characterized by 95% of pods reaching a mature, brown color (Fehr and Caviness 1977).

Table 1.1 Vegetative and Reproductive Stages of Soybean

<table>
<thead>
<tr>
<th>Vegetative (V) Stages</th>
<th>Reproductive (R) Stages</th>
</tr>
</thead>
<tbody>
<tr>
<td>VE = emergence</td>
<td>R1 = beginning bloom</td>
</tr>
<tr>
<td>VC = cotyledon</td>
<td>R2 = full bloom</td>
</tr>
<tr>
<td>V1 = first node</td>
<td>R3 = beginning pod</td>
</tr>
<tr>
<td>V2 = second node</td>
<td>R4 = full pod</td>
</tr>
<tr>
<td>V3 = third node</td>
<td>R5 = beginning seed</td>
</tr>
<tr>
<td>V4 = fourth node</td>
<td>R6 = full seed</td>
</tr>
<tr>
<td>V5 = fifth node</td>
<td>R7 = beginning maturity</td>
</tr>
</tbody>
</table>

Modified from Fehr and Caviness 1977.

Pests of Soybean

Insect pests such as three-cornered alfalfa hopper, *Spissistilus festinus* (Say); bean leaf beetle, *Cerotoma trifurcate* (Forster); armyworm and stinkbug complexes, soybean looper, *Chrysodeixis includens* (Walker); and corn earworm, *Helicoverpa zea* (Boddie), cause significant impacts on mid-south soybean growers’ profitability (Musser et al. 2018). The seed-feeding stink
bug complex comprising of *Euschistus servus* (Say), *Nezara viridula* (L.), and *Chinavia hilare* (Say), continues to be the most economically damaging soybean pests in the mid-south (Musser et al. 2020).

**Stink Bug**

Brown stink bug, southern green stink bug, and green stink bug comprise the majority of the stink bug complex in the southern United States (Turnipseed and Kogan 1976). A survey by McPherson et al. (1993) conducted in Georgia found these three species comprised 98% of all stink bugs found in soybean. However, this was prior to the establishment of redbanded stink bug, *Piezodorus guildinii* (Westwood), in the southern U.S. Stink bugs feed by piercing plant tissue and removing fluids from the plant (McPherson and McPherson 2000c). Feeding damages plant tissues which may result in abortion of fruit, or pathogen transmission (Panizzi et al. 2000). Damaged pod hulls due to stink bug feeding may leave the seed exposed to pathogens that can ultimately reduce yield and grain quality (Russin et al. 1988). In addition to the stink bug complex, redbanded stink bug can also cause significant economic losses in soybean (Musser et al. 2018).

The redbanded stink bug is one of the most encountered stink bug pests in Brazil after replacing southern green stink bug in the 1970’s (Turnipseed and Kogan 1976). The redbanded stink bug was first reported in the United States in the early 1970s, but the time of its arrival in North America is still unclear (Panizzi 2004). As of 2013, the redbanded stink bug has been recorded in several southern states including Alabama, Florida, Louisiana, Mississippi, New Mexico, South Carolina, and Texas (Temple et al. 2013a). Like the traditional stink bug complex, feeding by redbanded stink bug can damage plant tissue and leave seed exposed to pathogens reducing the quality of grain (Panizzi et al. 2000).
The stink bug complex was the most economically damaging soybean pest for 17 reporting states in 2019 infesting over 85 million hectares with 22 million of those hectares warranting an insecticide application (Musser et al. 2020). In Mississippi, green stink bug, southern green stink bug, and brown stink bug collectively totaled 88% of all stink bug species in 2019 while infesting 98% of planted soybean hectares (Musser et al. 2020). In 2017, the stink bug complex caused more economic damage than any other year reported with 65% of stink bugs reported being redbanded stink bug in Mississippi (Musser et al. 2018). This pest accounted for the highest cost + yield loss compared to other pests in Mississippi soybean in 2017 (Musser et al. 2018).

**Corn Earworm**

Corn earworm is a lepidopteran insect pest that is polyphagous and feeds on many food and oil crops including soybean (Fitt 1989). Corn earworm is known to feed on more than 100 different host plants (King and Coleman 1989). In recent years, corn earworm has been a primary pest of soybean in Mississippi requiring frequent insecticide applications and causing significant economic damage (Musser et al. 2020). Larval feeding can decrease yield by reducing surface area of the leaf, delaying pod fill, and lessening the number of seeds per pod (Eckel et al. 1992b). However, the greatest economic loss is due to pod feeding (Eckel et al. 1992b). Open canopied soybean fields are most attractive to corn earworms (Alston et al. 1991). Early instar larvae (1-3) can normally be located on new foliage, while later instar larvae (4-6) prefer older vegetation to feed on (Eckel et al. 1992a). Soybean is most desirable for corn earworm oviposition throughout the R1 and R3 growth stages when soybeans are flowering (Johnson et al. 1975). However, infestations at R4 and R5 growth stages may be common in some areas (McPherson and Moss 1989). Feeding can be most economically damaging during the R3-R4...
growth stages because a greater number of small pods and undeveloped seeds can be consumed per larva, compared with larval feeding in the later growth stages (R5-R6) when larger pods are present (McWilliams 1983, Swenson et al. 2013).

Corn earworm infested over 2 million hectares of soybean in the 17 reporting states in the United States (Musser et al. 2020). In these reporting states, 7.5% of those hectares were above economic threshold (Musser et al. 2020). Corn earworm was the second most damaging pest in 2019 behind the seed-feeding stink bug complex in Mississippi grown soybean (Musser et al. 2020). In 2019, corn earworm was found in over 200,000 out of the 650,000 hectares of the soybeans planted in Mississippi (Musser et al. 2020). This pest accounted for 1.2% overall yield reduction in Mississippi soybeans alone in 2019 (Musser et al. 2020).

**Chemical Control in Soybean**

Several insects cause a reduction in yield and grain quality in soybean on a year-to-year basis. Chemical control with foliar applied insecticides is one of the primary methods used to combat economic injury from these pests. In 2019, Mississippi growers spent over $41 million on foliar insecticides and averaged 2.2 applications in soybean (Musser et al. 2020). Resistance development and inconsistent efficacy of several common insecticides classes is well documented (Sparks 1981, Brown et al. 1998, Jacobson et al. 2009).

**Management of Stink Bug**

The stink bug complex was the primary soybean pest in Mississippi in 2019 (Musser et al. 2020). Compared to redbanded stink bug, the stinkbug complex is more susceptible to insecticides commonly used in soybean. According to a study by Temple et al. (2013b), southern green stinkbug was very sensitive to currently recommended insecticides including acephate,
bifenthrin, cyfluthrin, cypermethrin, and lambda-cyhalothrin in vial-treated assays. Brown stinkbugs are generally more difficult to control with commonly used insecticides compared to green and southern green stink bug. Results from a study by Snodgrass et al. (2005) confirmed that the brown stink bug was less susceptible to pyrethroid and organophosphate insecticides compared to green and southern green stink bugs. Insecticides commonly recommended for control of the stinkbug complex include, pyrethroids (bifenthrin, cypermethrin, lambda-cyhalothrin, esfenvalerate) and neonicotinoids (imidacloprid, thiamethoxam) (Crow et al. 2021).

Studies conducted in Argentina suggests redbanded stink bug is more damaging to a soybean crop than other stink bug pest species common in South America (Vicentini and Jimenez 1977). Redbanded stink bug is inherently less susceptible to insecticides commonly used in controlling the brown, green, and southern green stink bug. Although no resistance to insecticides has been documented in redbanded stink bug, the pest’s high mobility makes it complex and difficult to control with a foliar application. Insecticides currently recommended in Mississippi soybean production for controlling redbanded stink bug include pyrethroids (bifenthrin, lambda-cyhalothrin), organophosphates (acephate) and neonicotinoids (clothianidin, imidacloprid, thiamethoxam) (Crow et al. 2021). These insecticides provide suppression of redbanded stink bugs when applied alone, but tank-mixing single active ingredients in combination with bifenthrin are often recommended to provide effective control (Crow et al. 2021).

Management of Corn Earworm

Corn earworm resistance to organophosphate, cyclodiene, organophosphate, carbamate, and pyrethroid classes of insecticides has been documented (Sparks 1981, Stadelbach et al. 1990, Brickle et al. 2001, Jacobson et al. 2009, Plapp 1971). Pyrethroid insecticides were the standard
control of corn earworm for many years in Mississippi, but they are no longer recommended due to resistant populations (Musser et. al 2010, Crow et al. 2021). More options are available for control of this pest in soybean than in cotton. Commonly recommended insecticidal control strategies for corn earworm are spinosyns (spinetoram, spinosad), insect growth regulators + spinosyns (methoxyfenozide + spinetoram), and diamides (chlorantraniliprole) (Crow et al. 2021).

Cotton

Cotton Production

Upland cotton, *Gossypium hirsutum* (L.), accounts for 97% of the United States cotton production with the remaining 3% grown in Pima cotton, *Gossypium barbadense* (L.). The United States ranks third in cotton producing countries behind India and China (USDA FAS 2022). In 2019, 4.7 million hectares of cotton were harvested in the United States valued at $6 billion dollars (USDA FAS 2022). In Mississippi, cotton ranked as the fourth most valuable agricultural commodity in 2019 valued at $585 million (Mississippi Department of Agriculture 2019). Mississippi ranked third in cotton producing states in 2019 behind Texas and Georgia.

Cotton Growth and Development

Forty different species of cotton are available worldwide, but only four species are of economic importance- *G. arboretum* (L.), and *G. herbaceum* (L.), *G.hirsutum* (L.), and *G. barbadense* (L.) (YARA World Cotton Production 2015). Upland cotton is an important fiber and oilseed crop grown in the United States. Cotton is botanically a perennial shrub, but through years of successful breeding and management, it is grown as a pseudo-annual shrub. In early growth stages, cotton appears to develop slowly above ground, but root growth is rapid. Colder
temperatures and poor environmental conditions can delay the growing process of cotton (Ritchie et al. 2004). Growth stages for cotton are monitored in several ways: plant height, total number of nodes, fruiting structure formation, nodes above white flower (NAWF), and days after planting. Heat units, or DD$_{60}$, can be a consistent and predictable measurement for predicting growth and development in cotton (Oosterhuis 1990). Daily heat units can be calculated by adding daytime high and low temperatures, diving the sum by two, and subtracting the base temperature, 15.5°C (Oosterhuis 1990) (Table 1.2).

Table 1.2  Cotton Development Stages

<table>
<thead>
<tr>
<th>Growth Stage</th>
<th>Days</th>
<th>Heat Units-DD$_{60}$s</th>
</tr>
</thead>
<tbody>
<tr>
<td>Planting to Emergence</td>
<td>4 to 9</td>
<td>50 to 60</td>
</tr>
<tr>
<td>Emergence to First Square</td>
<td>27 to 38</td>
<td>425 to 475</td>
</tr>
<tr>
<td>Square to Flower</td>
<td>20 to 25</td>
<td>300 to 350</td>
</tr>
<tr>
<td>Planting to First Flower</td>
<td>60 to 70</td>
<td>775 to 850</td>
</tr>
<tr>
<td>Flower to Open Boll</td>
<td>45 to 65</td>
<td>850 to 950</td>
</tr>
<tr>
<td>Planting to Harvest Ready</td>
<td>130 to 160</td>
<td>2200 to 2600</td>
</tr>
</tbody>
</table>


Cotton requires 2,600 heat units from planting to harvest to reach full maturity, which is normally 130-160 days (Oosterhuis 1990). Emergence and unfolding of cotyledons occur 4-14 days after planting (Robertson et al. 2007). Reproductive stages generally occur within 40 days after planting, and the first square will appear (Ritchie et al. 2014). Appearance of a white flower occurs around 65 days after planting (Oosterhuis 1990). The flowering period generally last about 6-7 weeks depending on environmental conditions (Mauney 2012). In the mid-south, the flowering period can last up to eight weeks under favorable climate. This long flowering period prolongs the attractiveness of insect pests to the crop, therefore effective management strategies are recommended in order to avoid delaying maturity and to maximize yield.
Pests of Cotton

Throughout the growing season, cotton is intensely scouted and managed for insect pests. During the seedling stage, cotton is susceptible to thrips species including tobacco thrips, *Frankliniella fusca* (Hinds); flower thrips, *Frankliniella tritici* (Fitch); and western flower thrips *Frankliniella occidentalis* (Pergande) (Albeldano et al. 2008). Mid to late season pests of cotton produced in the mid-south include twospotted spider mite, *Tetranychus urticae* (Koch); cotton aphid, *Aphis gossypii* (Glover); corn earworm, *Helioconverpa zea* (Boddie); and tobacco budworm, *Heliothis verescens* (Fab). An additional mid to late season pest, tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), is the most economically damaging insect pest of cotton in the mid-south causing significant yield loss every year (Cook and Threet 2020).

Tarnished Plant Bug

The tarnished plant bug is an economically important insect pest of cotton grown in the mid-south. A member of the Miridae (Hemiptera) family, tarnished plant bug is classified as a polyphagous species (Triplehorn and Johnson 2005). The tarnished plant bug has over 700 wild and cultivated host plants and can be found across the United States and into Canada (Young 1986). Under normal environmental conditions, the life cycle of a tarnished plant bug typically lasts 30-40 days (Fleischer and Gaylor 1988). The tarnished plant bug is one of several plant bug species that feeds on cotton in the U.S. and has the widest host range of any of the other species (Young 1986).

Tarnished plant bug damage can appear as early as emergence, with most of the economic damage occurring from first square into the early flowering stages (Scales and Furr 1968). Damage from tarnished plant bug can reduce yield by aborted terminals, deformed leaves, delayed fruiting, and square loss (Hanny et al. 1977). Infestations that occur later into flower
stage exceeding economic threshold can also cause abscission of older squares, but generally the larger squares remain attached. Damage to the cotton flowers is determined by dark brown anthers termed “dirty blooms.” Although, little to no yield loss is attributed to discolored blooms until 30% of the anthers are damaged (Pack and Tugwell 1976).

Due to the risk of tarnished plant bug damage throughout the growing season, foliar insecticides are often used to control this pest in cotton. In the Mississippi Delta, 99% of cotton hectares were treated for this pest in 2020 (Cook and Threet 2020). This region averaged four foliar applications totaling near $18 per hectare cost of control and accounted for a 4% loss in overall yield (Cook and Threet 2020).

**Corn Earworm**

Corn earworm is an economically important lepidopteran pest and is often referred to as bollworm when feeding in cotton. It is a polyphagous species that feeds on several important crops causing significant economic loss. Over 100 plant species have been documented to host bollworm (King and Coleman 1989). Pearly-white colored eggs are laid individually on cotton plants and take about three to four days to hatch (King and Coleman 1989). Upon hatching, younger larvae feed on smaller fruiting structures or anthers near the oviposition site (Reese et al. 1981). Small bolls, fresh flowers and wilting flower corollas (bloom tags) are preferred feeding structures of bollworm larvae (Farrar and Bradley 1985). Severe damage can cause abscission of fruiting structures (Gore et al. 2000). Older bollworm larvae (> third instar) prefer feeding on bolls as opposed to younger fruiting structures (Wilson and Gutierrez 1980).

Corn earworm infested 2.6 million hectares or 50% of U.S. planted cotton in 2019 with 900,000 of those hectares being treated with an insecticide (Cook and Threet 2019). In Mississippi alone, 93% of cotton hectares were infested and 85% of the hectares requiring an
insecticidal treatment in 2019 (Cook and Threet 2019). Corn earworm was the second most economically damaging pest in Mississippi cotton in 2019 behind tarnished plant bug (Cook and Threet 2019). In Mississippi, 1.7 average foliar insecticide applications were needed for corn earworm (Cook and Threet 2019). There were approximately 46,000 U.S. bales lost to bollworm feeding alone in Mississippi in 2019 (Cook and Threet 2019).

**Chemical Control in Cotton**

Numerous pests can infest cotton fields in the mid-south and cause economic loss. Foliar applied insecticides are a very important part of an integrated pest management approach to controlling pests in cotton. In 2019, cotton growers in Mississippi spent over $58 million on foliar insecticides averaging over six applications (Cook and Threet 2019). Resistance development in insects and inconsistencies of common cotton insecticides have been well documented (Sparks 1981, Brown et al. 1998, Jacobson et al. 2009). Therefore, newer chemistries and rotating insecticide classes is needed for resistance management.

**Management of Tarnished Plant Bug**

The adoption of Bt (*Bacillus thuringiensis*) cotton has contributed to a decline of insecticide applications targeting caterpillar pest that consequently controlled non-caterpillar pest such as tarnished plant bug (Beenbrook 2012). The reduction in pesticide applications due to Bt technology and completion of the of the boll weevil (*Anthonomus grandis*) eradication program has allowed tarnished plant bug to be the primary insect pest in cotton in Mississippi. Populations of tarnished plant bugs have become resistant to organophosphate and pyrethroid classes of insecticides (Snodgrass 1996, Zhu and Snodgrass 2003, Zhu et al. 2004, Dorman et al. 2020). Mississippi current recommendations include organophosphates (acephate, dicrotophos,
diamethoate), pyrethroids (bifenthrin), neonicotinoids (acetamiprid, imidacloprid, thiamethoxam), carbamates (oxamyl), sulfoximines (sulfoxaflor), and insect growth regulators (novaluron) (Crow et al. 2021). Rotating insecticide classes with differing modes of action is recommended in Mississippi to help avoid further tarnished plant bug resistance issues (Crow et al. 2021).

Management of Corn Earworm

Corn earworm is second only to tarnished plant bug in terms of economic loss in Mississippi-grown cotton. Transgenic Bt cotton is the primary source of control of bollworm in cotton, but due to resistance issues, supplemental control is often needed in the form of a foliar insecticide. Insecticide resistance issues and commonly used chemical control methods previously mentioned for bollworm as a pest in soybean are also applicable in cotton. Products containing chlorantraniliprole are often recommended as supplemental control of bollworm in cotton when damaged fruiting structures are present in the field (Crow et al. 2021).

Residual and Systemic Activity of Insecticides

Insecticides can be classified as either a contact or a residual insecticide. Contact insecticides have little to no residual activity and are only effective if the insect is directly contacted with the insecticide (Potts and Vanderplank 1945). Pyrethroids, one of the most common chemical classes in the world, are characterized by high knockdown and lethal activity, but a short residual activity (Hirano 1989). Organophosphates such as acephate readily decompose when exposed to ultraviolet light ultimately reducing the insecticide’s already low residual activity (Szeto 1978). Residual insecticides remain effective on plant tissue for some period of time, but generally residual decreases over time as compounds degrade and dilute
through the plant (Bennett 1957, Schmidt-Jeffries and Nault 2017). Many of the newer products commonly used offer longer residual control compared to older chemistries. Selection of an appropriate insecticide is often largely dependent on the chemical’s efficacy and residual activity. Multiple applications every four to five days is often recommended to control high population of tarnished plant bug in the mid-south (Crow et al. 2021). Often higher application rates of insecticides are recommended to achieve a longer residual life (Crow et al. 2021).

Novaluron, an insect growth regulator targeting tarnished plant bug, was limitedly affected by a rainfall event suggesting the insecticide could provide effective control over time despite rainy conditions (Barrett et al. 2021). Determining residual life of popular insecticides against a variety of pest in both soybean and cotton would be beneficial in making pest management decisions.

Systemic uptake and translocation of active ingredient inside the plant has been observed in newer insecticide classes such as neonicotinoids and diamides as well as older chemistries such as organophosphates (Adams et al. 2016, Simon-Delso et al. 2015, Lahm et al. 2007).

Neonicotinoid pesticides have become the most widely adopted class of chemistries in the world, and the success of this chemistry is arguably due to its systemic nature (Simon-Delso et al. 2015). In 2008, the diamide insecticide, chlorantraniliprole, was introduced to market. When soil applied, the insecticide can be taken up by the root system and move upwards in the plant via the xylem (Cameron et al. 2015). Based on research by Adams et al. (2016), chlorantraniliprole, when foliar applied, moved systemically to vegetative structures of soybean, but no concentrations were found in reproductive structures. Adams et al. (2016) conducted bioassays targeting corn earworm in soybean and discovered when soybeans are infested at R1 to R3 the systemic efficacy of chlorantraniliprole can provide some control in the foliage. According to field bioassays conducted by Babu et al. (2021), chlorantraniliprole had lethal and sublethal
effects on corn earworm feeding on fresh cotton leaf tissue for up to 22 days, which was the last time period tested. No research has been conducted on activity of chlorantraniliprole in fresh flowering structures of cotton or soybean.

**Justification**

The use of insecticides for crop protection emphasizes effectiveness against target pests, effects on beneficial insects, and long residuals to limit pesticide applications. Residual efficacy and systemic activity of foliar insecticides can be highly variable and difficult to quantify due to several factors such as application method, coverage, pest pressure, insecticide degradation, and rainfastness. The purpose of this research was to determine if several commonly used insecticides in Mississippi cotton and soybean production systems had any residual activity, while also determining if chlorantraniliprole was detected in fresh flowering structures.
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CHAPTER II
DETERMINING RESIDUAL CONTROL AND CONCENTRATIONS OF
CHLORANTRANILIPROLE IN SOYBEAN LEAVES AND FLORETS

Abstract
Studies were conducted in 2020 and 2021 at the Delta Research and Extension Center in Stoneville, MS to determine concentrations of chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA) in soybean leaves and florets. Chlorantraniliprole was applied as a foliar spray at four rates (0.028, 0.053, 0.078, 0.103 kg ai ha\(^{-1}\)) for leaves and two rates (0.053, 0.078 kg ai ha\(^{-1}\)) for florets. Leaf bioassays with corn earworm, Helicoverpa zea (Boddie), were conducted concurrently to determine mortality within three plant zones to evaluate chlorantraniliprole distribution throughout the canopy. For the leaf study, plants were partitioned into three zones consisting of a top (18\(^{th}\) node), middle (13\(^{th}\) node), and bottom (9\(^{th}\) node) zone. Leaf samples from each zone were analyzed for chemical concentrations and bioassays were conducted at 1, 7, 14, 21, and 28 days after treatment (DAT). Floret samples were analyzed at 4, 7, 10, and 14 DAT. Concentrations of chlorantraniliprole, though variable, provided >71% control through all sampling dates, application rates, and canopy zones tested.

Chlorantraniliprole was viable up to 28 DAT. Results from the soybean floret study suggested chlorantraniliprole was detected in florets up to 14 DAT. An additional leaf bioassay was conducted using concentrations detected in the floret study. Concentrations in florets provided mortality of corn earworm up to 48% out to 14 DAT. With a long residual expected,
chlorantraniliprole applications should continue to be used to control corn earworm infestations in soybean and some additional control could be expected in florets.

**Introduction**

Corn earworm, *Helicoverpa zea* (Boddie), is a detrimental pest of mid-south soybean if left uncontrolled (Musser et al. 2021). In 2020, corn earworm was the third most economically important soybean pest in Mississippi behind stink bugs (Hemiptera: Pentatomidae) and soybean looper (*Chrysodeixis includens*, Walker) in terms of yield loss plus cost of control (Musser et al. 2021). Open canopied soybeans that are between the R1 and R3 growth stage are most attractive for corn earworm oviposition (Eckel 1992a). Infestations at the R4 and R5 growth stages may also occur and are common in some regions (Eckel 1992a). Larval feeding can limit yield potential by reducing leaf surface area and negatively impacting photosynthetic processes in the plant (Eckel et al. 1992b). Pod feeding can delay pod fill and reduce the number of seeds per pod resulting in the greatest economic loss (Eckel et al. 1992b). Feeding at R3 to R4 on numerous small, undeveloped seeds may impact yield (McWilliams 1983, Swenson et al. 2013). A simulated corn earworm injury study found significant yield losses were not observed until fruit was removed at the R5 growth stage (Coelho et al. 2020). Cultural control such as early planting is often implemented to reduce the risk of corn earworm pressure. In addition to cultural control, foliar insecticides are the most common strategy to manage corn earworm infestations.

Synthetic insecticides have been widely adopted for corn earworm control, but resistance issues have been well documented in cyclodienes, carbamates, organophosphates, and pyrethroids (Plapp 1971, Sparks 1981, Stadelbach et al. 1990). Chlorantraniliprole, a diamide insecticide with a novel mode of action, was released in 2008 and is highly toxic to corn earworm and other lepidopteran species (Temple et al. 2009, Hardke et al. 2011, Adams et al.
Research conducted in soybean, cotton, and brassica species has shown potential for chlorantraniliprole to move systemically and provide a long residual control (Lahm et al. 2007, Cameron et al. 2015, Adams et al. 2016, Babu et al. 2021). Furthermore, in cabbage, soil applied chlorantraniliprole moved systemically in the xylem and provided control of lepidopteran pests in the foliage (Cameron et al. 2015). Previous research using fresh tissue bioassays in soybean suggested that chlorantraniliprole applied to foliage translocated to vegetative structures but not the reproductive structures (Adams et al. 2016). When soybeans were infested at R1 to R3 growth stages the systemic efficacy of chlorantraniliprole provided some control of corn earworm in fresh leaf tissue (Adams et al. 2016). Additionally, the greatest mortality was recorded when chlorantraniliprole was applied to the whole plant or just stems (Adams et al. 2016). Limited research has been conducted on systemic activity and residual control of chlorantraniliprole in soybean. The objectives of this study were to determine residual concentrations of chlorantraniliprole and mortality of corn earworm in soybean leaves throughout the canopy, as well as the lethal concentrations in florets.

**Materials and Methods**

**Field Experiment Details**

Three hybrid lab-field studies were conducted at the Delta Research and Extension Center in Stoneville, MS in 2020 and 2021. The field experiments utilizing chlorantraniliprole (Prevathon®; FMC Corporation, Philadelphia, PA) consisted of a soybean leaf study with standard rates, a soybean floret study, and a soybean leaf study with reduced rates. Field based experiments were arranged as a randomized complete block design with four replications. Soybean variety (Asgrow 46x6®, Bayer CropScience, St. Louis, Mo) was planted at 294,600 seed ha⁻¹ into conventionally tilled raised beds between 5 May and 12 June in 2020 and 2021.
Plots were 6 rows wide with 1.02 m row spacing and 12.2 m long. 3.04 m long fallow alleys separated the replications. All standard production practices were made according to Mississippi State University Extension Service recommendations, and insects were managed based on economic thresholds (Crow et al. 2021) using insecticides without lepidopteran activity. The center four rows of each plot were sprayed to minimize drift between plots. Canopeo (Mathworks, Inc., Natick MA), a mobile device application developed to measure percent canopy coverage, was used to determine 64 to 75% canopy coverage in plots one day prior to application. Gloves were worn and changed between plots to reduce contamination when handling sprayed material. For studies that included a chemical analysis, samples were kept in a freezer set to 18° C until samples could be transported to the Mississippi State University Chemical Analysis Lab.

**Insect Rearing**

Laboratory colonies of corn earworm used in the leaf bioassays were maintained at the Mississippi State University insect rearing facility. The colony originated from individuals collected from non-Bt corn ears in 2006, and wild individuals were added to the colony on a biannual basis to maintain genetic diversity. The colony was reared under recommended conditions of 25°C, 80% relative humidity, and 16:8 (L:D) photoperiod. Upon collection, larvae were placed in 59.2 mL cups (Solo®, Dart Container Corp., Mason, MI) containing Stonefly *Heliothis* diet (Product No. 38-0600, Ward’s Science, Rochester, NY) with matching lids. At pupation, approximately 40 pupae were placed into 3.79 L cardboard buckets and covered with cheesecloth, which acted as a detachable oviposition substrate location for moths. Egg sheets were collected and placed into 3.79 L self-sealing bags (Ziploc®, S.C. Johnson & Son, Inc., Racine, WI). Upon hatching, approximately 100 larvae were transferred into 473 mL plastic deli
containers (Fabr-Kai Corp, Kalamazoo, MI) filled with Stonefly *Heliothis* diet and covered. Transferred larvae were returned to the rearing room until larvae reached second instar, the appropriate size for assays.

**Soybean Leaf- Standard Rates Study**

In 2020, research was conducted to determine residual concentrations of chlorantraniliprole when applied as a foliar spray to soybean at four standard rates and to assess corn earworm mortality on soybean leaves at different vertical zones in the canopy. The rates of chlorantraniliprole included 0.028, 0.053, 0.078, and 0.103 kg ai ha\(^{-1}\). One day prior to application, total node counts for 10 random soybean plants per plot were taken to determine the partitioning of zones. These vertical zones consisted of a top (18\(^{th}\) node), middle (13\(^{th}\) node), and bottom (9\(^{th}\) node) zone. Plants were partitioned in this manner to evaluate the distribution of insecticide throughout the canopy. Four rates of chlorantraniliprole and an untreated control were applied to plots at the R5 growth stage. Experimental treatments were applied with a John Deere 6000 High clearance sprayer (John Deere, Moline, IL) calibrated to deliver 93.5 L ha\(^{-1}\) at 350 kPa through TX-6 ConeJet® VisiFlo® Hollow Cone Spray Tip nozzles (2 nozzles per row) (TeeJet® Technologies, Glendale Heights, IL)

At 1, 7, 14, 21, and 28 days after treatment (DAT), 20 soybean plants from the center two rows were clipped at the base of the soil line, and cables ties were used to bundle the plants. Each plant bundle was placed into 113 L waste bags (Hefty, Reynolds Consumer Products LLC, Lake Forest, IL) and transported to the laboratory. Upon returning to the lab, 20 center leaflets from the trifoliate within each zone were pulled from the plant. Ten leaflets were placed into 946 mL self-sealed plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) for chemical
analysis. The other ten leaflets were used for bioassays. To reduce contamination, gloves were worn and changed between plots when handling sprayed material.

**Soybean Floret Study**

In 2020, trials were conducted to determine concentrations of chlorantraniliprole in soybean florets. At the R1 growth stage, two rates of chlorantraniliprole (0.053 and 0.078 kg ai ha\(^{-1}\)) and an untreated control was applied to plots using the methods described previously.

Using forceps, 100 soybean florets per plot were removed from the center two rows at 4, 7, 10, and 14 DAT. Floret samples were placed into 50 mL Falcon® Conical Centrifuge Tubes (Corning, Corning, NY, USA) for chemical analysis.

**Soybean Leaf- Reduced Rates Study**

This study was conducted in 2021 to determine susceptibility of corn earworm to concentrations of chlorantraniliprole discovered in the soybean floret study. Soybean florets were unable to be used for bioassays, so the test was conducted using soybean leaves. Five concentrations (5, 25, 75, 125 PPB) were selected for this study to represent what was found in the floret study and converted to kg ai ha\(^{-1}\) rates that needed to be sprayed to yield those concentrations on leaves at 1 DAT (Table 2.1). Commercial formulation of chlorantraniliprole (Prevathon®; 5 SC; 41.5 g ai/L, FMC Corporation, Philadelphia, PA) was used for this study. Dilutions were prepared by adding 0.1 g of formulated chlorantraniliprole to 1000 mL of distilled water. Serial dilutions of the desired concentrations were diluted into 3785 mL of distilled water to yield five concentrations an untreated check. The mixture was deposited into 3785 mL spray canisters that were mounted into the high clearance sprayer and applications were made at R5 growth stage.
Table 2.1  Rates of chlorantraniliprole and PPB equivalents used in the soybean reduced rate bioassay study to determine susceptibility of corn earworm to concentrations of chlorantraniliprole found in soybean floret chemical analysis study.

<table>
<thead>
<tr>
<th>Rate (kg ai ha$^{-1}$)</th>
<th>PPB Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00002244</td>
<td>5</td>
</tr>
<tr>
<td>0.00011222</td>
<td>25</td>
</tr>
<tr>
<td>0.00036661</td>
<td>75</td>
</tr>
<tr>
<td>0.00044888</td>
<td>100</td>
</tr>
<tr>
<td>0.00056109</td>
<td>125</td>
</tr>
</tbody>
</table>

At 1 DAT, 10 sprayed leaflets were removed from the upper one-third of the soybean plant, placed into 946 mL self-sealed plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) and transported back to the lab for bioassays.

**Leaf Bioassays- Standard and Reduced Rates**

Leaf bioassays from the standard and reduced rate soybean leaf studies were set up in a similar manner. In the laboratory, 15 mm soybean leaf disk were placed into 59.2 mL cups (Solo®, Dart Container Corp., Mason, MI) and labeled by plot and zone for each study. Lids were placed upside down on the table and filled with 1% water agar (Product No. 7060, Frontier Agricultural Sciences. Newark, DE) solution to prevent desiccation. One second instar lab colony corn earworm from the laboratory colony was placed in each cup and lids with agar were attached. Mortality was rated 4 days after infestation (DAI), and larvae were considered dead if they could not right themselves when rolled on their dorsal surface.

**Chemical Analysis**

Soybean leaf and floret samples were analyzed using a modified QuEChERS by LC/MS/MS and GC/MS/MS procedure developed by Anastassiades and Lehotay (2003). Samples were ground into a fine powder and 5 g of the sample was deposited into a 50 mL
polypropylene tube. Two tubes of clean, lab grown samples were measured with 5 g placed into 50 mL polypropylene tubes for a “blank” and “spike” sample. The “blank” represented an untreated control while “spike” received adequate insecticide concentrations to be accurately tested by the mass spectrometer. All tubed samples received a ceramic bead for homogenization and 10 mL of liquid chromatography water before centrifuging. Samples were placed into a GenoGrind (SPEX Sample Prep, Metuchen, NJ) plant tissue homogenizer for five minutes at 1000 RPM. After the first round of centrifuging, 10 mL of acetonitrile (ACN), which allows for active ingredient extraction, was deposited into samples. Additional centrifuging was conducted for five minutes. Samples were removed from the centrifuge and 4 g of MgSO₄ (anhydrous magnesium sulfate) was added and placed back into the centrifuge for five minutes. After separation of water and ACN, samples were placed into a larger centrifuge for ten minutes and RPM was increased to 4000 to separate materials entirely. The samples were removed with a layer of active ingredient on top. The insecticide layer was extracted and deposited into a new 15 mL polypropylene tube. 1 mL of extracted liquid material was placed into an auto sampler vial with a PTFE/PVDF filter and analyzed using a LC/MS/MS or GC/MS/MS for GC-amenable pesticides. Recovery of residual insecticide ranged between 85-101% (mostly >95%) (Anastassiades and Lehotay 2003). Analysis conclusions were displayed in parts per billion (PPB) of active ingredient.

**Data Analysis**

For standard rate leaf bioassays, data were corrected for control mortality using Abbott’s formula (Abbott 1925). Trace amounts of drift in the untreated controls was observed in the leaf chemical analysis study and was omitted from statistical procedures. Chemical analysis data were transformed using log transformation prior to statistical analysis and non-transformed
means and standard errors were reported. The analysis included zone, rate, DAT, and their interactions were considered fixed effects in the model for the standard rate bioassay and chemical analysis study. For the soybean floret chemical analysis study, rate, DAT and their interaction was considered fixed effects in the model. For the soybean leaf reduced rates study, rate was considered a fixed effect. Replication was established as the random effect for all studies. All data were subjected to analysis of variance using generalized linear mixed model procedures (Proc Glimmix, SAS 9.4, SAS Institute Inc. Cary, NC). Kenward-Roger method was used to calculate degrees of freedom. Means and standard errors were calculated using PROC MEANS statement. LS means were separated using Fisher’s Protected LSD \( \alpha = 0.05 \).

**Results**

**Soybean Leaf- Standard Rates Bioassay**

There was an interaction between rate and DAT observed for the top \( (F= 2.2; \text{df}= 12, 56; P=0.02) \), middle \( (F= 1.9; \text{df}= 12, 57; P= 0.05) \) and bottom \( (F= 2.9; \text{df}= 12, 57; P= 0.01) \) zones of the plant for percent mortality of corn earworm (Table 2.2). Variability in mortality of corn earworm was noticed in all zones of the plant (Table 2.2). In the top zone, rate \( (F= 2.7; \text{df}= 3, 56; P=0.05) \), days after treatment \( (F= 2.9; \text{df}= 4, 56; P=0.03) \) and an interaction between the two \( (F= 2.2; \text{df}= 12, 56; P=0.02) \) was observed. Excellent control ranging from 78.1 to 97.5% mortality was recorded across all rates and days after treatment in the top zone. At 28 DAT, plots that received 0.053 kg ai\(^{-1}\), a recommended use rate in Mississippi soybean, achieved 85.3% control for leaves the top zone. Main effects of rate \( (F= 3.7; \text{df}= 3, 57; P= 0.02) \), days after treatment \( (F= 3.8; \text{df}= 4, 57; P< 0.01) \) and a two-way interaction between rate and DAT \( (F= 1.9; \text{df}= 12, 57; P=0.05) \) were significant in the middle of the plant. In the middle zone, corn earworm control ranged from 71.2 to 97.5%. Additionally in the middle zone, a reduction in mortality with the
lowest use rate (0.028 kg ai⁻¹) at 28 DAT was observed. An effect of rate (F= 8.2; df= 3, 57; P< 0.01), days after treatment (F= 6.6; df= 4, 57; P< 0.01) and the interaction between the two (F= 2.9; df= 12, 57; P< 0.01) was significant for mortality in the bottom zone. 73.1 to 97.5% control was observed in the bottom zone. Within the bottom zone, plots that received a 0.028 kg ai⁻¹ at 28 DAT provided a reduced mortality compared to all other rates and days after treatment. Although variable, chlorantraniliprole was highly lethal, >71.2%, at all zones, rates, and sampling dates tested.

**Soybean Leaf- Standard Rates Chemical Analysis**

There was not a significant interaction between rate and DAT observed for the top (F= 0.79; df= 12, 38; P=0.7), middle (F= 0.89; df= 12, 38; P= 0.6) and bottom (F= 1.0; df= 12, 34.1; P= 0.5) zones of the plant for concentrations of chlorantraniliprole. However, main effects of rate were observed in the top, middle, and bottom zone (Table 2.3). For application rates in the top zone, concentrations decreased as rates were reduced (F= 26.1; df= 3, 38; P< 0.01). Concentrations detected with 0.028 kg ai ha⁻¹ rates in the top zone were significantly lower compared to what was found using the other rates. 0.078 and 0.103 kg ai ha⁻¹ rates had similar concentrations but were significantly higher than 0.028 and 0.053 kg ai ha⁻¹. Additionally for rates in the middle zone, differences in concentrations were observed (F= 49.6; df= 3, 38; P< 0.01). Reduced concentrations were observed in the middle leaves with 0.028 kg ai ha⁻¹ rate compared to 0.053, 0.078 and 0.103 kg ai ha⁻¹ rates. Use rates of 0.078 and 0.103 kg ai ha⁻¹ recorded higher concentrations than all other rates for middle leaves. Differences in concentrations for rates in the bottom zones were observed (F= 19.3; df= 3, 38; P= 0.03). Concentrations associated with 0.078 and 0.103 kg ai ha⁻¹ rates were significantly higher than the
lowest use rate (0.028 kg ai ha\(^{-1}\)). 0.028 kg ai ha\(^{-1}\) rate of chlorantraniliprole recorded the lowest concentrations compared to all other rates for leaves in the bottom zone.

Additionally, main effects of DAT were significant for concentrations in the top zone (F= 17.1; df= 4, 38; P< 0.01) (Table 2.3). Concentrations at 1 DAT were higher compared to all other sampling dates in the top zone. No differences in concentrations were observed between 21 and 28 DAT; however, a reduction in concentrations were observed at 21 and 28 DAT compared to 7 DAT. At 1 DAT, concentrations in the middle zone were higher compared to all other sampling dates (F= 38.1; df= 4, 38; P< 0.01). Higher concentrations were detected at 1, 7, and 14 DAT compared to concentrations at 28 DAT. No differences in concentrations were observed at 7 and 14 DAT. Additionally in the middle zone, concentrations at 21 and 28 DAT were similar.

Variability in chlorantraniliprole concentrations was observed at sampling dates in the bottom zone (F= 8.7; df= 4, 34.1; P< 0.01). Detections at 1 DAT yielded higher concentrations compared to all other sampling dates in the bottom zone. Differences in concentrations were observed between concentrations at 14 and 21 DAT but not for 7 and 28 DAT.

**Soybean Floret- Chemical Analysis**

The interaction between rate and DAT (F=0.71; df= 3, 14; P= 0.5) was not significant for chlorantraniliprole concentrations in soybean florets. However, significant differences in rate (F= 5.8; df= 1, 14; P= 0.03) and DAT (F= 15.3; df= 3, 14; P< 0.01) were observed. Concentrations of chlorantraniliprole were lower when applied at a rate of 0.053 kg ai ha\(^{-1}\) compared to a rate of 0.078 kg ai ha\(^{-1}\) (Table 2.4). Additionally, concentrations at 4 DAT were statistically greater than what was found at 7, 10, and 14 DAT. No significant differences were observed between chlorantraniliprole concentrations at 4, 7, 10, and 14 DAT. Concentrations decreased over time and as sampling date increased.
Soybean Leaf- Reduced Rates Bioassay

When similar chlorantraniliprole rates observed in the soybean floret study were tested in a bioassay against corn earworm, mortality was observed. Results from the leaf bioassay indicated significant differences between rates of chlorantraniliprole (Figure 2.1). Mortality was similar with 0.00002244 kg ai ha\(^{-1}\) (5 PBB equivalent) and 0.00011222 kg ai\(^{-1}\) (25 PPB equivalent) rates, and the untreated control. There was a drastic increase in mortality of corn earworm from 0.00011222 (25 PPB equivalent) to 0.00036661 kg ai ha\(^{-1}\) (75 PPB equivalent). The three highest use rates (0.00036661, 0.0044888, and 0.0056109 kg ai ha\(^{-1}\)) were not significantly different and resulted in >50% mortality.

Discussion

Control of corn earworm in soybean is highly dependent on chemical control, but due to resistance to older chemical classes (Sparks 1981, Brown et al. 1998, Jacobson et al. 2009), new insecticides with novel modes of action and long residuals are essential for effective control and overall IPM programs. Diamides, including chlorantraniliprole, are a recently developed class of insecticide that provide highly effective control of corn earworm and other lepidopteran pests (Herbert et al. 2011, Adams et al. 2016). Additionally, chlorantraniliprole showed reduced mammalian toxicity and low activity on non-target insects (Bassie et al. 2009, Brugger et al. 2010). Insecticides that provide long residual control, like chlorantraniliprole, remain effective on plant tissue for extended periods of time compared to more traditional insecticides (Bennett 1957). Research conducted in soybean has shown potential for chlorantraniliprole to move systemically, possibly through the xylem, and provide long residual control (Adams et al. 2016, Lahm et al. 2007). In the current study, concentrations of chlorantraniliprole were detected up to 74.3 PPB in soybean florets out to 14 DAT. As rates were reduced and sampling date increased,
concentrations decreased. Concentrations in florets could possibly be from systemic movement or due to flower buds being present at the time of application.

Additional bioassay studies using reduced rates were conducted in 2021 to determine mortality of corn earworm on soybean leaves based on concentrations detected in the soybean floret study. This study confirmed concentrations in florets could provide mortality of corn earworm up to 64%. However, Coelho et al. (2020) demonstrated that there were minimal yield penalties from flower and small pod removal during early reproductive stages due to the capability of soybean to compensate. Additionally, compensation is dependent on the plant’s developmental stage, environmental conditions, and the severity of pressure (Thomas et al. 1974, Swenson et al. 2013). Nevertheless, some control of corn earworm due to concentrations of chlorantraniliprole in soybean florets can be expected out to 4 DAT.

Based on the soybean leaf study using standard rates, concentrations of foliar applied chlorantraniliprole were detected in or on leaves out to 28 DAT, and mortality did not significantly decrease over time. In general, as rates decreased, and as leaf zones changed from top to bottom, then concentrations decreased over time. However, even with concentrations reducing over time as well as throughout the canopy, greater than 71% mortality was recorded in all sampling dates, no matter the rate or zone of the plant. This suggests all concentrations detected were in high enough levels to be extremely lethal to corn earworm larva. For the leaf chemical analysis study using standard rates, at 1 DAT, concentrations were relatively high in leaves in the top of the plant compared to other sampling dates, more than likely due to good spray coverage in the uppermost zone in the canopy. Leaves from the bottom of the canopy indicated reduced concentrations compared to leaves in the top and middle zone, possibly a result of poor spray coverage deep in the canopy (64 to 75% canopy coverage). However, the
lower concentrations did not negatively impact corn earworm mortality. Residual concentrations of chlorantraniliprole could be dependent on plant size and canopy closure at the time of applications. Nevertheless, chlorantraniliprole concentrations in leaves, though variable, were present and highly lethal through all sampling dates, rates, and zones tested.

For corn earworm, soybean is most attractive for oviposition during R1 to R3 growth stages (Eckel 1992a). Singular or small groups of scattered eggs are mostly found in the upper two-thirds of soybean plants (Quaintance and Brues 1905, Hillhouse and Pitre 1976, Eckel et al. 1992a). With the lasting residual efficacy throughout the canopy found in this study, highly effective control should be expected out to 28 DAT on corn earworms migrating from leaves to fruiting structures. While chlorantraniliprole is highly lethal to lepidopteran pests, a continued reliance on this product could result in resistance issues because the long residual could cause low-dose exposure to multiple generations in some situations. Fortunately, in soybean, other effective chemistries such as methoxyfenozide + spinetoram are on the market. Heligen® (AgBiTech, Fort Worth, TX), a highly selective nuclear polyhedrosis virus that can be sprayed on foliage, is another control option for corn earworm in soybean. With other chemical control options available, rotation of insecticides and spraying only when action threshold is met is essential to a sustainable IPM program. Based on these studies, chlorantraniliprole is highly lethal in soybean leaves out to 28 DAT, and added control due to chlorantraniliprole might be expected when feeding occurs in florets up to 4 DAT.
Table 2.2  Mean (SEM) percent mortality of corn earworm in leaf bioassays conducted in 2020 to determine mortality on soybean leaves throughout the canopy.

<table>
<thead>
<tr>
<th>Rate 1</th>
<th>DAT 2</th>
<th>Top 3</th>
<th>Middle</th>
<th>Bottom</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.028</td>
<td>1</td>
<td>93.5ab (3.9)</td>
<td>94.0ab (3.3)</td>
<td>83.6de (4.6)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>86.6bcd (5.0)</td>
<td>91.9ab (5.2)</td>
<td>86.3cde (6.2)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>94.7ab (3.1)</td>
<td>91.2ab (2.9)</td>
<td>91.7abcd (4.9)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>85.7bcd (2.7)</td>
<td>91.4ab (5.1)</td>
<td>88.3bcd (0.9)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>78.1d(1.2)</td>
<td>71.2c (5.5)</td>
<td>73.1f (2.8)</td>
</tr>
<tr>
<td>0.053</td>
<td>1</td>
<td>95.0ab (5)</td>
<td>84.4b (9.7)</td>
<td>87.5bcd (4.8)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>89.2abc (0.3)</td>
<td>94.3ab (3.3)</td>
<td>89.4abcd (4.1)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>97.5a (2.4)</td>
<td>97.5a (2.5)</td>
<td>97.5a (2.5)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>94.4ab (3.3)</td>
<td>91.1ab (3.1)</td>
<td>91.9abcd (5.2)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>85.3bcd (1.9)</td>
<td>87.5ab (5.2)</td>
<td>86.6cd (5.1)</td>
</tr>
<tr>
<td>0.078</td>
<td>1</td>
<td>91.9abc (4.2)</td>
<td>87.7ab (5.7)</td>
<td>97.5a (2.5)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>94.7ab (3.1)</td>
<td>97.5a (2.4)</td>
<td>88.8abcd (4.2)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>95.0ab (5.0)</td>
<td>97.8a (2.5)</td>
<td>97.5a (2.5)</td>
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<tr>
<td></td>
<td>21</td>
<td>88.1abc (1.9)</td>
<td>97.3a (2.5)</td>
<td>94.4abc (3.3)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>92.2abc (2.6)</td>
<td>94.3ab (3.3)</td>
<td>92.2abcd (2.6)</td>
</tr>
<tr>
<td>0.103</td>
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<td>83.6cd (8.4)</td>
<td>89.3ab (7.1)</td>
<td>77.6ef (4.3)</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>97.5a (2.5)</td>
<td>97.5a (2.5)</td>
<td>91.6abcd (2.8)</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>94.7ab (3.5)</td>
<td>97.3a (2.4)</td>
<td>97.5a (2.5)</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>94.4ab (3.3)</td>
<td>94.7ab (3.1)</td>
<td>97.5a (2.5)</td>
</tr>
<tr>
<td></td>
<td>28</td>
<td>94.3abc (3.1)</td>
<td>96.7a (3.3)</td>
<td>96.1ab (4.1)</td>
</tr>
</tbody>
</table>

| F      | 2.2   | 1.9   | 2.9   |
| d.f.   | 12, 56| 12, 57| 12, 57|
| P>F    | 0.02  | 0.05  | 0.01  |

Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05).
1Rate of chlorantraniliprole expressed in kg ai ha⁻¹.
2DAT= Days after treatment.
3Plants were partitioned into three “zones” consisting of leaves from a top (18th node), middle (13th node), and bottom (9th node) zone.
Table 2.3  Mean (SEM) of chlorantraniliprole concentrations (PPB) by zone in soybean leaves in the top, middle, and bottom of the plant to determine the residual activity throughout the canopy in 2020 study.

<table>
<thead>
<tr>
<th>Rate 1</th>
<th>Top 2</th>
<th>Middle</th>
<th>Bottom</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (± S.E.)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.028</td>
<td>1404c (657)</td>
<td>877c (338)</td>
<td>171c (30)</td>
</tr>
<tr>
<td>0.053</td>
<td>2967b (969)</td>
<td>1685b (649)</td>
<td>321b (32)</td>
</tr>
<tr>
<td>0.078</td>
<td>5902a (1564)</td>
<td>3693a (990)</td>
<td>1010a (403)</td>
</tr>
<tr>
<td>0.103</td>
<td>8173a (2054)</td>
<td>4576a (1068)</td>
<td>814a (222)</td>
</tr>
<tr>
<td>F</td>
<td>26.1</td>
<td>49.6</td>
<td>19.3</td>
</tr>
<tr>
<td>d.f.</td>
<td>3, 38</td>
<td>3, 38</td>
<td>4, 34.3</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DAT 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>14</td>
</tr>
<tr>
<td>21</td>
</tr>
<tr>
<td>28</td>
</tr>
<tr>
<td>F</td>
</tr>
<tr>
<td>d.f.</td>
</tr>
<tr>
<td>P&gt;F</td>
</tr>
</tbody>
</table>

Letters assigned based on log transformed statistics.
Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05).
Means and standard errors are expressed as concentrations (PPB) of chlorantraniliprole.
Trace amounts of drift was detected in untreated check plots.
1Rate of chlorantraniliprole expressed in kg ai ha⁻¹.
2Plants were partitioned into three “zones” consisting of leaves from a top (18th node), middle (13th node), and bottom (9th node) zone.
3DAT= Days after treatment.
Table 2.4  Mean (SEM) of chlorantraniliprole concentrations (PPB) detected in soybean florets.

<table>
<thead>
<tr>
<th>Rate(^1)</th>
<th>Mean (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.078</td>
<td>17.7b (9.2)</td>
</tr>
<tr>
<td>0.103</td>
<td>37.8a (11.1)</td>
</tr>
<tr>
<td>F</td>
<td>5.8</td>
</tr>
<tr>
<td>d.f.</td>
<td>1, 14</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>0.03</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DAT(^2)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>74.3a (17.7)</td>
</tr>
<tr>
<td>7</td>
<td>25.8b (5.3)</td>
</tr>
<tr>
<td>10</td>
<td>7.8b (5.7)</td>
</tr>
<tr>
<td>14</td>
<td>3.1b (1.9)</td>
</tr>
<tr>
<td>F</td>
<td>15.4</td>
</tr>
<tr>
<td>d.f.</td>
<td>4, 14</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Letters assigned based on log transformed statistics. Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05). Means and standard errors are expressed as concentrations (PPB) of chlorantraniliprole. \(^1\)Rate of chlorantraniliprole expressed in kg ai ha\(^{-1}\). \(^2\)DAT= Days after treatment.
Figure 2.1  Mean (SEM) percent mortality of corn earworm in reduced rate bioassay study at 1 DAT to determine susceptibility to concentrations of chlorantraniliprole found in soybean floret chemical analysis study. Means with like letters do not differ significantly according to Fisher’s Protected LSD (α=0.05).
References


Plapp, Jr., F. W. 1971. Insecticide resistance in *Heliothis*: tolerance in larvae of *H. virescens* as compared with *H. zea* to organophosphate insecticides. J. Econ. Entomol. 64: 999-1002.


CHAPTER III
DETERMINING RESIDUAL CONTROL AND CONCENTRATIONS OF CHLORANTRANILIPROLE IN COTTON LEAVES AND FLOWERS

Abstract
Studies were conducted in 2020 and 2021 at the Delta Research and Extension Center in Stoneville, MS to determine the residual concentrations of chlorantraniliprole (Prevathon®, FMC Corporation, Philadelphia, PA) in cotton leaves, as well as the concentrations in petals and anthers that developed after the time of application. Foliar applications of chlorantraniliprole were applied at four rates (0.028, 0.053, 0.078, 0.103 kg ai ha\(^{-1}\)) for leaves and two rates (0.053, 0.078 kg ai ha\(^{-1}\)) for petals and anthers at the second week of bloom. Additional bioassays were conducted to determine mortality of corn earworm (Helicoverpa zea, Boddie) in anthers. For the leaf study, plants were partitioned into three zones consisting of a top (16\(^{th}\) node), middle (13\(^{th}\) node), and bottom (8\(^{th}\) node) zone. Zones were established to determine the distribution of insecticide throughout the canopy. Leaf samples from each zone were analyzed for chemical concentrations at 1, 7, 14, 21, and 28 days after treatment (DAT). Residual concentrations, though variable, persisted through all sampling dates, rates, and zones tested. In this study, chlorantraniliprole remained detectable up to 28 DAT. Results from the cotton flower petal and anther studies detected concentrations of chlorantraniliprole in petals at 4, 7, 10 and 14 DAT, but no concentrations were detected in anthers. Therefore, no mortality of corn earworm was recorded in the anther bioassays. A series of diet incorporated bioassays were conducted using
concentrations previously found in the petal study to determine base-line susceptibilities of field and laboratory colonies of corn earworms and predicted mortality. Results from the diet incorporated bioassays showed similar susceptibility in field and lab colony corn earworms. Concentrations of chlorantraniliprole could provide up to 64% control of corn earworm when feeding occurs on the petals.

Introduction

Corn earworm, *Helicoverpa zea*, (Boddie), is an economically important pest of both soybean and cotton in the mid-south. If left uncontrolled, corn earworm can be detrimental to cotton production systems. Behind tarnished plant bug *[Lygus lineolaris* (Palisot de Beauvois)], corn earworm was the second most economically damaging pest of Mississippi grown cotton in 2020 (Cook and Threet 2020). Small corn earworm larvae feed on young fruiting structures near the oviposition site and move to maturing fruiting structures as the insect molts to later instars (Leigh et al. 1996). Preferred feeding structures include flowers and wilting flower corollas (bloom tags) which can lead to abscission of fruiting and/or a reduction in yield (Adkisson et al. 1964, Farrar and Bradley 1985, Gore et al. 2000). Planting of transgenic Bt technology is the primary management practice used in cotton to control corn earworm infestations, but due to Bt resistance supplemental foliar insecticides may be needed (Sparks 1981, Brown et al. 1998, Jacobson et al. 2009, Reisig and Kurtz 2018, Crow et al. 2021).

The use of insecticides targeting corn earworm has become even more prominent in recent years due to documented failures of two gene Bt cotton and insecticide resistance (Sparks 1981, Brown et al. 1998, Jacobson et al. 2009, Reisig and Kurtz 2018, Crow et al. 2021). Chlorantraniliprole, *(Prevathon®* FMC Corporation, Philadelphia, PA), a diamide insecticide, was released in 2008. This insecticide has proven to be very effective at controlling lepidopteran
pests such as corn earworm, fall armyworm, and soybean looper (Temple et al. 2009, Hardke et al. 2011, Adams et al. 2016). Research has shown systemic and translocative movement of chlorantraniliprole along with a long residual (Lahm et al. 2007, Cameron et al. 2015, Adams et al. 2016, Babu et al. 2021). When applied to the soil, the insecticide was taken up by the root systems of cabbage or other plants and moves upwards in the plant’s xylem (Cameron et al. 2015). Research by Adams et al. (2016) found when chlorantraniliprole was foliar applied it moved systemically to vegetation in soybean, but no concentrations were detected in reproductive structures. Adams et al. (2016) also conducted bioassays targeting corn earworm in soybean and discovered when soybeans are infested at R1-R3 the systemic efficacy of chlorantraniliprole can provide some control in the foliage. According to field bioassays conducted by Babu (2021), chlorantraniliprole had lethal and sublethal effects on corn earworm feeding on fresh cotton leaf tissue for up to 22 days. Limited research has been conducted on the systemic activity of chlorantraniliprole in fresh flowering structures of cotton or soybean (Babu 2021). The objective of this study was to determine the residual concentrations of chlorantraniliprole in cotton leaves, as well as petals and anthers that developed after the time of application.

**Materials and Methods**

**Field Experiment Details**

Three field studies were conducted in 2020 and 2021 at the Delta Research and Extension Center in Stoneville, MS. All field trials were conducted with the same plot layout and experimental design. Non-Bt cotton (Deltapine 1822XF, Bayer CropScience, St. Louis, MO) was planted on raised beds at the Delta Research and Extension Center in Stoneville, MS between 8 to 20 May in 2020 and 2021. Plots were 6 rows wide with 1.02m row spacing and 12.2m long.
Plots were separated by fallow alleys 3.04m long. All standard production practices were done according to Mississippi State University Extension Service recommendations. Insects were managed based on economic thresholds (Crow et al. 2021) using insecticides without lepidopteran activity. Field studies were arranged as a randomized complete block design with four replications. In order to minimize the risk of drift between plots, only the center four rows were sprayed. Canopeo (Mathworks, Inc., Natick MA), a mobile device application developed to measure green canopy coverage, was used to determine >90% canopy closure in all plots one day prior to application. When handling sprayed material for all field tests, gloves were worn and changed between plots to reduce the risk of insecticide contamination. Samples were kept frozen at -18° C until samples could be transported to the Chemical Analysis Laboratory at Mississippi State University.

**Cotton Leaf Application**

This study was conducted in 2020 to determine residual concentrations of foliar applied chlorantraniliprole in cotton leaves throughout the plant canopy. Prior to insecticide application, 10 plants per plot were sampled to calculate the average number of nodes. The data was used to determine the partitioning of zones. These zones consisted of a top (16th node), middle (13th node), and bottom (8th node) zone. Plants were partitioned accordingly to determine the distribution of insecticide throughout the canopy to better estimate potential insect mortality.

Four rates of chlorantraniliprole (0.028, 0.053, 0.078, 0.103 kg ai ha⁻¹) and an untreated control were applied to plots at the second week of bloom with a John Deere 6000 Hi clearance sprayer (John Deere, Moline, IL) calibrated to deliver 93.5 L ha⁻¹ at 350 kPa through TX-6 ConeJet® VisiFlo® Hollow Cone Spray Tip nozzles (2 nozzles per row) (TeeJet® Technologies, Glendale Heights, IL).
At 1, 7, 14, 21, 28 days after treatment (DAT), 15 cotton plants from the center two rows were clipped at the base of the soil line and bundled together with cable ties. Each bundle was placed in 113L trash bags (Hefty, Reynolds Consumer Products LLC, Lake Forest, IL) and transported to the laboratory. Within each zone, 15 leaves were removed and placed in 946mL self-sealed plastic bags and placed in the freezer (Ziploc, S. C. Johnson & Son, Inc., Racine, WI). Leaf bioassays were conducted concurrently with the previous test. In the laboratory, 15 mm cotton leaf disk were placed into 59.2 mL cups (Solo®, Dart Container Corp., Mason, MI). Lids were placed upside down on the table and filled with 1% water agar (Product No. 7060, Frontier Agricultural Sciences. Newark, DE) solution to prevent desiccation. One second instar lab colony corn earworm larva was placed in each cup and lids with agar were attached. Mortality was recorded 4 days after infestation (DAI), and larvae were recorded dead if they could not right themselves when rolled on to their dorsal surface.

**Cotton Flower and Anther Application**

In 2020 and 2021, studies were conducted to determine chlorantraniliprole concentrations and mortality of corn earworm in cotton petals and anthers that were undeveloped prior to the application. Treatments consisted of two rates of chlorantraniliprole (0.053 and 0.078 kg ai ha⁻¹) and an untreated control. Applications were made at the 2nd week of bloom.

At 4, 7, 10, and 14 DAT, forty-five cotton flowers per plot were removed from the upper one-third of plants in the center two rows. Samples were placed in 946mL self-sealed plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) and transported back to the laboratory. Bracts were removed from each flower, and fifteen flowers with anthers were placed in clean bags for chemical analysis. Anthers were removed from the remaining flowers. Twenty anthers
were placed in separate clean bags for the additional chemical analysis. The other ten anthers were used for bioassays with corn earworm larvae.

**Insect Rearing**

Laboratory-reared (lab colony) corn earworms, originating from larvae collected from non-Bt corn in 2006, were maintained at the Mississippi State University insect rearing facility. Wild individuals were added to the colony on a biannual basis to maintain genetic diversity within the colony. This colony was reared under recommended conditions of 25°C, 80% relative humidity, and 16:8 (L:D) photoperiod. Wild (field colony) corn earworms were collected 15 May 2021 from crimson clover, *Trifolium incarnatum* L. near Vicksburg, Mississippi and placed into 59.2 mL cups (Solo®, Dart Container Corp., Mason, MI) containing Stonefly *Heliothis* diet (Product No. 38-0600, Ward’s Science, Rochester, NY) with matching lids. Larvae from the field were maintained in a climate-controlled room set to 26.7°C, 80% humidity, and (L:D) photoperiod of 16:8 hours at the Mississippi State University Delta Research and Extension Center insect rearing facility in Stoneville, MS. At pupation, approximately 40 pupae from each colony were placed in 3.79L cardboard buckets and covered with cheesecloth, which acted as a detachable oviposition location for moths. Cheesecloth with eggs were placed into 3.79L self-sealing bags (Ziploc®, S.C. Johnson & Son, Inc., Racine, WI). Once neonates appeared, approximately 100 larvae were transferred into 473mL plastic deli containers (Fabr-Kai Corp, Kalamazoo, MI) filled with a thin layer of Stonefly *Heliothis* diet (Product No. 38-0600, Ward’s Science, Rochester, NY) and covered. Transferred larvae were placed back into the climate-controlled rearing room until larvae reached second instar when they were used for bioassays.
Cotton Anther Bioassay

Bioassays were conducted in 2021 at the Mississippi State University Delta Research and Extension Center in Stoneville, Mississippi to determine efficacy of chlorantraniliprole in cotton anthers. Ten anthers from each of the four reps for a total of forty per treatment were placed individually into 59.2 mL plastic cups (Solo®, Dart Container Corp., Mason, MI). One second instar lab colony corn earworm larva was placed in each cup and matching lids were attached. Mortality was evaluated 2 days after infestation and larvae were recorded dead if they could not right themselves when rolled on their dorsal surface.

Diet Incorporated Bioassays

Diet incorporated concentration-mortality bioassays were conducted in 2021 at the Mississippi State University Delta Research and Extension Center in Stoneville, Mississippi to compare the susceptibility of lab and field colony corn earworms to concentrations of chlorantraniliprole found in the cotton flower chemical analysis study. Methods for preparation of diet incorporated bioassays were similar to Temple et al. (2009). A commercial formulation of chlorantraniliprole (Prevathon®; 5 SC; 41.5 g ai/L, FMC Corporation, Philadelphia, PA) was used for this study. Dilutions were prepared by adding 0.1g of formulated chlorantraniliprole to 1000mL of distilled water. Serial dilutions of the desired concentrations of chlorantraniliprole were diluted in 200mL of distilled water. Five concentrations (5, 25, 75, 100, 125 PPB) and an untreated check were combined with Stonefly Heliothis Diet (Product No. 38-0600, Ward’s Natural Science, Rochester, NY) to yield 200mL of insecticide-treated diet for each concentration. To distribute insecticide evenly, treated diet was hand-agitated for 60 s, and gloves were worn and changed between concentrations to reduce the risk of cross contamination. Treated diet was placed into 59.2 mL cups (Solo®, Dart Container Corp., Mason, MI) to yield 20
cups per treatment. One second instar corn earworm larvae was infested per cup. Cups were covered with matching lids and placed into a rearing chamber maintained at 26.7°C, 80% humidity, and a light/dark cycle of 16:8 hours. Insects were evaluated 5 days after infestation (DAI) for mortality. Intoxicated larvae were recorded dead if they could not right themselves when rolled to their dorsal surface. Lab colony corn earworms were replicated eight times (160 larvae per concentration) while field colony corn earworms were replicated three times (60 larvae per concentration).

**Chemical Analysis**

Cotton leaf, flower, and anther samples were analyzed using a modified QuEChERS by LC/MS/MS and GC/MS/MS procedure developed by Anastassiades and Lehotay (2003), and results were displayed in parts per billion (PPB) of active ingredient. Leaf, flower, or anther samples were ground into a powder and 5g of the sample was deposited into a 50mL polypropylene tube. 5g of clean, lab grown samples were placed into two 50mL polypropylene tubes for a “blank” and “spike” sample. Spike samples were given adequate concentrations of insecticides to be tested to ensure concise readings and the blank sample was left clean. Ceramic beads were placed in each tube for homogenizing the samples when centrifuging. Additionally, 10mL of high-performance liquid chromatography water were deposited in the tubes. A GenoGrind (SPEX Sample Prep, Metuchen, NJ) plant tissue homogenizer was used to centrifuge all samples at 1000 RPM for five minutes. Following the first round of centrifuging, each sample received 10mL of acetonitrile (ACN), which allows extraction of the active ingredient, and were centrifuged again for five minutes. MgSO$_4$ (anhydrous magnesium sulfate) was then added to samples to separate the active ingredient from plant material. Additional five minutes of centrifuging was needed to separated water and ACN. Samples were placed back into the
GenoGrind and centrifuging time and RPM was increased to ten minutes and 4000, respectively. Following this final round of centrifuging, complete separation of the mixture was achieved with the top layer of liquid containing the residual active ingredient. 1mL of the extracted liquid was placed into 15mL polypropylene tubes. Tubes containing the extracted liquid were placed into an auto sampler vial with a PTFE/PVDF filter and analyzed using a LC/MS/MS or GC/MS/MS for GC-amenable pesticides. Recovery of residual insecticide ranged between 85-101% (mostly >95%) (Anastassiades and Lehotay 2003).

**Data Analysis**

Trace amounts of drift in the untreated controls was observed in the cotton leaf chemical analysis study and was omitted from statistical procedures. Chemical analysis data were transformed using log transformation prior to statistical analysis and non-transformed means and standard errors were reported. The analysis for the cotton leaf study included zone, rate, DAT, and their interactions were considered fixed effects in the model. For the cotton flower chemical analysis study, rate, DAT and their interaction was considered fixed effects in the model. Replication was established as the random effect for all studies. Chemical analyses for cotton leaves, petals and anthers were analyzed with a mixed model analysis of variance (PROC GLIMMIX, SAS 9.4, SAS Institute Inc. Cary, NC). Kenward-Roger method was used to calculate degrees of freedom. Means and standard errors were calculated using PROC MEANS statement. LS means were separated using Fisher’s Protected LSD $\alpha=0.05$. Results of concentrations found in cotton flowers were used for diet incorporated bioassays.

Data from diet incorporated bioassays were analyzed using probit analysis in SAS 9.4 (PROC PROBIT, SAS Institute Inc. Cary, NC) to calculate LC$_{50}$ values (PPB). LC$_{50}$ values were considered different when 95 percent confidence intervals did not overlap. Regression equation

50
was determined from the cotton petal chlorantraniliprole concentrations which is a function of application rate and sample timing (DAT). For the LC₅₀ regression equation, concentration and mortality were used to determine the percent predicted mortality at the various sample dates.

**Results**

**Diet Incorporated Bioassays**

Similar LC₅₀ values were observed between field colony (LC₅₀ = 30.1 PPB) and lab colony (LC₅₀ = 30.0 PPB) corn earworms in the diet incorporated bioassays. Confidence intervals overlapped among the two populations and were considered not significantly different [Field Colony (8.9-56.03) and Lab Colony (10.8-56.80)]. Since no differences in responses between colonies was observed, lab colony corn earworms were used for the remainder of bioassays.

**Chemical Analysis of Cotton Leaves**

There was not a significant interaction between rate and DAT for the top (F= 0.23; df=12, 37; P= 0.1), middle (F= 1.1; df= 12, 38; P< 0.01), and bottom (F= 0.59; df= 12, 37; P< 0.01) zones of the plant for chlorantraniliprole concentrations. However, main effects of rate were observed for concentrations in the top, middle, and bottom zone of the plant (Table 3.1). In the top zone, concentrations detected with 0.078 and 0.103 kg ai ha⁻¹ rate were similar but significantly higher than what was found with the 0.028 and 0.053 kg ai ha⁻¹ rate (F= 8.3; df= 3, 37; P< 0.01). Additionally, 0.028 and 0.053 kg ai ha⁻¹ rates recorded similar chlorantraniliprole concentrations for leaves in the top zone. For leaves in the middle zone, concentrations declined as rates were reduced (F= 33.7; df= 3, 38; P< 0.01). Mean concentrations for the lowest use rate (0.028 kg ai ha⁻¹) were significantly lower compared to all other rates in the middle of the plant. Differences in concentrations for rates in the bottom zone were observed (F= 3.4; df= 4, 37; P=
Concentrations associated with the 0.028 and 0.053 kg ai ha\(^{-1}\) rates were not significantly different but were lower than the higher use rates (0.103 and 0.078 kg ai ha\(^{-1}\)) in the bottom of the plants.

Additionally, main effects of DAT were significant for concentrations in the top of the plant (\(F=12.9; \text{df}=4, 37; P<0.01\)) (Table 3.1). Significantly higher concentrations were recorded at 1 DAT compared to all other sampling dates in the top zone. At 28 DAT, concentrations were reduced 98\% compared to concentrations at 1 DAT. No differences in concentrations were recorded between 21 and 28 DAT in the top zone. Concentrations in leaves in the middle zone were significantly different from each other and decreased over time (\(F=91.1; \text{df}=4, 38; P<0.01\)). Although chlorantraniliprole was present throughout all sampling dates, concentrations decreased 96\% from 1 to 28 DAT in the middle of the plant. At 1 DAT, concentrations in the bottom zone were higher compared to all other sampling dates (\(F=10.8; \text{df}=4, 37; P<0.01\)). Reduced concentrations were recorded at 21 and 28 DAT compared to 7 DAT.

**Chemical Analysis of Cotton Petals and Anthers**

The interaction between rate and DAT (\(F=2.1; \text{df}=3, 14; P<0.15\)) was not significant for chlorantraniliprole concentrations in cotton petals. However, main effects of rate (\(F=12.4; \text{df}=1, 14; P<0.01\)) and DAT (\(F=92.4; \text{df}=3, 14; P<0.01\)) were observed. For rate, concentrations were lower when applied at a rate of 0.053 kg ai ha\(^{-1}\) compared to a rate of 0.078 kg ai ha\(^{-1}\) (Table 3.2). Additionally, concentrations detected at 4, 7, 10, and 14 DAT were significantly different from each other and decreased over time. However, no chlorantraniliprole concentrations were detected when testing anthers alone.
Discussion

Corn earworm control is largely dependent on Bt cotton varieties, but due to resistance issues foliar applications of insecticides are often needed (Sparks 1981, Brown et al. 1998, Jacobson et al. 2009, Reisig and Kurtz 2018, Crow et al. 2021). The use of insecticides in today’s world emphasizes effectiveness against target pests, effects on beneficial insects, mammalian toxicity, and long residuals to combat resistance and limit pesticide applications. Diamides, including chlorantraniliprole, showed low activity on non-target beneficial insects and reduced mammalian toxicity (Bassie et al. 2009, Brugger et al. 2010). Additionally, chlorantraniliprole is active against lepidopteran pests and provides effective residual control of caterpillar species in crops such as cabbage, soybean, and cotton (Cameron et al. 2015, Adams et al. 2015, Babu et al. 2021). Based on the cotton leaf chemical analysis study, concentrations of foliar applied chlorantraniliprole remained in or on leaves out to 28 DAT. Generally, as sampling date increased and rate decreased, concentrations decreased. Concentrations at 1 DAT were relatively high in leaves in the top of the plant compared to other sampling dates, more than likely due to good spray coverage in the uppermost zone in the canopy. In a study in snap beans, residual activity decreased over time as the compound degraded and diluted through the plant (Schmidt-Jefferies and Nault 2017). In this study, in cotton, environmental factors such as rainfall events and degradation possibly contributed to concentrations in the top of the plant decreasing rapidly over time. Leaves from the bottom of the canopy had lower concentrations of chlorantraniliprole compared to leaves in the top and middle zone, possibly a result of poor spray coverage deep in the canopy (>90% canopy closure). Residual concentrations of chlorantraniliprole could be dependent on plant size and canopy closure at the time of applications. Nonetheless, chlorantraniliprole concentrations, though variable, persisted through all sampling dates, rates,
and zones tested. Data from cotton leaf bioassays conducted concurrently with the chemical analysis of leaves were unusable due to high untreated control mortality. However, nearly all concentrations of chlorantraniliprole detected in the cotton leaf chemical analysis were in greater concentrations than what was used in the diet incorporated assays- where mortality occurred.

Typically, corn earworm feeding is of most concern on marketable structures of cotton such as squares and bolls. In a study by Braswell et al. (2019), leaves of cotton plants were the most attractive for corn earworm oviposition, and many eggs were found on leaves deep in the canopy. Additionally, upon hatching on leaves, first instar corn earworm larvae were observed feeding on fruiting structures near the oviposition site (Braswell et al. 2019). With the known preferred oviposition and feeding site documented and the lasting residual concentrations of chlorantraniliprole detected throughout the canopy in this study, this compound shows potential to cause residual mortality up to 28 DAT of corn earworms migrating from leaves to fruiting structures. Based on this study, chlorantraniliprole should continue to be used in lepidopteran insect pest management in cotton and a long residual could be expected.

Chlorantraniliprole appears to be absorbed through the stem in soybean and transported through the xylem (Lahm et al. 2007, Adams et al. 2016). Since chlorantraniliprole was applied to entire plants with immature flower buds present, we cannot confidently assume concentrations in cotton flower petals were systemic. Based on research by Adams et al. (2016), chlorantraniliprole moved systemically to vegetation in soybean and provided some control of corn earworm, but no mortality was recorded in reproductive structures. Results from the cotton anther study indicated no concentrations of chlorantraniliprole in anthers and no mortality of corn earworm was recorded. Overall, concentrations of chlorantraniliprole were detected in cotton flower petals but not in anthers. In this study, in cotton, concentrations of
chlorantraniliprole ranged from 0-50.9 parts per billion (PPB) in flower petals out to 14 DAT. However, based on field observations, corn earworm feeding is almost always exclusive to the cotton anthers and not the petals themselves because survival rates are generally greater on anthers than petals (Gore et al. 2001). Additional studies were conducted to determine mortality of corn earworm using diet incorporated bioassay method. Similar LC$_{50}$ values for both lab and field colony corn earworms suggest susceptibility is comparable in the two tested populations. Since no statistical differences in LC$_{50}$ values were observed, lab colony corn earworms were used for the remaining assays. Some corn earworm control due to chlorantraniliprole might be excepted up to 14 DAT in flower petals. Based on this study, bollworm mortality up to 47% might be expected in cotton flowers undeveloped at the time of a chlorantraniliprole application assuming feeding was occurring on the petals and not anthers (Figure 3.1). Insecticides, primarily diamides, are a primary management option for corn earworm control in cotton, but now serve as a supplemental control in combination with Bt cotton varieties (Crow et al. 2021, Reisig and Kurtz 2018). Although, flowering structures are most susceptible to corn earworm feeding in Bt cotton (Gore et al. 2020). The use of Bt cotton varieties supplemented by diamide insecticide applications should continue, and increased control might be expected in flowering structures due to the possible systemic nature of chlorantraniliprole.
Table 3.1  Mean (SEM) of chlorantraniliprole concentrations (PPB) by zone in cotton leaves in the top, middle, and bottom of the plant to determine the residual activity throughout the canopy in 2020 study.

<table>
<thead>
<tr>
<th>Rate&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Top&lt;sup&gt;2&lt;/sup&gt; Mean (± S.E.)</th>
<th>Middle Mean (± S.E.)</th>
<th>Bottom Mean (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.028</td>
<td>1067b (452)</td>
<td>817d (281)</td>
<td>396b (147)</td>
</tr>
<tr>
<td>0.053</td>
<td>1227b (625)</td>
<td>1473c (446)</td>
<td>594b (203)</td>
</tr>
<tr>
<td>0.078</td>
<td>2661a (938)</td>
<td>1787b (491)</td>
<td>1038a (247)</td>
</tr>
<tr>
<td>0.103</td>
<td>3879a (1305)</td>
<td>2874a (772)</td>
<td>1480a (500)</td>
</tr>
<tr>
<td>F</td>
<td>8.3</td>
<td>33.7</td>
<td>3.4</td>
</tr>
<tr>
<td>d.f.</td>
<td>3, 37</td>
<td>3, 38</td>
<td>4, 37</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.03</td>
</tr>
</tbody>
</table>

**DAT<sup>3</sup>**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>7471a (1406)</td>
<td>4815a (759)</td>
<td>2303a (519)</td>
</tr>
<tr>
<td>7</td>
<td>2253b (404)</td>
<td>1882b (352)</td>
<td>986b (275)</td>
</tr>
<tr>
<td>14</td>
<td>1001bc (251)</td>
<td>1345c (267)</td>
<td>663bc (242)</td>
</tr>
<tr>
<td>21</td>
<td>302cd (82)</td>
<td>450d (99)</td>
<td>318cd (78)</td>
</tr>
<tr>
<td>28</td>
<td>102d (34)</td>
<td>196e (41)</td>
<td>148d (37)</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>F</td>
<td>12.9</td>
<td>91.1</td>
<td>10.8</td>
</tr>
<tr>
<td>d.f.</td>
<td>4, 37</td>
<td>4, 38</td>
<td>4, 37</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Letters assigned based on log transformed statistics. Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05).

Means and standard errors are expressed as concentrations (PPB) of chlorantraniliprole. Trace amounts of drift were detected in untreated plots.

<sup>1</sup>Rate of chlorantraniliprole expressed in kg ai ha<sup>−1</sup>.

<sup>2</sup>Plants were partitioned into three “zones” consisting of leaves from a top (16<sup>th</sup> node), middle (13<sup>th</sup> node), and bottom (8<sup>th</sup> node) zone.

<sup>3</sup>DAT= Days after treatment
Table 3.2  Mean (SEM) of chlorantraniliprole concentrations (PPB) detected in cotton flower petals.

<table>
<thead>
<tr>
<th>Rate&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Mean (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.078</td>
<td>25.5b (4.0)</td>
</tr>
<tr>
<td>0.103</td>
<td>31.8a (5.9)</td>
</tr>
<tr>
<td>F</td>
<td>12.4</td>
</tr>
<tr>
<td>d.f.</td>
<td>1, 14</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DAT&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Mean (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>46.4a (2.9)</td>
</tr>
<tr>
<td>7</td>
<td>39.3b (3.1)</td>
</tr>
<tr>
<td>10</td>
<td>20.2c (1.8)</td>
</tr>
<tr>
<td>14</td>
<td>8.7d (1.1)</td>
</tr>
<tr>
<td>F</td>
<td>93.4</td>
</tr>
<tr>
<td>d.f.</td>
<td>3, 14</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Letters assigned based on log transformed statistics.
Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05).
Means and standard errors are expressed as concentrations (PPB) of chlorantraniliprole.
<sup>1</sup>Rate of chlorantraniliprole expressed in kg ai ha<sup>-1</sup>.
<sup>2</sup>DAT = Days after treatment.
Figure 3.1  Relationship between chlorantraniliprole concentration and corn earworm mortality in diet incorporated bioassays and predicted mortality based on concentrations in cotton flower petals.
References


CHAPTER IV

EVALUATING EFFICACY, RESIDUAL CONTROL, AND CHEMICAL CONCENTRATIONS COMMONLY USED INSECTICIDES TARGETING TARNISHED PLANT BUG IN MID-SOUTH COTTON

Abstract

Several studies were conducted from 2017 to 2021 at nine locations across Arkansas, Louisiana, Mississippi, and Tennessee to evaluate efficacy, residual control, and chemical concentrations of commonly used insecticides targeting nymph tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) in mid-south cotton. Foliar applications of imidacloprid, flonicamid, thiamethoxam, oxamyl, dicrotophos, acephate, novaluron, and sulfoxaflor were applied at locally recommended rates. Plots were sampled for nymphs at 2 to 4, 5 to 8, and 9 to 14 days after treatments (DAT), and leaves were analyzed for concentrations of active ingredient from plots located in Stoneville, Mississippi in 2021 at 4, 7, 10, and 14 DAT. Across all sampling dates, all insecticide treatments reduced nymph infestations compared to the untreated control, except for imidacloprid at 9 to 14 DAT. All insecticide treatments resulted in significantly higher lint yields compared to the untreated control. Overall, sulfoxaflor, novaluron, and acephate offered adequate residual control of nymphs and provided the greatest yield protection among treatments. Moderate residual control was achieved with thiamethoxam, oxamyl, and dicrotophos. Imidacloprid and flonicamid resulted in poor residual control. Results from chemical analysis indicated concentrations generally decreased over time. Concentrations
of flonicamid, thiamethoxam, dicrotophos, acephate, and novaluron in leaves persisted out to 14 DAT. No concentrations of imidacloprid or oxamyl were detected at 7, 10 and, 14 DAT, and sulfoxaflor concentrations were not detected at 14 DAT. In these studies, across the mid-south, good (>75%) control of tarnished plant bug nymphs was never reached regardless of insecticide or sampling date. Results from these studies further support recommendations by extension services in the mid-south to continue to use subsequent applications within shorter intervals to manage heavy tarnished plant bug populations.

**Introduction**

Tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois), has been the most destructive insect pest of mid-south cotton for several years (Cook and Threet 2021). Management of this pest is essential in all mid-southern cotton producing states. Tarnished plant bugs can appear as early as cotton plant emergence, but most economic damage occurs from first square to early flowering stages (Scales and Furr 1968). Immature squares are preferred feeding sites of this destructive pest (Layton 1995). Early season feeding by tarnished plant bug causes abscission of squares, which can cause direct yield loss (Pack and Tugwell 1976). Infestations later into the flowering stages can also cause abscission of older squares, but generally these squares remain attached to the plant (Pack and Tugwell 1976). If abscission does not occur, the injury may result in malformed flowers, termed “dirty blooms,” that do not pollinate properly. Little to no yield penalty is attributed to the malformed flowers until 30% of anthers are harmed (Pack and Tugwell 1976).

In the mid-south, tarnished plant bug thresholds change according to current growth stages of cotton. Generally, thresholds are more aggressive during the first week of squaring and become more relaxed after that time (Crow et al. 2021). In addition to thresholds, sampling
methods vary based on the cotton’s developmental stage. During the pre-flowering stage, a sweep net is recommended because adults are usually more common (Musser et al. 2009). Nymphs are predominately found in cotton during flowering stages of cotton development, so a drop cloth is the more accurate sampling technique (Musser et al. 2009). Cultural practices including planting early, choosing short maturing varieties, and removing wild host plants from field edges are effective at managing in tarnished plant bug populations (Adams et al. 2013). However, management of this pest often requires insecticide applications to maintain yield potential.

Populations of tarnished plant bug have become resistant to organophosphate and pyrethroid classes of insecticides making control options for this pest even more limited (Snodgrass 1996, Zhu and Snodgrass 2003, Zhu et al. 2004, Dorman et al. 2020). The development of resistance to these insecticides is likely the main contributing factor of the increasing number of foliar applications needed for control. Thus, increasing input costs across the mid-south. However, some effective insecticides are available in today’s market. Foliar insecticides recommended for control of tarnished plant bug in the mid-south include organophosphates (acephate, dicrotophos, dimethoate), carbamates (oxamyl), neonicotinoids (thiamethoxam, imidacloprid), pyridinecarboxamides (flonicamid), pyrethroids (bifenthrin) insect growth regulators (novaluron), and sulfoximines (sulfoxaflor) (Crow et al. 2021). Tank-mixing and rotating insecticides are recommended to effectively control this pest and limit resistance concerns (Crow et al. 2021). Throughout the growing season, multiple applications are often needed every four to five days to control heavy tarnished plant bug populations (Cook and Threet. 2021, Crow et al. 2021).
Additionally, residual activity of insecticides used to control tarnished plant bug is often short. Pyrethroids, one of the most common chemical classes in the world, are characterized by fast knockdown and lethal activity, but a substandard residual activity (Hirano 1989). Organophosphates, such as acephate, readily decompose when exposed to ultraviolet light ultimately reducing the insecticide’s residual activity (Szeto 1965). However, novaluron, an insect growth regulator targeting tarnished plant bug, was limitedly affected by a rainfall event suggesting the insecticide could provide effective control over time despite rainy conditions (Barrett 2021). The objective of this study was to evaluate residual control and determine chemical concentrations of several commonly used insecticides in mid-south cotton targeting tarnished plant bug.

**Materials and Methods**

**Field Experiment Details**

From 2017 to 2021, several experiments were conducted at nine locations across Arkansas, Louisiana, Mississippi, and Tennessee to determine residual control of commonly used insecticides targeting tarnished plant bug in mid-south cotton. Eight commercially available insecticides were used at the locally recommended rates (Table 4.1). Cotton varieties planted and plot dimensions for these experiments varied across locations and years. Varieties were two or three gene Bt cottons that included either Bollgard II® (Bayer CropScience, St. Louis, MO), Bollgard III®, (Bayer CropScience, St. Louis, MO) or WideStrike III™ (Corteva, Wilmington, DE). Cotton was cultivated and managed according to recommendations of Extension Services in each region. Plots were four rows wide (3.9 to 4.1 m) and 9.1 to 15.2 m in length. All experiments were conducted as a randomized complete block design with four replications. Applications were made with a locally available compressed air sprayer calibrated to deliver 28.1
to 112.2 L per hectare through TX-6 or TX-8 hollow cone nozzles at 4.8 to 8.0 km h⁻¹ when tarnished plant bug populations were at or above economic threshold established by Extension Services for each state.

**Insecticide Efficacy**

Efficacy of treatments was evaluated with a 0.762 m black drop by sampling the center two rows for tarnished plant bug nymphs. The black drop cloth was laid on the ground between two rows, and cotton plants were vigorously shaken to dislodge nymphs unto the cloth. Two samples were collected per plot at 2-4, 5-8, and 9-14 days after treatment (DAT). Sampling dates varied across locations, so this range of dates were established. The center two rows of each plot were harvested with a mechanical cotton picker, weighed, and converted to kilograms per hectare (kg ha⁻¹). Yields were converted to kg lint per hectare based on a 40% lint turnout.

**Chemical Analysis**

The insecticide efficacy trial conducted at the Delta Research and Extension Center in Stoneville, Mississippi during 2021 was used to also determine chemical concentrations. 15 leaves per plot were removed from the center two rows at 4, 7, 10, and 14 DAT. Leaves were removed by counting three nodes down from the top of the plant to ensure leaf tissue collected was present at the time of the spray. Leaf samples were placed in 946mL self-sealed plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) and transported back to the laboratory. Samples were kept in -18°C freezer until samples from three replications could be transported to the Chemical Analysis Lab at Mississippi State University. Cotton leaf samples were analyzed using a modified QuEChERS by LC/MS/MS and GC/MS/MS procedure established by Anastassiades and Lehotay (2003), and concentrations were presented in parts per billion (PPB) of active
ingredient. Leaf tissues were emulsified into a powder, and 5g of the sample was placed into a 50mL polypropylene tube. 5g of clean, lab grown samples were placed into two 50mL polypropylene tubes for a “blank” and “spike” sample. Spike samples were given sufficient concentrations of insecticides to be tested to ensure concise readings, and the blank sample was left clean. Ceramic beads were placed in each tube for homogenizing the samples when centrifuging. Additionally, 10mL of high-performance liquid chromatography water were deposited in the tubes. A GenoGrind (SPEX Sample Prep, Metuchen, NJ) plant tissue homogenizer was used to centrifuge all samples at 1000 RPM for five minutes. Following the first round of centrifuging, samples were centrifuged again for five minutes after each sample received 10mL of acetonitrile (ACN), which allows extraction of the active ingredient, MgSO₄ (anhydrous magnesium sulfate) was added to samples to separate the active ingredient from leaf material. Additional five minutes of centrifuging was needed to separate water and ACN. Samples were placed back into the GenoGrind and centrifuging time was increased to ten minutes and RPM was raised 4000. After this final round of centrifuging, complete separation of the mixture was achieved with the top layer of liquid containing the residual active ingredient. 1mL of the extracted liquid was placed into 15mL polypropylene tubes. Tubes containing the extracted liquid were placed into an auto sampler vial with a PTFE/PVDF filter and analyzed using a LC/MS/MS or GC/MS/MS for GC-amenable pesticides. Recovery of residual insecticide ranged between 85-101% (mostly >95%) (Anastassiades and Lehotay 2003).

**Data Analysis**

All data were subjected to analysis of variance using generalized linear mixed model procedures (Proc Glimmix, SAS 9.4, SAS Institute Inc. Cary, NC). For the insecticide efficacy study, treatment was considered a fixed effect. Year, location, and replication nested within year
by location were designated as random effects. Chemical analysis data were transformed using log transformation, and non-transformed means and standard errors were reported. For chemical analysis data, days after treatment was considered a fixed effect while replication was a random effect. Untreated controls were omitted from chemical analysis study. Kenward-Roger method was used to calculate degrees of freedom. Means and standard errors were calculated using PROC MEANS statement. LS means were separated using Fisher’s Protected LSD $\alpha=0.05$.

**Results**

**Insecticide Efficacy**

At 2 to 4 DAT, all of the insecticide treatments significantly reduced tarnished plant bug nymph numbers compared to the untreated control ($F= 39.6; \text{df}= 8, 890; P< 0.01$) (Table 4.2). However, no differences in densities were observed in imidacloprid, flonicamid, and thiamethoxam treatments. Thiamethoxam and oxamyl provided similar control ranging from 47 to 52%. Dicrotophos, acephate, and novaluron treatments had similar nymph densities at 2 to 4 DAT and provided 61, 65, and 58% control, respectively. Additionally, sulfoxaflor resulted in a reduction in tarnished plant bug nymph densities equivalent to 70% control. Significant differences among treatments for plant bug nymph densities at 5 to 8 DAT were observed ($F= 51.1; \text{df}= 8, 968; P< 0.01$). All insecticide treatments provided control of nymphs compared to the untreated control. Tarnished plant bug nymph densities remained consistent in untreated plots from 2 to 8 DAT. Imidacloprid provided significantly lower efficacy compared to all other treatments, providing only 25% control. Additionally at 5 to 8 DAT, similar densities were recorded in oxamyl, 36% control, and flonicamid, 44% control, treatments. Acephate and novaluron provided similar percent control ranging from 60% to 61%. However, sulfoxaflor resulted in the greatest reduction of nymph densities, 67% control. All insecticide treatments,
except for imidacloprid, significantly reduced tarnished plant bug nymphs at 9 to 14 DAT (F = 25.1; df = 8, 584; P < 0.08). No differences were observed between flonicamid, thiamethoxam, oxamyl, and dicrotophos treatments, and these treatments only provided nymph control ranging from 30 to 38%. Additionally at 9 to 14 DAT, acephate, 58% control, novaluron, 65% control, and sulfoxaflor, 65% control, significantly reduced populations compared to any other insecticide treatments and the untreated check.

All of the insecticide treatments resulted in significantly higher lint yields compared to the untreated control (Table 4.2) (F = 11.1; df = 8, 422; P < 0.01). Imidacloprid, flonicamid, and thiamethoxam resulted in an 8 to 12% lint yield protection compared to the untreated control. A 16 to 18% yield protection was observed in oxamyl and dicrotophos treatments. Acephate, novaluron and sulfoxaflor were the only treatments to result in yields >1,100 kg ha⁻¹ of lint. These three insecticides resulted in significantly greater lint yield protection, 32 to 35%, compared to imidacloprid, flonicamid, or thiamethoxam.

Chemical Analysis

Concentrations of imidacloprid were not detected after 4 DAT (Table 4.3). Differences in flonicamid concentrations were observed across sampling dates (F= 9.6; df= 3, 7; <0.01). Concentrations of flonicamid persisted out to 14 DAT and generally decreased over time. Thiamethoxam concentrations were detected in all sampling dates (F= 6.3; df= 3, 7; P< 0.01). Concentrations of thiamethoxam were significantly greater at 4 DAT compared to 7, 10, and 14 DAT. Concentrations of oxamyl were not detected after 4 DAT. Dicrotophos concentrations were present out to 14 DAT (F= 16.7; df= 3, 5.4; P< 0.01). Significantly higher dicrotophos concentrations were detected at 4 DAT compared to all other sampling dates. Dicrotophos decreased 99% from 4 to 14 DAT. Differences in acephate concentrations were observed across
sampling dates (F= 6.3; df= 3, 7; P<0.03). Significantly higher concentrations of acephate were detected at 4 and 7 DAT compared to what was found at 14 DAT. Although no differences were observed (F= 1.3; df= 3, 6; P=0.3), novaluron was present in cotton leaves out to 14 DAT. Sulfaxaflor concentrations were detected at 4, 7, and 10 DAT but not at 14 DAT. Concentrations of sulfoxaflor were higher at 4 DAT compared to detections at 7 and 10 DAT (F= 14.4; df= 3, 6; P< 0.01).

**Discussion**

Management of tarnished plant bug is essential in all cotton producing states in the mid-south. The number of sprays has increased considerably over the last two decades ultimately resulting in higher total costs of control (Gore et al. 2014). In Mississippi, from years 2000 to 2004, 2.44 average insecticide applications per hectare were needed to control tarnished plant bugs increasing to 5.08 average applications in years 2010 to 2014 (Cook and Threet 2020). Few insecticides are available to control this pest, and residual control is often short. Applications every four to five days are needed to control heavy tarnished plant bug densities (Cook and Threet 2020, Crow et al. 2021). In these studies, all insecticides generally reduced tarnished plant bug nymph populations out to 14 DAT. However, all insecticides except for acephate, novaluron and sulfoxaflor provided <38% control at 9 to 14 DAT. Imidacloprid had almost no residual control, 5% at 9 to 14 DAT. Although singular applications of imidacloprid may not be sufficient, subsequent applications made within 10 days of the initial spray provided good control of tarnished plant bugs in a study in Arkansas (Taillon et al. 2019). Due to its low-cost compared to other insecticides, multiple imidacloprid applications in a short time period are often recommended in controlling tarnished plant bugs in pre-flowering cotton. Results from the chemical analysis supports imidacloprid’s poor residual activity since no concentrations were
detected after 4 DAT. Overall, thiamethoxam, flonicamid, and dicrotophos provided some control of nymphs across sampling dates, but control was poor at 9 to 14 DAT, < 37%. However, dicrotophos resulted in adequate control at 2 to 4 and 5 to 8 DAT, 61% and 56%, respectively. Oxamyl also provided similar control at 9 to 14 DAT to thiamethoxam, flonicamid, and dicrotophos, but it is unclear why no concentrations of oxamyl were detected after 4 DAT.

Although organophosphate resistant populations of tarnished plant bugs have been documented (Snodgrass 1996), acephate provided adequate residual control of nymphs, 58%, and concentrations persisted out to 14 DAT in these studies. This is contradictory to bioassay results of Barrett et al. (2021), where acephate had poor control of tarnished plant bugs after a rainfall event suggesting mortality can be variable and highly dependent on rainfastness and resistant populations present at a given region. In addition to acephate, novaluron and sulfoxaflor provided adequate residual control, 58% to 65%, of tarnished plant bug nymphs out to 14 DAT. Similarly, studies by Gore et al. (2018) revealed suppression of nymphs >2 weeks when novaluron was singularly applied. Results from Taillon et al. (2019) indicated good control of tarnished plant bugs out to 11 DAT with sulfoxaflor, supporting the findings in this study.

However, at 14 DAT, no sulfoxaflor was detected in the chemical analysis study. Yield results were variable in these trials, but all insecticide treatments resulted in greater yield compared to the untreated control. Acephate, novaluron, and sulfoxaflor resulted in yield greater than imidacloprid, flonicamid, and thiamethoxam. During third week of square or peak migration of adults into cotton, novaluron applications has shown yield protecting benefits (Crow et al. 2021).

In these studies across the mid-south, good, >75%, control of tarnished plant bug nymphs was never reached no matter the insecticide or sampling date. However, rather than assuming insecticides provided long residual control, treatments possibly killed a large percentage of the
initial densities of tarnished plant bug nymphs, and the perceived control was likely due to lack of reinfestation. Results from these studies further support recommendations by extension services in the mid-south to continue to use subsequent applications within a short time period to manage heavy tarnished plant bug populations (Crow et al. 2021). Continued resistance monitoring and insecticide screening trials are important to optimize the success of controlling this pest. Rotating and tank-mixing insecticides with differing modes of actions are recommended to provide effective control of tarnished plant bug and limit resistance concerns (Crow et al. 2021). In the mid-south, a significant reduction in tarnished plant bug populations in cotton fields by spraying herbicide on key hosts plants on roadsides and ditches has been documented (Abel et al. 2007). Alternative control methods such as planting early, removal of host plants, and proper fertility should be incorporated into overall IPM plans so there is not a complete reliance on insecticides.
Table 4.1  Class, common names, trade names, and rates evaluated for control of tarnished plant bug nymphs from years 2017 to 2021 in the mid-south.

<table>
<thead>
<tr>
<th>Class</th>
<th>Common Name</th>
<th>Trade Name</th>
<th>Rate (kg ai ha(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonicotinoid</td>
<td>Imidacloprid</td>
<td>Admire Pro (Bayer CropScience, St. Louis, MO)</td>
<td>0.06</td>
</tr>
<tr>
<td>Pyridinecarboxamide</td>
<td>Flonicamid</td>
<td>Carbine (FMC, Corporation, Philadelphia, PA)</td>
<td>0.099</td>
</tr>
<tr>
<td>Neonicotinoid</td>
<td>Thiamethoxam</td>
<td>Centric (Syngenta, Greensboro, NC)</td>
<td>0.056</td>
</tr>
<tr>
<td>Carbamate</td>
<td>Oxamyl</td>
<td>Vydate (Corteva, Wilmington, DE)</td>
<td>0.40</td>
</tr>
<tr>
<td>Organophosphate</td>
<td>Dicrotophos</td>
<td>Bidrin (Amvac, Newport Beach, CA)</td>
<td>0.56</td>
</tr>
<tr>
<td>Organophosphate</td>
<td>Acephate</td>
<td>Orthene (Amvac, Newport Beach, CA)</td>
<td>0.84</td>
</tr>
<tr>
<td>Insect Growth Regulator</td>
<td>Novaluron</td>
<td>Diamond (ADAMA USA, Raleigh, NC)</td>
<td>0.065</td>
</tr>
<tr>
<td>Sulfoximines</td>
<td>Sulfoxaflor</td>
<td>Transform (Corteva, Wilmington, DE)</td>
<td>0.053</td>
</tr>
</tbody>
</table>
Table 4.2  Impact of selected insecticides on mean (SEM) number of tarnished plant bug nymphs per 3.048 row m and mean (SEM) cotton lint in the mid-south from 2017 to 2021.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2 to 4 DAT&lt;sup&gt;1&lt;/sup&gt;</th>
<th>5 to 8 DAT&lt;sup&gt;2&lt;/sup&gt;</th>
<th>9 to 14 DAT&lt;sup&gt;3&lt;/sup&gt;</th>
<th>Yield&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (± S.E.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>19.8a (1.4)</td>
<td>19.8a (1.3)</td>
<td>17.6a (1.8)</td>
<td>907e (66.5)</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>11.9b (1.0)</td>
<td>14.5b (0.9)</td>
<td>16.7a (1.8)</td>
<td>983d (68.4)</td>
</tr>
<tr>
<td>Flonicamid</td>
<td>11.3bc (0.9)</td>
<td>12.4c (0.9)</td>
<td>11.6b (1.3)</td>
<td>1062bcd (69.3)</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>10.5bc (0.9)</td>
<td>10.3d (0.8)</td>
<td>11.8b (1.3)</td>
<td>1012cd (69.2)</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>9.4cd (0.9)</td>
<td>10.7cd (1.0)</td>
<td>10.9b (1.1)</td>
<td>1098ab (66.4)</td>
</tr>
<tr>
<td>Dicrotophos</td>
<td>7.7ef (0.7)</td>
<td>8.4e (0.6)</td>
<td>12.3b (1.3)</td>
<td>1135abc (65.8)</td>
</tr>
<tr>
<td>Acephate</td>
<td>6.9ef (0.6)</td>
<td>7.6ef (0.5)</td>
<td>7.3c (0.8)</td>
<td>1146a (67.9)</td>
</tr>
<tr>
<td>Novaluron</td>
<td>8.3ed (0.5)</td>
<td>7.5ef (0.5)</td>
<td>6.1c (0.7)</td>
<td>1129a (66.0)</td>
</tr>
<tr>
<td>Sulfoxaflor</td>
<td>5.8f (0.5)</td>
<td>6.4f (0.5)</td>
<td>6.0c (0.7)</td>
<td>1137a (68.4)</td>
</tr>
<tr>
<td>F</td>
<td>39.6</td>
<td>51.1</td>
<td>25.1</td>
<td>11.1</td>
</tr>
<tr>
<td>d.f</td>
<td>8, 890</td>
<td>8, 968</td>
<td>8, 584</td>
<td>8, 422</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05).

<sup>1</sup>2 to 4 Days after treatment. Data from 8 locations across 5 years.

<sup>2</sup>5 to 8 Days after treatment. Data from 9 locations across 5 years.

<sup>3</sup>9 to 14 Days after treatment. Data from 8 locations across 5 years.

<sup>4</sup>Yield expressed in kg ha<sup>-1</sup> of lint based on 40% lint turnout. Data from 8 locations across 5 years.
Table 4.3  Mean (SEM) of concentrations of selected tarnished plant bug insecticides in cotton leaf tissue to determine residual activity in 2021 study conducted at the Delta Research and Extension Center in Stoneville, MS.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4 DAT(^1)</th>
<th>7 DAT</th>
<th>10 DAT</th>
<th>14 DAT</th>
<th>F</th>
<th>d.f.</th>
<th>P&gt;F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidacloprid</td>
<td>19.3a (10.3)</td>
<td>0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
<td>3.4</td>
<td>3, 7</td>
<td>0.08</td>
</tr>
<tr>
<td>Flonicamid</td>
<td>7687.3a (3663.0)</td>
<td>2176.7ab (1576.0)</td>
<td>323.5bc (144.9)</td>
<td>615.6c (1.2)</td>
<td>9.6</td>
<td>3, 7</td>
<td>0.01</td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>193.2a (86.6)</td>
<td>29.6b (15.4)</td>
<td>12.1b (1.1)</td>
<td>15.7b (1.3)</td>
<td>6.3</td>
<td>3, 7</td>
<td>0.03</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>200.7a (133.2)</td>
<td>0.0a</td>
<td>0.0a</td>
<td>0.0a</td>
<td>4.0</td>
<td>3, 8</td>
<td>0.06</td>
</tr>
<tr>
<td>Dicrotophos</td>
<td>22,445.0a (11,894.0)</td>
<td>3341.7b (2900.0)</td>
<td>253.1bc (117.4)</td>
<td>44.9c (1.8)</td>
<td>16.7</td>
<td>3, 5.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Acephate</td>
<td>35,407.0a (17,520.4)</td>
<td>13,419.8ab (6996.0)</td>
<td>535.0bc (176.6)</td>
<td>338.0c (74.5)</td>
<td>6.3</td>
<td>3, 7</td>
<td>0.03</td>
</tr>
<tr>
<td>Novaluron</td>
<td>2350.8a (1181.1)</td>
<td>829.2a (575.5)</td>
<td>144.9a (64.2)</td>
<td>137.5a (92.4)</td>
<td>1.3</td>
<td>3, 6</td>
<td>0.3</td>
</tr>
<tr>
<td>Sulfoxaflor</td>
<td>1279.5a (655.6)</td>
<td>150.3b (104.0)</td>
<td>19.1bc (1.1)</td>
<td>0.0c</td>
<td>14.4</td>
<td>3, 6</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Letters assigned based on log transformed statistics.

Means within a row followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05).

\(^1\) Days after treatment

\(^2\) Means and standard errors are expressed as concentrations of active ingredient in parts per billion (PPB). Trace amounts of acephate, novaluron, and thiamethoxam drift detected in untreated controls.
References


CHAPTER V
EVALUATING EFFICACY, RESIDUAL CONTROL, AND CHEMICAL
CONCENTRATIONS OF COMMONLY USED INSECTICIDES
TARGETING STINK BUG IN MISSISSIPPI SOYBEAN

Abstract

In 2020 and 2021, studies were conducted at the Delta Research and Extension Center in Stoneville, MS and R.R. Foil Plant Science Research Center in Starkville, MS to evaluate efficacy, residual control, and chemical concentrations of commonly used insecticides targeting brown stink bug, *Euschistus servus* (Say); southern green stink bug, *Nezara viridula* (L.); green stink bug, *Chinavia hilare* (Say); and redbanded stink bug, *Piezodorus guildinii* (Westwood) in Mississippi soybean. Foliar applications of clothianidin at 0.119 kg ai ha\(^{-1}\), bifenthrin at 0.112 kg ai ha\(^{-1}\), acephate at 0.84 kg ai ha\(^{-1}\), and acephate + bifenthrin at 0.84 + 0.112 kg ai ha\(^{-1}\) were applied at R5 growth stage. Plots were sampled by taking 25 sweeps per plot at 3, 7, 14, 21, and 28 days after treatment (DAT) with 38.1 cm diameter sweep net. Active ingredient chemical analysis was evaluated at 4, 7, 10, 14, 21, and 28 DAT. All insecticides reduced stink bug populations out to 21 DAT. At 3 DAT, acephate + bifenthrin and acephate alone provided 86 to 89% control. Clothianidin and bifenthrin reduced stink bug densities between 67 and 69% at 3 DAT. At 7 DAT, all treatments reduced populations compared to the untreated control with acephate + bifenthrin resulting in the greatest control, 75%. Similar to the findings at 7 DAT, all insecticide treatments controlled densities at 14 DAT while 71% control was reached with acephate + bifenthrin. At 21 DAT, 34 to 58% control was observed with all treatments. No
reduction in stink bug populations were observed at 28 DAT. For the chemical analysis, clothianidin concentrations failed to persist in soybean leaves after 7 DAT, while acephate was present up to 21 DAT. Bifenthrin was present until 28 DAT, and concentrations decreased over time.

**Introduction**

The seed feeding stink bug complex found in the mid-south soybean is comprised of the brown stink bug, *Euschistus servus* (Say); southern green stink bug, *Nezara viridula* (L.); and green stink bug, *Chinavia hilare* (Say) (Musser et al. 2020). Collectively, brown, green, and southern green stink bug make up the majority of the stink bug complex in the southern United States accounting for 98% of all stink bugs found in soybean (Turnipseed and Kogan 1976, McPherson et al. 1993). In addition to the native stink bug complex, redbanded stink bug, *Piezodorus guildinii* (Westwood), is another detrimental pest to mid-south soybean production (Musser et al. 2020). Although the actual time of its arrival is unclear, the redbanded stink bug was first reported in the United States sometime in the early 1970’s (Panizzi 2004). As of 2013, Alabama, Florida, Louisiana, Mississippi, New Mexico, South Carolina, and Texas have documented populations of redbanded stink bug (Temple et al 2013a). Phytophagous stink bug feed by piercing plant tissue and removing fluids from soybean plants (McPherson and McPherson 2000). This feeding can cause seed to be exposed to pathogens that may reduce yield and grain quality (Russin et al. 1988).

In 2019, the stink bug complex was the primary pests of soybean in Mississippi (Musser et al. 2020). Compared to redbanded stink bug, the stink bug complex is generally more susceptible to commonly used soybean insecticides. According to a study by Temple et al. (2013b), southern green stink bug was very sensitive to currently recommended insecticides.
including acephate, bifenthrin, cyfluthrin, cypermethrin, and lambda-cyhalothrin in vial-treated assays. Brown stink bugs are generally more difficult to control with commonly used insecticides compared to green and southern green stink bug. Results from a study by Snodgrass et al. (2003) confirmed that the brown stink bug was less susceptible to pyrethroid and organophosphate insecticides. Although no resistance to insecticides has been documented in redbanded stink bug, the pest’s high mobility makes it complex and difficult to control with a foliar application. Insecticides currently recommended in Mississippi soybean production for controlling redbanded stink bug and the stink bug complex include pyrethroids (bifenthrin, lambda-cyhalothrin), organophosphates (acephate) and neonicotinoids (clothianidin, imidaclorpid, thiamethoxam) (Crow et al. 2021).

Residual activity of insecticides commonly used to control stink bug is often believed to be short. Pyrethroids, are characterized by high knockdown and lethal activity, but low residual activity (Hirano 1989). Organophosphates, such as acephate, readily decompose when exposed to ultraviolet light ultimately reducing the insecticide’s residual activity (Szeto 1965). Without rain, the half-life of organophosphates is 3.0 days, and pyrethroids is 5.3 days (Boyd and Boethel 1998). Rainfall events have been shown to negatively impact the efficacy of foliar insecticides (Barrett et al. 2021). The objective of this study was to evaluate the efficacy and residual control of commonly used soybean insecticides while determining chemical concentrations.

**Materials and Methods**

**Field Experiment Details**

In 2020 and 2021, three experiments were conducted at the R.R Foil Plant Science Research Center in Starkville, MS and at the Delta Research and Extension Center in Stoneville, MS to determine residual activity and chemical concentrations of commonly used insecticides.
targeting the stink bug complex, and redbanded stink bug. Field studies were arranged as a randomized complete block design with four replications. Plots were 4 rows wide by 15.24 m. These experiments were conducted using soybean variety Asgrow 46x6® (Monsanto Company, St. Louis, Mo) planted at 312,100 seeds ha\(^{-1}\). Soybeans were planted into raised conventionally tilled beds with a 0.97 m row spacing in Starkville, MS and a 1.02 m row spacing in Stoneville, MS between 29 May and 12 June in 2020 and 2021. Treatments included clothianidin (Belay® 2.13 SC, Valent U.S.A, Walnut Creek, CA) at 0.119 kg ai ha\(^{-1}\), bifenthrin (Brigade® 2EC, FMC Corporation, Philadelphia, PA) at 0.112 kg ai ha\(^{-1}\), acesphate (Orthene® 97S, AMVAC Chemical Corporation, Axis, AL) at 0.84 kg ai ha\(^{-1}\), and acesphate + bifenthrin (Orthene® 90S + Brigade® 2EC) at 0.84 + 0.112 kg ai ha\(^{-1}\). Insecticides were applied with a John Deere 6000 Hi clearance sprayer (John Deere, Moline, IL) calibrated to deliver 93.5 L/ha\(^{-1}\) at 350 kPa through TX-6 ConeJet® VisiFlo® Hollow Cone Spray Tip nozzles (2 nozzles per row) (TeeJet® Technologies, Glendale Heights, IL). To evaluate residual activity applications were made when soybean reached R5 growth stage but prior to stink bug infestations. All standard production practices were made according to Mississippi State University Extension Service recommendations.

**Insecticide Efficacy**

Insecticide efficacy was evaluated at 4, 7, 14, 21, and 28 days after treatment (DAT) and at the same intervals leaf samples were pulled for chemical analysis. The number of stink bugs per plot were determined using a 38.1 cm diameter sweep net to take 25 sweeps from one of the center two rows. At the time of sampling, each species was counted individually; however, since population densities of each individual stink bug were not high enough to count separately, counts were combined and evaluated as a complex.
**Chemical Analysis**

For the chemical analysis component, 15 leaves per plot were removed from the center two rows by counting three nodes down from the top of the soybean plant to ensure sprayed leaf tissue was being sampled. Samples were placed in 946mL self-sealed plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) and brought back to the laboratory. Samples remained frozen at \(-18^\circ\)C until samples from three replications could be transported to the Chemical Analysis Lab at Mississippi State University. Leaf samples were analyzed using a modified QuEChERS by LC/MS/MS and GC/MS/MS procedure established by Anastassiades and Lehotay (2003), and concentrations were presented in parts per billion (PPB) of active ingredient. Leaf tissues were emulsified into a powder, and 5g of the sample was placed into a 50mL polypropylene tube. 5g of clean, lab grown samples were placed into two 50mL polypropylene tubes for a “blank” and “spike” sample. Spike samples were given sufficient concentrations of insecticides to be tested to ensure concise readings, and the blank sample was left clean. Ceramic beads were placed in each tube for homogenizing the samples when centrifuging. Additionally, 10mL of high-performance liquid chromatography water were deposited in the tubes. A GenoGrind (SPEX Sample Prep, Metuchen, NJ) plant tissue homogenizer was used to centrifuge all samples at 1000 RPM for five minutes. Following the first round of centrifuging, samples were centrifuged again for five minutes after each sample received 10mL of acetonitrile (ACN), which allows extraction of the active ingredient, MgSO₄ (anhydrous magnesium sulfate) was added to samples to separate the active ingredient from leaf material. An additional five minutes of centrifuging was needed to separated water and ACN. Samples were placed back into the GenoGrind, and centrifuging time was increased to ten minutes and RPM was raised 4000. After this final round of centrifuging, complete separation of the mixture was achieved with the top layer of liquid containing the
residual active ingredient. 1mL of the extracted liquid was placed into 15mL polypropylene tubes. Tubes containing the extracted liquid were placed into an auto sampler vial with a PTFE/PVDF filter and analyzed using a LC/MS/MS or GC/MS/MS for GC-amenable pesticides. Recovery of residual insecticide ranged between 85-101% (mostly >95%) (Anastassiades and Lehotay 2003).

**Data Analysis**

All data were subjected to analysis of variance using generalized linear mixed model procedures (Proc Glimmix, SAS 9.4, SAS Institute Inc. Cary, NC). For the insecticide efficacy study, treatment was considered a fixed effect. Site year (combination of year and location) and site year nested within replication were considered random effects. For the chemical analysis study, treatment was considered a fixed effect while replication was a random effect. Untreated controls were omitted from chemical analysis study and data were transformed before statistical analysis. Degrees of freedom were estimated using the Kenward-Rodger method. Means and standard errors were estimated using the LSMEANS statement and separated according to Fisher’s Protected LSD ($\alpha=0.05$).

**Results**

**Insecticide Efficacy**

Total densities were 5% green, 31% southern green, 8% brown, and 54% redbanded stink bugs at both Starkville and Stoneville locations. All insecticide treatments significantly reduced stink bug densities compared to the untreated control from 3 to 21 DAT. All treatments at 3, 7, 10, and 14 DAT reduced densities below the published economic threshold for Mississippi of 9 per 25 sweeps for the stink bug complex (Crow et al. 2021). By 28 DAT, no treatment separated
from the untreated control (Table 5.1). >62% control was recorded for all insecticide treatments at 3 DAT (F = 5.7; df = 4, 44; P < 0.01). While there was no significant difference among insecticide treatments at 3 DAT, acephate and acephate + bifenthrin treatments resulted in <1 stink bug per 25 sweeps. By 7 DAT, control ranging from 48 to 75% was observed (F = 5.1; df = 4, 113; P < 0.01). Efficacy of treatments were similar at 14 DAT with control averaging between 51 and 87% (F = 5.3; df = 4, 113; P < 0.01). 71% control was observed for acephate + bifenthrin applications at this sampling date. The efficacy decreased to 34 to 58% control by 21 DAT (F = 7.2; df = 4, 93; P < 0.01). While the level of control remained low at 21 DAT all treatments except for acephate reduced stink bug populations below the established economic threshold. Finally at 28 DAT, there were no significant differences among treatments and the untreated control (F = 1.9; df = 4, 93; P < 0.01). <37% control was documented for all insecticides at 28 DAT.

**Chemical Analysis**

When evaluating the presence of active ingredient at 4 DAT, significant differences in concentrations were detected among treatments (F = 13.8; df = 2, 4; P = 0.02) (Table 5.2). Acephate treatments recorded the highest concentrations compared to all other treatments. Clothianidin and bifenthrin treatments resulted in similar concentrations. Differences in concentrations were observed among insecticides at 7 DAT (F = 240; df = 2, 4; P < 0.01). Clothianidin, bifenthrin, and acephate were significantly different from each other. Additionally at 7 DAT, clothianidin was detected in reduced concentrations compared to what was found at 4 DAT. At 10 DAT, there were significant differences among treatments for concentrations (F = 31.7; df = 2, 4.9; P < 0.01). Acephate concentrations were significantly lower than concentrations of bifenthrin. No detections of clothianidin were observed. Greater concentrations of bifenthrin were recorded compared to all other treatments at 14 DAT (F = 23.8; df = 2, 4.9; P < 0.01).
Additionally, bifenthrin concentrations were significantly higher than acephate concentrations. There were no detections of concentrations for clothianidin at 14 DAT. At 21 DAT, concentrations of acephate were significantly reduced compared to bifenthrin \((F= 20.3; \text{df}= 2, 4; \ P< 0.01)\). A 39% reduction in acephate concentrations was recorded from 14 to 21 DAT. Only bifenthrin was detected at 28 DAT \((F= 33.0; \text{df}= 2, 6; \ P< 0.01)\).

**Discussion**

Stink bug management is essential in all soybean producing states in the mid-south. Similar to previous years, the seed-feeding stink bug complex was the costliest pest in 17 reporting states in 2020 in terms of cost of control and yield loss (Musser et al. 2021). In the past five years, at least one application was needed to control stink bug in Mississippi soybean (Musser et al. 2021). Although several insecticides are available to effectively control stink bugs, residual control is often believed to be short. Redbanded stink bug is inherently less susceptible to insecticides commonly used in controlling southern green and green stink bug, while brown stink bugs tend to be less susceptible than green and southern green stink bug (Temple et al. 2013b, Snodgrass et al. 2013). Redbanded stink bugs are largely more difficult to control compared to the native stink bug complex (Akin et al. 2011). Generally, in the current study, all insecticides used reduced stink bug populations out to 21 DAT. Acephate + bifenthrin provided the greatest efficacy among treatments out to 21 DAT with control never falling below 56%. At 3 DAT, good stink bug control, 90%, was observed with acephate treatments, but only a 35% reduction in populations were documented at 21 DAT suggesting acephate provided poor residual control. Results from the chemical analysis supports poor residual control acephate provides, since little to no concentrations were detected after 21 DAT. Overall, bifenthrin and clothianidin provided some control of stink bug at 3 DAT, <62%, but residual control was
moderate at 14 and 21 DAT, <46%. Clothianidin concentrations decreased greatly from 4 to 7 DAT and did not persist after 10 DAT. Clothianidin, a neonicotinoid insecticide, is systemic by nature (Simon-Delso et al. 2015) and possibly translocated away from leaves used for the chemical analysis study.

Vial treated bioassays conducted by Temple et al. (2013b) estimated LC$_{50}$ values for southern green and redbanded stink bug testing various concentrations of organophosphate and pyrethroid insecticides. Concentrations used in those studies were lower than what was found in the current leaf study, and mortality still occurred. This suggest concentrations of organophosphate and pyrethroids that persist in leaves should be in high enough levels to provide control assuming that stink bugs are actively feeding. Additionally, trials conducted by Cook et al. (2021) indicated similar efficacy of tested insecticides at 14 DAT to what was found in our study. In the current studies, good, >85%, control on stink bug was reached with acephate and acephate + bifenthrin at 3 DAT. Generally, all insecticides reduced stink bug densities at all sampling dates except at 28 DAT. Control of stink bugs and concentrations of insecticide decreased over time for all treatments. However, rather than assuming insecticides provided long residual control, treatments possibly killed a large percentage of the initial population, and the perceived control was likely due to lack of reinfestation. Under heavy stink bug populations rapid reinfestation can occur and can appear as control failures. However, under light pressure situations as observed in these studies, effective control of the initial stink bug population can limit reinfestation and keep densities below economic injury level. Rotation of insecticides, tank-mixing different chemistries, and timely applications are key to effectively reduce stink bug populations in mid-south soybean.
Table 5.1  
Impact of selected insecticides on mean (SEM) number of stink bugs per 25 sweeps at the Delta Research and Extension Center in Stoneville, MS and the R.R. Foil Plant and Soil Sciences Research Center in Starkville, MS in 2020.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>3 DAT</th>
<th>7 DAT</th>
<th>14 DAT</th>
<th>21 DAT</th>
<th>28 DAT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (± S.E.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>5.8a (1.7)</td>
<td>5.2a (0.8)</td>
<td>10.5a (1.9)</td>
<td>18.6a (2.5)</td>
<td>13.3a (2.5)</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>1.9b (0.5)</td>
<td>2.4b (0.6)</td>
<td>5.1b (0.8)</td>
<td>7.7b (1.3)</td>
<td>11.2a (2.8)</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>2.2b (0.7)</td>
<td>2.7b (0.7)</td>
<td>5.6b (1.7)</td>
<td>8.3b (1.5)</td>
<td>7.2a (1.5)</td>
</tr>
<tr>
<td>Acephate</td>
<td>0.6b (0.2)</td>
<td>2.2b (0.6)</td>
<td>4.7b (1.0)</td>
<td>12.2b (1.4)</td>
<td>13.7a (2.9)</td>
</tr>
<tr>
<td>Acephate + Bifenthrin</td>
<td>0.8b (0.2)</td>
<td>1.3b (0.4)</td>
<td>3.0b (0.6)</td>
<td>8.1b (1.4)</td>
<td>8.4a (1.5)</td>
</tr>
<tr>
<td><strong>F</strong></td>
<td>5.7</td>
<td>5.1</td>
<td>5.3</td>
<td>7.2</td>
<td>1.9</td>
</tr>
<tr>
<td>d.f</td>
<td>4, 44</td>
<td>4, 113</td>
<td>4, 113</td>
<td>4, 93</td>
<td>4, 93</td>
</tr>
<tr>
<td>P&gt;F</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>0.11</td>
</tr>
</tbody>
</table>

Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05).

\(^{1}\text{DAT}= \text{Days after treatment.}\)
Table 5.2  Mean (SEM) of concentrations of selected stink bug insecticides in soybean leaf tissue to determine chemical concentrations for study conducted at the Delta Research and Extension Center in Stoneville, MS in 2021.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4 DAT$^1$ Mean (± S.E.)</th>
<th>7 DAT Mean (± S.E.)</th>
<th>10 DAT Mean (± S.E.)</th>
<th>14 DAT Mean (± S.E.)</th>
<th>21 DAT Mean (± S.E.)</th>
<th>28 DAT Mean (± S.E.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clothianidin</td>
<td>463.0b (210) 9.7c (5)</td>
<td>0.0b</td>
<td>0.0b</td>
<td>0.0b</td>
<td>0.0b</td>
<td>0.0b</td>
</tr>
<tr>
<td>Bifenthrin</td>
<td>4195.0b (2146) 2290.0b (1108) 1797.7b (1048)</td>
<td>2416.6a (1219) 1236.9a (662)</td>
<td>1470.0a (709)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acephate</td>
<td>24,023.3a (9021) 9778.3a (5767) 3059.0a (1048)</td>
<td>293.7b (163) 24.5b (14)</td>
<td>0.0b</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

F 13.8 240 31.7 23.8 20.3 33

d.f 2, 4 2, 4 2, 4.9 2, 4.9 2, 4 2, 6

P>F 0.02 <0.01 <0.01 <0.01 <0.01 0.04

Means within a column followed by the same letter are not significantly different according to Fisher’s Protected LSD (α=0.05).
Means and standard errors are expressed as concentrations of active ingredient in parts per billion (PPB).
$^1$DAT= Days after treatment
References


