

1-1-2016

## **Baseline Susceptibility of Selected Lepidopteran Pests to Diamides and Use Strategies in Mississippi Soybean**

Charles Andrew Adams

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

---

### **Recommended Citation**

Adams, Charles Andrew, "Baseline Susceptibility of Selected Lepidopteran Pests to Diamides and Use Strategies in Mississippi Soybean" (2016). *Theses and Dissertations*. 851.  
<https://scholarsjunction.msstate.edu/td/851>

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

Baseline susceptibility of selected lepidopteran pests to diamides  
and use strategies in Mississippi soybean

By

Charles Andrew Adams

A Dissertation  
Submitted to the Faculty of  
Mississippi State University  
in Partial Fulfillment of the Requirements  
for the Degree of Doctor of Philosophy  
in Life Sciences  
in the Department of Biochemistry, Molecular Biology, Entomology and Plant Pathology

Mississippi State, Mississippi

May 2016

Copyright by

Charles Andrew Adams

2016

Baseline susceptibility of selected lepidopteran pests to diamides  
and use strategies in Mississippi soybean

By

Charles Andrew Adams

Approved:

---

Jeffrey Gore  
(Co-Major Professor)

---

Angus L. Catchot, Jr.  
(Co-Major Professor)

---

Donald Cook  
(Committee Member)

---

Fred R. Musser  
(Committee Member)

---

Natraj Krishnan  
(Committee Member)

---

Trent Irby  
(Committee Member)

---

Michael A. Caprio  
(Graduate Coordinator)

---

George M. Hopper  
Dean  
College of Agriculture and Life Sciences

Name: Charles Andrew Adams

Date of Degree: May 6, 2016

Institution: Mississippi State University

Major Field: Life Sciences

Major Professors: Jeffrey Gore and Angus L. Catchot, Jr.

Title of Study: Baseline susceptibility of selected lepidopteran pests to diamides and use strategies in Mississippi soybean

Pages in Study 89

Candidate for Degree of Doctor of Philosophy

Insecticides in the diamide class have a novel mode of action and have become a key component for management of agriculturally important lepidopteran pests since their introduction in 2008. Corn earworm, *Helicoverpa zea* (Boddie); and the armyworm complex including fall armyworm, *Spodoptera frugiperda* (J.E. Smith); and *Spodoptera exigua* (Hübner); are significant pests of agroecosystems in the Mid-southern and Southeastern regions of the United States. They have developed resistance to, and/or inconsistent control has occurred with most chemical classes. The objectives of this study were to establish susceptibility levels of field populations of *H. zea*, *S. frugiperda*, and *S. exigua* collected in the Mid-southern and Southeastern regions of the United States to flubendiamide and chlorantraniliprole. To achieve equivalent levels of mortality for each species, a higher concentration of flubendiamide was required compared to chlorantraniliprole. Furthermore, two experiments were conducted to determine the systemic and residual efficacy of chlorantraniliprole and flubendiamide against *H. zea* on vegetative and reproductive structures of soybean. Chlorantraniliprole moved systemically and had significantly greater control than flubendiamide in the systemic and

residual study out to 31 DAT. Flubendiamide did not move systemically but provided significant residual control out to 31 DAT compared with the untreated control. Neither insecticide was detected in reproductive structures. Finally, to determine the risk of resistance development, a *S. exigua* colony, originating from a field collection in 2013, was separated into three cohorts that were independently selected with three concentrations (0.016, 0.020, and 0.025 ppm) of flubendiamide incorporated into a meridic diet. These concentrations were chosen from the LC<sub>30</sub>, LC<sub>60</sub> and LC<sub>90</sub> of the original colony. Resistance ratios never increased past 2.11-fold. The highest resistance ratios occurred after 18 generations for the LC<sub>30</sub> colony, 19 generations for the LC<sub>60</sub> colony, and 13 and 15 generations for the LC<sub>90</sub> colony. After reaching their highest point of resistance, the colonies began to decline in egg production and larval survivability and did not recover. After 22 generations the selected colonies were terminated. The results from this portion of the study suggest that the potential for resistance development of beet armyworm to flubendiamide is unclear.

## DEDICATION

I dedicate this dissertation to my parents Donny and Donna Adams. I would not be where I am today without them. They supported me unconditionally through this journey and made all of this possible. I hope that this dissertation reflects the love and gratitude I have for you.

## ACKNOWLEDGEMENTS

First and foremost, I would like to thank God. Without his strength, guidance, grace, and mercy I would have never made it. He is the reason for my success. Secondly, I would like to thank my beautiful fiancé who supported me through all of this and made all of it worthwhile. I would like to thank Dr. Jeff Gore and Dr. Angus Catchot who believed in me and provided me with this great opportunity. I would also like to thank my committee members Dr. Fred Musser, Dr. Don Cook, Dr. Trent Irby, and Dr. Natraj Krishnan for their support and guidance throughout my time at Mississippi State University. My major professors and committee members are second to none and made my graduate experience very meaningful. Their doors were always open and they were never too busy to help with whatever was needed. The wisdom and guidance that I gained from these men allowed me to develop personal and professional skills that will benefit me for the rest of my life. I would also like to thank Dr. Gordon Snodgrass, Dr. Ryan Jackson, and Dr. Melissa Siebert for allowing me to work in their programs and provided me with valuable insight and experience in the field of entomology. I would also like to thank my fellow graduate students that helped me throughout my time here. There are too many of you to name but you are all greatly appreciated. I hope that this dissertation reflects the gratitude and appreciation that I have for everyone involved and is representative of the valuable education that I received during my time here.

## TABLE OF CONTENTS

DEDICATION .....	ii
ACKNOWLEDGEMENTS .....	iii
LIST OF TABLES .....	vi
LIST OF FIGURES .....	vii
CHAPTER	
I. INTRODUCTION .....	1
Soybeans .....	1
Mississippi Production Practices .....	4
Lepidopteran Pests of Soybean .....	5
Life Cycles .....	6
Geographic Distribution .....	8
Pests in Agroecosystems .....	9
Damage in <i>G. max</i> .....	11
Chemical Control .....	14
Diamides .....	17
Literature Cited .....	23
II. SUSCEPTIBILITY OF SELECTED LEPIDOPTERAN PESTS TO THE DIAMIDES IN THE MID-SOUTHERN AND SOUTHEASTERN UNITED STATES .....	33
Abstract .....	33
Introduction .....	34
Materials and Methods .....	36
Insect rearing .....	36
Bioassays .....	38
Data Analysis .....	39
Results and Discussion .....	39
References .....	46
III. RESIDUAL AND SYSTEMIC EFFICACY OF CHLORANTRANILIPROLE AND FLUBENDIAMIDE IN MISSISSIPPI SOYBEAN .....	50

Abstract.....	50
Introduction .....	51
Materials and Methods .....	53
Insect Rearing.....	54
Field Plot Details .....	55
Leaf Assays .....	56
Pod and Seed Assays.....	58
Greenhouse Study.....	59
Results .....	61
Leaf Assays at the V4 and R3 Applications.....	61
Pod and Seed Assays at the R3 Application.....	62
Greenhouse Study.....	62
Discussion.....	63
Literature Cited.....	70
IV.    LABORATORY SELECTION FOR BEET ARMYWORM (LEPIDOPTERA: NOCTUIDAE) RESISTANCE TO FLUBENDIAMIDE.....	73
Abstract.....	73
Introduction .....	74
Materials and Methods .....	75
Results and Discussion.....	79
Literature Cited.....	85
V.    SUMMARY AND CONCLUSION .....	87

## LIST OF TABLES

2.1	Description of <i>Helicoverpa zea</i> , <i>Spodoptera frugiperda</i> , and <i>Spodoptera exigua</i> , field derived populations and laboratory colonies by identification code, collection host, month collected, and collection location.....	43
2.2	Comparative susceptibility of <i>H. zea</i> , <i>S. frugiperda</i> , and <i>S. exigua</i> larvae to chlorantraniliprole in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet.....	44
2.3	Comparative susceptibility of <i>H. zea</i> , <i>S. frugiperda</i> , and <i>S. exigua</i> larvae to flubendiamide in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet.....	45
3.1	Mean (SEM) levels of mortality of <i>H. zea</i> exposed to <i>G. max</i> leaves that developed after application and leaves present at time of application when treated with chlorantraniliprole or flubendiamide at the R3 growth stage during 2013-2015.....	67
4.1	Susceptibility of <i>S. exigua</i> reared on non-treated diet measured in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet through 18 generations .....	81
4.2	Susceptibility of <i>S. exigua</i> exposed to flubendiamide at a concentration of 0.016 ppm measured in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet through 19 generations.....	82
4.3	Susceptibility of <i>S. exigua</i> exposed to flubendiamide at a concentration of 0.020 ppm measured in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet through 19 generations.....	83
4.4	Susceptibility of <i>S. exigua</i> exposed to flubendiamide at a concentration of 0.025 ppm measured in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet through 18 generations.....	84

## LIST OF FIGURES

- 3.1 Mean (SEM) levels of mortality of *H. zea* exposed to leaves that developed after application of chlorantraniliprole at the V4 growth stage during 2013-2015. ....66
- 3.2 Mean (SEM) levels of mortality of *H. zea* larvae exposed to *G. max* reproductive structures sprayed with chlorantraniliprole or flubendiamide at the R3 growth stage during 2014-2015.....68
- 3.3 Mean (SEM) levels of mortality of *H. zea* larvae exposed to *G. max* leaf material in laboratory assays with chlorantraniliprole applied to specific vegetative structures at V4 growth stage in a controlled environment during 2014-2015. ....69

## CHAPTER I INTRODUCTION

### **Soybeans**

Soybean, *Glycine max* (L) Merr., was domesticated in the eastern half of North China in the eleventh century B.C. and introduced into the American colonies in 1765 as “Chinese vetches” (North Carolina Soybean Producers Association 2011). The first documented research reports came from Rutgers Agricultural College in New Jersey in 1879 (North Carolina Soybean Producers Association 2011). Initially, *G. max* production increased slowly. By 1924, only 727,200 ha were planted in the US. During this time, *G. max* production was primarily for use as a forage crop rather than seed production (North Carolina Soybean Producers Association 2011). George Washington Carver discovered the use of *G. max* as an oil crop and a protein source for food products (North Carolina Soybean Producers Association 2011). Disruption of trade routes during World War II increased the demand for *G. max* oil as a lubricant and *G. max* meal for food products (North Carolina Soybean Producers Association 2011). This disruption resulted in the rapid expansion of *G. max* production in the US (North Carolina Soybean Producers Association 2011). As a result, production rapidly expanded after World War II. During 2015, 33.1 million ha were planted in the US producing 106 billion kg of grain (NASS 2014). The main *G. max* production areas in the US are the Midwestern states of Iowa, Illinois, Indiana, and Minnesota. However, production in the Mid-southern states has

increased following the decline in cotton, *Gossypium hirsutum* L., production in 2007 (NASS 2007).

*Glycine max* is a short-day annual plant species that initiates flowering with decreasing day length (Purcell et al. 2014). It is divided into maturity groups ranging from 00 to VIII based on the amount of daylight required to initiate flowering (Hartwig 1973). Furthermore, *G. max* growth habits can also be divided into determinate and indeterminate varieties (Fehr and Caviness 1977). Indeterminate varieties are characterized as achieving less than half of their final plant height and node count at flower initiation and continue vegetative growth while seed and pods develop (Fehr and Caviness 1977). In contrast, determinate varieties grow very little in height after flowering begins (Fehr and Caviness 1977).

For both determinate and indeterminate varieties, plant growth progress through a set of ordered growth stages. Several methods for describing *G. max* phenology have been published, but the method most commonly used was developed by Fehr and Caviness (1977). *Glycine max* is generally planted at a depth of 2.5 cm depending on soil temperature and soil moisture (Purcell et al. 2014). Proper germination requires the appropriate soil moisture, temperature, and oxygen (Purcell et al. 2014). Emergence occurs approximately four days after planting when soil temperatures are between 27.7 and 29.4°C (Purcell et al. 2014). When soil temperatures are less than 10°C, emergence can take up to two weeks (Purcell et al. 2014). The hypocotyl is the first structure to emerge from the soil followed by the cotyledons (Purcell et al. 2014). Emergence of cotyledons is considered the VE stage of development (Fehr and Caviness 1977). Following emergence, the cotyledons begin to unroll and a pair of unifoliate leaves

develop directly opposite of each other on the mainstem (Purcell et al. 2014). When these leaves are no longer touching (completely unrolled) the growth stage is termed VC or cotyledon stage (Fehr and Caviness 1977). Following the VC stage, *G. max* puts on trifoliolate leaves in an alternating pattern (Fehr and Caviness 1977, Purcell et al. 2014). Vegetative development is identified beginning at V2 (Purcell et al. 2014). The remaining stages of vegetative growth are designated by the number of trifoliolate leaves to the n<sup>th</sup> degree, or Vn (Fehr and Caviness 1977, Purcell et al. 2014).

Flower initiation is the beginning of the reproductive growth stages (Purcell et al. 2014). They progress from R1 to R8 and are based on flowering, pod development, seed development, and plant maturation (Fehr and Caviness 1977). The R1 growth stage is designated as beginning bloom and occurs when one open flower can be found on any node on the main stem (Fehr and Caviness 1977). The R2 growth stage is designated as full bloom and occurs when an open flower is at one of the two uppermost nodes on the main stem with a fully developed leaf (Fehr and Caviness 1977). Depending on environmental conditions, the time for *G. max* to progress from R1 to R2 growth stage is approximately three days (Fehr and Caviness 1977). During the R2 growth stage, rapid nutrient accumulation occurs throughout the entire plant. (Scott and Aldrich 1983). Nutrient accumulation shifts from the vegetative structures to the reproductive structures for pod and seed development (Scott and Aldrich 1983). The R3 growth stage is designated as the beginning pod stage and occurs when pods reach 5 mm (3/16 inch) in length at one of the four uppermost nodes on the main stem with a fully developed leaf (Fehr and Caviness 1977). The R4 growth stage is designated as the full pod stage and is described as a pod 2 cm (3/4 inch) in length at one of the four uppermost nodes on the

main stem with a fully developed leaf (Fehr and Caviness 1977). Pods will reach full size prior to seed development and are measured from the calyx to the tip of the pod to differentiate between the R3 and R4 growth stages (Fehr and Caviness 1977). The R5 growth stage is designated as the beginning seed stage and occurs when seed development begins within the pod (Fehr and Caviness 1977). Seed 3mm (1/8 inch) in length in a fully developed pod can be found at one of the four uppermost nodes on the main stem with a fully developed leaf in the R5 growth stage (Fehr and Caviness 1977). The pod and seed development growth stages (R3-R5) are the most critical growth stages for yield (Scott and Aldrich, 1983).

The number of pods and seeds that a plant will produce are determined during the R1 to early R5 growth stages. Seed size is determined during the later R5 to R7 growth stages (Pedersen 2004). The R6 growth stage is designated as the full seed stage and is described as when a pod containing a green seed fills the pod cavity at one of the four uppermost nodes on the main stem with a fully developed leaf (Fehr and Caviness 1977). The R7 growth stage is when plants begin maturity and is described as when one normal pod on the main stem has reached its mature pod color (Fehr and Caviness 1977). The R8 growth stage is full maturity and is characterized by when ninety-five percent of the pods have reached their mature pod color (Fehr and Caviness 1977). At R8 an additional five to ten days may be required to reach a harvestable moisture of 15%.

### **Mississippi Production Practices**

*Glycine max* is the most valuable row crop commodity in Mississippi in terms of planted area and total commodity value. During 2014, *G. max* accounted for 898,402 planted ha valued at US\$1,113,200,000 in Mississippi (NASS 2014). Maturity Groups IV

and V varieties are the most commonly grown in Mississippi (Heatherly et al. 1999). In general, planting in Mississippi occurs from March to June with the majority of *G. max* plantings occurring from April to mid-May. *Glycine max* planted at the end of May and into June are typically part of a double crop system that is planted following wheat, *Triticum aestivum* (L), harvest. *Glycine max* production in Mississippi comprises a variety of agronomic practices (Heatherly et al. 1999). *Glycine max* is grown in irrigated and non-irrigated conditions (Heatherly et al. 1999). Row spacing varies and ranges from as narrow as 0.19 m up to 1.02 m wide, with plant populations generally ranging from 247,100-494,200 seed per hectare (Heatherly et al. 1999).

### **Lepidopteran Pests of Soybean**

The order Lepidoptera is the most damaging insect order in the southern United States attacking corn, *Zea mays* (L); grain sorghum, *Sorghum bicolor* (L); cotton, *Gossypium hirsutum* (L); *G. max* and vegetable crops (Fitt 1989, Sparks 1979, Moulton et al. 2000). Corn earworm, *Helicoverpa zea* (Boddie); beet armyworm, *Spodoptera exigua* (Hübner); and fall armyworm, *Spodoptera frugiperda* (Smith) are widely distributed polyphagous pests of numerous cultivated crops throughout the Mid-Southern and Southeastern United States (Fitt 1989, Sparks 1979, Moulton et al. 2000). During 2014, these insects resulted in a combined economic loss of USD\$138,874,796 in soybean alone across Alabama, Arkansas, Louisiana, Mississippi, North Carolina, Tennessee, and Virginia in soybean (Musser et al. 2015).

## Life Cycles

Adult female *H. zea* can lay 500 to 3000 eggs in the field during their 8 to 12 day reproductive lifetime; however, most numbers range from 1,000 to 1,500 eggs per female (Fye et al. 1972, Hartstak et al. 1976, Knipling and Stadelbacher 1983). Eggs are described as small, spherical in shape with a flattened base, yellowish-white, developing a red band during incubation and darkening (Neunzig 1964). Eggs are 0.5-0.6 mm in diameter and are laid singly or in groups of two to three near growing points or buds (Fitt 1989, Hardwick 1965). In suitable environments, eggs generally hatch in 3-4 days at 25°C. Larvae progress through 5 to 6 instars in a 12 to 16 day period (Hardwick 1965). Larvae pupate in the soil in close proximity of the host plant at a depth of 2 to 18 cm (Quaintance and Brues 1905). The pupal stage lasts approximately 12 days (Quaintance and Bruce 1905). Adult moths emerge from the soil and have a lifespan of 5-17 days (Quaintance and Brues 1905). Total time to complete one generation is approximately 30 days (Quaintance and Brues 1905).

Diapause begins in late-instar larval and pre-pupal development stages (Fitt 1989). It is triggered when daylength declines to 11.5 to 12.5 hours and is accompanied by mean temperatures approaching 19 to 23°C (Hardwick 1965). The duration of diapause ranges from 187 to 243 days in Mississippi (Stadelbacher and Pfrimmer 1972). Under unseasonably warm conditions, populations of corn earworm will not enter diapause (Hardwick 1965). Diapause is a key physiological adaptation that allows the corn earworm to be a primary insect pest of multiple crops (Fitt 1989). In Mississippi, *H. zea* generally progress through 4 to 6 generations; however an exact number is difficult to determine as generations begin to overlap (Quaintance and Brues 1905, Neunzig 1969).

Adult female *S. frugiperda* prefer to oviposit on the underside of leaves in the lower portions (0 to 15 nodes) of the plant canopy (Ali et al. 1989). Eggs are laid in a cluster ranging in size from a few to greater than 100 and are typically found densely covered in scales and silken threads (Dew 1913, Sparks 1979). Female moths lay approximately 1500 to 2000 eggs over their lifetime. *Spodoptera frugiperda* eggs are oblate-spheroidal in shape and are initially greenish gray in color, becoming progressively darker with age (Luginbill 1928). Eggs hatch in approximately 2 to 4 days at 21.1 to 26.67°C and progress through 5 to 6 larval instars (Luginbill 1928). Pupation occurs in the soil at a depth of 2 to 8 cm with a duration of 7 to 37 days when soil temperatures are 15 to 28.89°C (Sparks 1979).

Adult moths will migrate up to 480 km prior to mating and oviposition (Robinson 1999). *Spodoptera frugiperda* do not enter diapause and instead overwinter in tropical and subtropical environments of the Western Hemisphere where temperatures rarely drop below 10°C (Sparks 1979). As many as ten generations per year can occur within the southern US (Robinson 1999). The number of generations per year decline as populations migrate to northern latitudes (Robinson 1999). Environmental conditions influence the generation times and the number of generations that occur in a season (Sparks 1979). One generation can require as little as 30 days to as long as 90 days for completion (Sparks 1979).

The life cycle of *S. exigua* and *S. frugiperda* are similar. Adult female *S. exigua* moths prefer to oviposit on the underside of leaves in clusters ranging in size from 50 to 150 eggs per mass (Capinera 2014). Female moths can produce up to 2000 eggs but most often produce an average of 300 to 600 eggs during their reproductive life cycle of three

to seven days (Tisdale and Sappington 2001, Capinera 2014). The egg cluster is densely covered in scales and silken threads (Capinera 2014). The shape of the egg is spherical when viewed from above, yet is slightly peaked, tapering to a point, when viewed from the side (Capinera 2014). The eggs are greenish to white in color when initially laid but grow darker as age increases (Capinera 2014). Eggs hatch in two to three days. Larvae progress through five instars in approximately 10 days at 30°C (Fye and McAda 1972). Larvae then move from the host plant to the soil for pupation. Pupation lasts six to seven days when temperatures are optimum. As with *S. frugiperda*, *S. exigua* do not enter diapause and primarily overwinter in continuous generations in the southern most regions of Arizona, Florida, and Texas (Kim and Kim 1997, Capinera 2014).

### **Geographic Distribution**

*Helicoverpa zea* is distributed throughout North, Central, and South America (Kogan et al. 1989). Facultative diapause allows permanent populations to occur in most areas within latitudes 40° north and south (Fitt 1989). In unseasonably warm environments, generations can continue throughout the year without entering diapause (Hardwick 1965).

*Spodoptera frugiperda* is a migratory insect pest of tropical and sub-tropical origins in the Western Hemisphere (Luginbill 1928, Sparks 1979). It has no diapause mechanism, and overwinters in southern regions of Florida and Texas, as well as Mexico and South America (Sparks 1979). Fall armyworm have been documented overwintering along the Gulf of Mexico in milder winters (Sparks 1979, Ashley et al. 1989). The following growing season, fall armyworm disperses through the Eastern and Central US

(Sparks 1979). The northern and western dispersion boundaries appear to be southern Canada and the Rocky Mountains (Pair et al. 1986, Ashely et al. 1989).

*Spodoptera exigua* is documented in 101 countries ranging from 64°N to 45°S (Zheng et al. 2011). It is a temperate species and has no known photoperiod or temperature induced diapause (Kim and Kim 1997). Overwintering populations migrate according to seasonal changes in temperature. The northern boundary for overwintering populations is unclear. It is better defined as an overwintering range of 37.6° to 44°N latitudes (Zheng et al. 2011, Adamczyk et al. 2003). This range is variable based on temperature patterns within regions.

### **Pests in Agroecosystems**

*Helicoverpa zea*, *S. frugiperda*, and *S. exigua* share several ecological attributes that contribute to their pest status. However, *H. zea* is a primary insect pest of agroecosystems in the United States; whereas, *S. frugiperda* and *S. exigua* are sporadic insect pests of agroecosystems in the United States (Sparks 1979, Fitt 1989, Zheng et al. 2011). The reason *S. frugiperda* and *S. exigua* are classified as sporadic insect pests is due to their migratory behavior; whereas, *H. zea* overwinters in harsher environments due to facultative diapause (Sparks 1979, Fitt 1989, Zheng et al. 2011, Hardke et al. 2015). However, *H. zea*, *S. frugiperda*, and *S. exigua* do share three attributes that contribute to their success as insect pests in agroecosystems.

First, all three species are highly polyphagous insects that feed on a wide range of food, fiber, oil, horticultural crops, and wild hosts (Fitt 1989, Pearson 1982, Stadelbacher et al. 1986, Pashley 1988). *H. zea* has been documented on over 100 plant species in the US (Stadelbacher et al. 1986). *S. frugiperda* has been documented on more than 80

species of plants comprising 23 families (Pashley 1988). *S. exigua* has been documented on more than 90 plant species in at least 18 families (Pearson 1982). The polyphagous nature of these species allows them to persist and increase in diverse environments. This effect is threefold. First, it allows populations to develop on a range of diversified hosts simultaneously. Second, populations can develop continuously during suitable periods by exploiting multiple cultivated and uncultivated hosts. Finally, small populations can survive in unsuitable environments because their broad host range increases the likelihood that the female can find a suitable host for oviposition (Fitt 1989).

Mobility is the second factor that contributes to the pest status of *H. zea*, *S. frugiperda*, and *S. exigua*. These species have the ability to travel locally and interregionally (Sparks 1979, Mitchell 1979, Fitt 1989). The ability to travel within cropping systems, between cropping systems, and if necessary to travel regionally in migratory flight allows coincidence with the spatial and temporal distribution of hosts (Fitt 1989). Short and long range movements occur just above the host canopy up to 10 meters and can be in response to needs based on feeding, oviposition, mating, and shelter (Fitt 1989, Sparks 1979, Mitchell 1979). Migration, or regional travel, generally occurs at an altitude of 1-2 km and can result in a migration of hundreds of kilometers (Fitt 1989, Ashley et al. 1989). Regional migration primarily occurs when larval and adult nutrition is lacking or when ovipositional hosts senesce (Fitt 1989). Modern agroecosystems provide continuous feeding and oviposition sites throughout the growing season. Mobility allows the movement to available hosts through successively planted agroecosystems and is a key attribute to the seasonal dynamics of these insect pest species.

High fecundity and quick generational turnover is the third key contributor to the pest status of these insect species. The host selection behavior of *H. zea* is not greatly understood, but the females prefer to oviposit on hosts in the flowering stage (Johnson et al. 1975). It is further suggested that females will oviposit on additional hosts that would otherwise not be selected for oviposition in the absence of flowering hosts (Parsons 1940). In *Spodoptera* spp, egg masses are laid on the bottom half of the plant canopy, thus they are not easily observed, allowing populations to develop rapidly (Smith 1989). Overall, oviposition relies on the spatial and temporal distribution of hosts at the appropriate growth stage (Fitt 1989). High numbers of eggs and multiple oviposition events during the lifecycle ensure the pest potential to some degree on an annual basis.

### **Damage in *G. max***

*Helicoverpa zea* is a major insect pest of *G. max* in the Mid-Southern and Southeastern US. Yearly infestations of *H. zea* result in millions of dollars in damage each year (Fitt 1989). It is a primary pest of *G. max*, resulting in economic damage in most years (Musser et al. 2015). In Mississippi, the first generation of corn earworm occurs in the spring on crimson clover, *Trifolium incarnatum* (L), and cut-leaf geranium, *Geranium dissectum* (L), (Snow and Brazzel 1965, Stadelbacher 1981). The second generation occurs primarily on its preferred host plant *Z. mays*, but will continue to develop on many wild hosts when *Z. mays* is not available (Johnson et al. 1975). When *Z. mays* senesces, the third and fourth generations migrate to *G. hirsutum* and *G. max* (Hartstack et al. 1973). Additional generations will migrate to wild hosts and volunteer corn that has emerged following *G. hirsutum* and *G. max* harvest (Hartstack et al. 1973).

*Helicoverpa zea* infestations generally occur during the R1 to R3 growth stages and in open canopied fields when *G. max* is most attractive for oviposition (Johnson et al. 1975, Swenson et al. 2013). Infestations at R4 and R5 can be common in some areas (McPherson and Moss 1989). Early instar larvae (1-3) can typically be found on young foliage; whereas, later instars (4-6) prefer to feed on older foliage when infestations occur during the vegetative stages (Eckel 1992a). Yield is limited by larval feeding. Larval feeding can reduce leaf surface area, delay pod fill, and reduce the number of seed per pod when populations are above the economic threshold (Eckel et al. 1992b, Swenson et al. 2013).

*Helicoverpa zea* is a direct pest of soybean because it prefers to feed on fruiting structures, so thresholds tend to be low (Hardwick 1965). Four factors contribute to the severity of damage from larval feeding; larval age, plant growth stage, timing of damage, and the ability of the plant to compensate from larval feeding (Swenson et al. 2013). All larval instars prefer to feed on blooms over leaves or pods (Mueller and Engroff 1980). When blooms are not readily available larvae prefer to feed on pods and seeds (Mueller and Engroff 1980, Swenson et al. 2013). Damage per larva can be most severe during the R3-R4 growth stages because a greater number of small pods and immature seeds can be consumed per larva, compared with larval feeding in the later growth stages (R5-R6) where more developed pods are common (McWilliams 1983, Swenson et al. 2013). *Glycine max* can compensate from damage incurred during early growth stages (R1-R3) (Eckel 1992b). However, the ability of *G. max* to compensate for larval damage is dependent on environmental conditions and early season damage may result in delayed pod set (Eckel 1992b). The ability of *G. max* to compensate in early growth stages is

important, but the possible delay in maturity may be problematic for *G. max* not planted in the optimal planting window. Damage incurred during later growth stages (R4-R5) limits time for compensation, and yield losses are more directly related to pod removal and seed consumption (Thomas et al. 1974, McPherson and Moss 1989).

*Spodoptera frugiperda* prefer to oviposit on *Z. mays*, but *G. max* is a suitable host when *Z. mays* is not available (Pitre and Hogg 1983). *Spodoptera frugiperda* is an occasional yet severe pest of *G. max* in large numbers (Pitre et al. 1983). Infestations during early vegetative development can reduce the number of plants per ha (Sparks 1979). Because *S. frugiperda* is a migratory insect pest, this situation in MS would most likely occur in *G. max* fields that were planted later in the growing season behind wheat (Sparks 1979). Adult moths prefer to oviposit on plants that are 54 to 64 days old versus plants that are 22 to 42 days old (Pitre et al. 1983). These older plants provide a greater level of protection, higher nutritional value, and ease of dispersion for larvae, and it is difficult for insecticides to penetrate the canopy (Pitre et al. 1983, Hardke et al. 2015). Later growth stage infestations result in defoliation and damage to pods (Sparks 1979). Recommendations for control of *S. frugiperda* in Mississippi are based on defoliation and stand reduction rather than insect numbers (Catchot et al. 2016).

*Spodoptera exigua* prefer to infest seedling *G. max* and primarily feed on foliage (Pearson 1982). However, infestations occurring during the reproductive growth stages can result in damage from feeding on fruiting structures (Huffman and Mueller 1983). Huffman and Mueller (1983) observed larval feeding preference to be on bloom petals, when available, and on fully expanded leaves. The first through third instars skeletonized leaf surfaces, whereas later stages ingest leaf margins, leaving veins intact (Pearson 1982,

Huffman and Mueller 1983). *Spodoptera exigua* prefer to feed on young tissue and population size decreases in the late R5 growth stage when leaves become leathery and tough (Huffman and Mueller 1983). Yield losses may occur when sequential infestations of large populations occur during the R3 to R7 growth stages (Thomas et al. 1974, Thomas et al. 1978). High populations are required to initiate control measures (Hoffman and Mueller 1983). As with *S. frugiperda*, recommendations for control of *S. exigua* in Mississippi are based on defoliation rather than actual insect numbers (Catchot et al. 2016).

The implementation of the early production system described by Heatherly et al. (1999) reduces late season insect pest problems in the Mid-South (Baur et al. 2000). However, the availability of hosts, high reproductive rate, short generation time, and mobility allow *H. zea*, *S. frugiperda*, and *S. exigua* to migrate to later *G. max* plantings that follow wheat harvest. Fields with later planting dates are the most prolific hosts and are prone to more severe pest outbreaks at times. In these instances, control of *H. zea*, *S. frugiperda*, and *S. exigua* is almost always achieved through the application of synthetic insecticides (Catchot et al. 2016).

### **Chemical Control**

Foliar application of synthetic insecticides to *G. max* is second to planting date as a control method for lepidopteran insect pests when they occur in Mississippi (Catchot et al. 2016). Widespread foliar applications of synthetic insecticides in multiple crops has led to resistance development and/or inconsistent control with most chemical classes (Sparks 1981, Brown et al. 1998, Temple et al. 2006, Jacobson et al. 2009, Lai and Su 2011).

Resistance to the carbamate, organophosphate, cyclodiene, organochlorine, and pyrethroid classes of insecticides has been documented for *H. zea* (IRAC 2015b). Historically, good control of *H. zea* was achieved with the use of DDT and other organochlorine insecticides (Brazzel 1964, Sparks 1981). However, the widespread use in cotton led to field control failures and documented resistance to the organochlorines, DDT, and organophosphates (Sparks 1981). Newer insecticides in the classes of organophosphates and carbamates were introduced, but the majority of applications targeting *H. zea* were replaced with the use of pyrethroid applications as the primary means of control during the late 1970's and early 1980's (Martinez-Carrillo and Reynolds 1983, Jacobson et al. 2009, Temple et al. 2009). Resistance to pyrethroids has been reported in Mississippi, Arkansas, South Carolina, Florida, Louisiana, Texas, Illinois, and Indiana (Stadlebacher et al. 1990, Hsu and Yu 1991, Abd-Elghafar et al. 1996, Kanga et al. 1996, Brown et al. 1998, Ottea and Holloway 1998, Jacobson et al. 2009, Temple et al. 2009). As a result, the recommended insecticides to control *H. zea* in *G. max* in Mississippi include carbamates (methomyl), spinosyns (spinetoram, spinosad), oxadiazines (indoxacarb), and diamides (chlorantraniliprole, flubendiamide) (Catchot et al. 2016, IRAC Mode of Action Classification Scheme 2015).

Resistance in *S. frugiperda* has been documented to carbamates, organophosphates, and pyrethroids (Wood et al. 1981, Yu 1992, Adamczyk et al. 1999, Al-Sarar et al. 2006, IRAC 2015c). Detecting *S. frugiperda* is difficult because they feed in the lower portion of the canopy (Ali et al. 1990). Oftentimes, *S. frugiperda* are generally at later developmental instars when applications are initiated (Ali et al. 1990, Sullivan et al. 1999). This inherently increases the difficulty in control because larger

larvae are more difficult to control than small larvae (Yu 1983). Furthermore, penetrating the plant canopy is difficult with insecticide applications (Mink and Luttrell 1989, Ali et al. 1990). *Spodoptera frugiperda* infestations generally require multiple applications to reduce populations to sub-economic levels (Sullivan et al. 1999, Hardke et al. 2015). Insecticides currently recommended in Mississippi *G. max* production targeting *S. frugiperda* are the pyrethroids (beta-cyfluthrin, bifenthrin, lambda-cyhalothrin, zeta-cypermethrin, etc.), organophosphates (acephate), spinosyns (spinetoram, spinosad), oxadiazines (indoxacarb), insect growth regulator (diacylhydrazines; methoxyfenozide) and diamides (chlorantraniliprole, flubendiamide (Catchot et al. 2016, IRAC Mode of Action Classification Scheme 2015)).

Layton (1994) described *S. exigua* as being inherently tolerant to most classes of insecticides. Resistance to the carbamate, organophosphate, phenylpyrazole, pyrethroid, neonicotinoid, spinosyn, avermectin-milbemycin, chlorfenapyr-DNOC-sulfuramid, benzoylurea, diacylhydrazine, and oxadiazine classes of insecticides has been documented for *S. exigua* (IRAC 2015a). In the mid 1980's thiodicarb and chlorpyrifos were the most effective insecticides for *S. exigua* control in Louisiana (Burriss 1983). Inconsistent and/or unsatisfactory control from these two insecticides were reported by the mid 1990's (Burriss et al. 1994, Layton et al. 1994, Graves et al. 1995). Insecticides currently recommended in Mississippi *G. max* production targeting *S. exigua* are the spinosyns (spinetoram, spinosad), oxadiazines (indoxacarb), insect growth regulators (diacylhydrazines; methoxyfenozide) and diamides (chlorantraniliprole, flubendiamide) (Catchot et al. 2016, IRAC Mode of Action Classification Scheme 2015).

In 2003, 75% of insecticide market shares acted on only four target sites (Casida 2009). The majority of insecticides in use today act on the nervous system at the synapse or the axon (Casida 2009). Though many classes are structurally diverse they still act at the same target site (Casida 2009). The majority of insecticide resistance events can be attributed to increased detoxification by microsomal oxidases and target site insensitivity (Yu 1992). Common target sites oftentimes result in the occurrence of cross resistance. Resistance to DDT extended to pyrethrins and pyrethroids through target site insensitivity (Casida 2009). Organophosphate resistant insects can also incur cross-resistance to other organophosphate insecticides and methylcarbamates by way of acetylcholinesterase modifications (Casida 2009). The development and use of new insecticide technologies is important for insecticide resistance management. Furthermore, the necessity for new insecticide technologies to be effective, selective, and safe has resulted in the introduction of new insecticides that are more potent and have a higher degree of organismal specificity (Casida 2009).

## **Diamides**

Insects have an innate ability to develop resistance to existing insecticides and there are a limited number of target sites that provide activity sufficient for crop protection (Cordova et al. 2006). The discovery and use of insecticides with novel modes of action are important for insecticide resistance management programs (Sparks 2013). In 2008, the diamide class of insecticides was introduced and is the newest class of insecticides (EPA 2008). It has a novel mode of action classified as ryanodine receptor modulators (MoA Group 28) (IRAC 2015). Ryanodine receptors (RyR) are intracellular calcium channels located in the sarcoplasmic reticulum that specialize in the rapid and

massive release of calcium from intracellular stores, which is necessary for excitation contraction coupling in striated muscle. (Ebbinghaus-Kintscher et al. 2006). Calcium serves as the primary physiological regulator of insect ryanodine receptors (Xu et al. 2000, Scott-Ward et al. 2001). Diamide insecticides directly activate the ryanodine receptor by binding to the ryanodine receptor complex, blocking the ryanodine receptor open (Cordova et al. 2006). The prolonged opening of the ryanodine receptor prompts the release of intracellular calcium stores, resulting in cessation of feeding and uncoordinated muscle contraction of intoxicated insects, resulting in death (Cordova et al. 2006, Ebbinghaus-Kintscher et al. 2006, Nauen et al. 2007, Hannig et al. 2009, Roditakis et al. 2015). The diamide insecticides are characterized by their low mammalian toxicity and are effective against a large number of lepidopteran species and other orders including Coleoptera, Diptera, Isoptera and Hemiptera. (Sattelle et al. 2008, Tohnishi et al. 2005, Lahm et al. 2009, Teixeira and Andaloro 2013, Qi et al. 2014). Two representatives from this class of insecticides are flubendiamide (Belt Bayer CropScience, Raleigh, NC), a phthalic acid diamide and chlorantraniliprole (Prevathon DuPont Crop Protection, Newark, DE), an anthranilic diamide (Lahm et al. 2009). Although they are structurally independent, these insecticides share the same target site (Lahm et al. 2009, Teixeira and Andaloro 2013). To date, there have been no reports of cross resistance with other classes of insecticides.

The diamides are highly toxic to lepidopteran insect pests. Cordova et al. (2006) reported that *S. frugiperda* was 50-fold more susceptible to chlorantraniliprole compared with cypermerthrin and 3-fold more susceptible compared with indoxacarb. Chlorantraniliprole provides longer residual efficacy than most standard insecticides.

Hardke et al. (2011) reported residual efficacy of greater than 21 days after treatment for *S. frugiperda* in sorghum treated with chlorantraniliprole. In Hardke et al. (2011), *S. frugiperda* mortality tested on grain sorghum tissue treated with chlorantraniliprole was 100, 96.9, 85.9, 82.8, and 63.1% at 0, 7, 14, 21, and 28 days after treatment, respectively. In comparison, *S. frugiperda* mortality tested on grain sorghum tissue treated with flubendiamide was 93.8, 53.1, 26.6, and 9.4% at 0, 7, 14, and 21 days after treatment (Hardke et al. 2011). Mortality of *S. frugiperda* in plots treated with flubendiamide was not different from plots treated with chlorantraniliprole at 0 days after treatment (Hardke et al. 2011). However, chlorantraniliprole provided significantly greater control of *S. frugiperda* compared with flubendiamide and the untreated control out to 28 days after treatment (Hardke et al. 2011). Flubendiamide was not significantly different compared with the untreated control at 21 days after treatment (Hardke et al. 2011). Hardke et al. (2011) reported the residual efficacy of chlorantraniliprole to be greater than flubendiamide but did not discuss or declare a hypothesis for this observation.

Chlorantraniliprole is xylem-mobile, allowing the insecticide to move upwards throughout the plant (Lahm et al. 2007). It is often applied to the soil as seed treatments, soil drenches, or through chemigation in multiple crops such as brassicas and other vegetables (Cameron et al. 2015, Lahm et al. 2007, Schuster et al. 2009, Ghidui et al. 2009, Khuhar et al. 2008, Palumbo 2008). With those applications, the insecticide is taken up by the roots and provides effective control of lepidopteran and other insect pests in the foliage. It is currently registered in the US for use as an in-furrow spray at planting, transplant water treatment, hill drench at planting, surface band at planting, soil shank injection at planting, through drip irrigation, and by foliar application (Lahm et al.,

2007; Cameron et al. 2015). Furthermore, chlorantraniliprole is also effective as a seed treatment to manage *Lissorhoptrus oryzophilus* (Kuschel) infestations in rice, *Oryza sativa* (L), (Adams et al. 2015).

The primary function of xylem is to transport water and minerals from the roots to aerial portions of the plant (Lucas et al. 2013). Chlorantraniliprole is used extensively in vegetable production (Ghidiu et al. 2009). The systemic efficacy of chlorantraniliprole against lepidopteran pest species when applied to the root zone has been well documented (Lahm et al. 2007, Schuster et al. 2009, Ghidiu et al. 2009, Khuhar et al. 2008, Palumbo 2008). Ghidiu et al. (2009) reported that two applications of chlorantraniliprole through drip irrigation provided season long control of European corn borer, *Ostrinia nubilalis* (Hübner), in bell peppers, *Capsicum annuum* (L), and was as effective as up to nine foliar applications of a standard insecticide program.

The use of chlorantraniliprole and flubendiamide has been readily adopted since their introduction. Eight years after their introduction to the global market, these two active ingredients comprise 7% of global insecticide use (Sparks 2013). Large global market shares result from the favorable biological, ecological, and toxicological attributes of this insecticide class (Tiexeira and Andaloro 2013). It is perceived that the use of this insecticide class will continue to increase globally on a wide variety of crops (Tiexeira and Andaloro 2013, Roditakis et al. 2015).

Repeated field applications of the diamide insecticides have resulted in numerous cases of resistance development for several lepidopteran species (Roditakis et al. 2015). To date, cross resistance between chlorantraniliprole and flubendiamide has been documented for diamondback moth, *Plutella xylostella* (L), smaller tea tortrix,

*Adoxophyes honmai* (Yasuda), and tomato borer, *Tuta absoluta* (Meyrick) (Wang and Wu 2012, Uchiyama and Ozawa 2014, Roditakis et al. 2015). Also, resistance to chlorantraniliprole has been documented for rice stem borer, *Chilo suppressalis* (Walker), cutworm, *Spodoptera litura* (F), and *S. exigua* (Su et al. 2012, Che et al. 2013, Gao et al. 2013). Furthermore, resistance to flubendiamide has been documented in *C. suppressalis* (Walker) (Wu et al. 2014).

All of the investment in developing a potent and safe insecticide can be lost if insect resistance management strategies are not used. Insecticide resistance management is growing increasingly more difficult by the optimization of target site potency and low doses (Casida 2009). This could lead to more rapid detoxification by the pest of the exceedingly small amount of pesticide (Casida 2009). Monitoring susceptibility levels of target pest species is important for pest management and resistance management efforts. Furthermore, development of successful insecticide resistance management strategies requires the establishment of baseline susceptibility levels of target pest species while resistant allele frequencies are low (French-Constant and Roush 1990, Cook et al. 2005). Baseline susceptibility to the diamide insecticide class was generated in Louisiana by Hardke et al. (2011) for *S. frugiperda* and Temple et al. (2009) for *H. zea*. However, baseline data have not been produced for these species and *S. exigua* populations for Mississippi or other states in the Mid-South and Southeastern US.

Baseline responses of laboratory and field strains of target pest populations to novel modes of action act as a historical reference, and are necessary to mitigate resistance development, prolonging the effectiveness of novel modes of action. Furthermore, chlorantraniliprole and flubendiamide have greater residual efficacy

compared to other insecticides (Hardke et al. 2011). The diamide insecticides have long residual efficacy and can expose multiple generations of insect pests to the insecticide class. The residual efficacy of chlorantraniliprole is greater than flubendiamide.

Chlorantraniliprole is xylem mobile and taken up through the root zone but it is not known if it is translocated when applied as a foliar application. This could explain the differences observed in the residual efficacy of these two insecticides. Furthermore, because of the long residual efficacy of these insecticides and their widespread use, it is important to understand the risk of resistance development with this insecticide class. Therefore it is necessary to understand the impact of selection pressure on the risk of resistance development. The objectives of these studies were:

- I. To generate baseline dose-mortality responses of *H. zea*, *S. frugiperda*, and *S. exigua* to flubendiamide and chlorantraniliprole for future resistance monitoring efforts and development of resistance management strategies.
- II. To determine the systemic and residual efficacy of chlorantraniliprole and flubendiamide against corn earworm, through laboratory bioassays, when applied as a foliar application to soybean.
- III. To determine the influence of selection pressure with flubendiamide on resistance development in *S. exigua*.

## Literature Cited

- Abd-Elghafar, S. F., and C. O. Knowles. 1996.** Pharmacokinetics of fenvalerate in laboratory and field strains of *Helicoverpa zea* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 89: 590-593.
- Adamczyk, Jr., J. J., B. R. Leonard, and J. B. Graves. 1999.** Toxicity of selected insecticides to fall armyworms (Lepidoptera: Noctuidae) in laboratory bioassay studies. *Florida Entomol.* 82: 231-236.
- Adamczyk, Jr., J. J., M. R. Williams, J. T. Reed, D. W. Hubbard, and D. D. Hardee. 2003.** Spatial and temporal occurrence of beet armyworm (Lepidoptera: Noctuidae) moths in Mississippi. *Florida Entomol.* 86: 229-232.
- Adams, A., J. Gore, F. Musser, D. Cook, A. Catchot, T. Walker, and C. Dobbins. 2015.** Efficacy of selected insecticides applied to hybrid rice seed. *J. Econ. Entomol.* 109: 200-206.
- Ali, A., R. G. Luttrell, H. N. Pitre, and F. M. Davis. 1989.** Distribution of fall armyworm (Lepidoptera: Noctuidae) egg masses on cotton. *Environ. Entomol.* 18: 881-885.
- Ali, A., R. G. Luttrell, and H. N. Pitre. 1990.** Feeding sites and distribution of fall armyworm (Lepidoptera: Noctuidae) larvae on cotton. *Environ. Entomol.* 19: 1060-1067.
- Al-Sarar, A., F. R. Hall, and R. A. Downer. 2006.** Impact of spray application methodology on the development of resistance to cypermethrin and spinosad by fall armyworm *Spodoptera frugiperda* (J.E. Smith). *Pest Management Sci.* 62: 1023-1031.
- Ashley, T. R., B. R. Wiseman, F. M. Davis, and K. L. Andrews. 1989.** The fall armyworm: a bibliography. *Florida Entomol.* 72: 152-202.
- Baur, M. E., D. J. Boethel, M. L. Boyd, G. R. Bowers, M. O. Way, L. G. Heatherly, J. Rabb, and L. Ashlock. 2000.** Arthropod populations in early soybean production systems in the Mid-South. *Enomol.* 29: 312-328.
- Brazzel, J. R. 1964.** DDT resistance in *Heliothis zea*. *J. Econ. Entomol.* 57: 454-457.
- Brown, T. M., P. K. Bryson, D. S. Brickle, J. T. Walker, and M. J. Sullivan. 1998.** Pyrethroid-resistant *Helicoverpa zea* in cotton in South Carolina. *Crop Prot.* 17: 441-445.
- Burris, E., J. B. Graves, B. R. Leonard, and C.A. White. 1994.** Beet armyworms (Lepidoptera: Noctuidae) in northeast Louisiana: observations on an uncommon insect pest. *Fla. Entomol.* 77: 454-459.

- Cameron R. A., C. J. Williams, H. E. Portillo, P. C. Marcon, and L. A. Teixeira. 2015.** Systemic application of chlorantraniliprole to cabbage transplants for control of foliar-feeding lepidopteran pests. *Crop Protect.* 67: 13-19.
- Capinera, J. L. 2014.** Featured Creatures, beet armyworm, *Spodoptera exigua* (Hubner). University of Florida. Publication EENY-105. Accessed: Jan. 2016.  
[http://entnemdept.ufl.edu/creatures/veg/leaf/beet\\_armyworm.htm](http://entnemdept.ufl.edu/creatures/veg/leaf/beet_armyworm.htm)
- Casida, J. E. 2009.** Pest toxicology: the primary mechanisms of pesticide action. *Chem. Res. Toxicol.* 22: 609-619.
- Catchot, A., C. Allen, D. Cook, D. Dodds, J. Gore, T. Irby, E. Larson, B. Layton, S. Meyers, and F. Musser. 2016.** Insect control guide for agronomic crops. Mississippi State University, Mississippi State, MS. Publication No. 2471.
- Che W., T. Shi, Y. Wu, and Y. Yang. 2013.** Insecticide resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) from China. *J. Econ. Entomol.* 106: 1855–1862.
- Cook, D.R., B.R. Leonard, J. Gore, and J.H. Temple, 2005.** Baseline responses of bollworm, *Helicoverpa zea* (Boddie), and Tobacco Budworm, *Heliothis virescens* (F.), to indoxacarb and pyridalyl. *J. Agric. Urban Entomol.* 22: 100-109.
- Cordova, D., E. A. Benner, M. D. Sacher, J. J. Rauh, J. S. Sopa, G. P. Lahm, T. P. Selby, T. M. Stevenson, L. Flexner, S. Gutteridge, D. G. Rhoades, L. Wu, R. M. Smith, and Y. Tao. 2006.** Anthranilic diamides: a new class of insecticides with a novel mode of action, ryanodine receptor activation. *Pest. Biochem. Phys.* 84: 196-214.
- Dew, J. A. 1913.** Fall armyworm. *J. Econ. Entomol.* 6: 361-366.
- Ebbinghaus-Kintscher U, P. Luemmen, N. Lobitz, T. Schulte, C. Funke, R. Fischer, T. Masaki, N. Yasokawa, and M. Tohnishi. 2006.** Phthalic acid diamides activate ryanodine-sensitive Ca<sup>2+</sup> release channels in insects. *Cell Calcium* 39: 21–33.
- Eckel, C. S., L. I. Terry, J. R. Bradley, Jr., and J. W. Van Duyn. 1992a.** Changes in within-plant distribution of *Helicoverpa zea* Boddie (Lepidoptera: Noctuidae) on soybean. *Environ. Entomol.* 21: 287–293.
- Eckel, C. S., J. R. Bradley, Jr., and J. W. Van Duyn. 1992b.** Soybean growth and development alterations caused by *Helicoverpa zea* (Boddie) feeding. *Agron. J.* 84: 813–820.
- EPA. 2008.** Pesticide Fact Sheet, Chlorantraniliprole,  
[http://www3.epa.gov/pesticides/chem\\_search/reg\\_actions/registration/fs\\_PC-090100\\_01-Apr-08.pdf](http://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-090100_01-Apr-08.pdf). Accessed: Jan. 2016.

- Fehr, W. R., and C. E. Caviness. 1977.** Stages of soybean development. Iowa St. University Ames, IA.
- French-Constant, R. H., and R. T. Roush. 1990.** Resistance detection and documentation: the relative roles of pesticidal and biochemical assays, pp 4-38. *In* R. T. Roush and B. E. Tabashnik [Eds.], Pesticide Resistance in Arthropods. Chapman and Hall, New York, New York and London, United Kingdom.
- Fitt, G.P. 1989.** The ecology of *Heliothis* species in relation to agroecosystems. *Ann. Rev. Entomol.* 34: 17-52.
- Fye, R. E. and W. C. McAda. 1972.** Laboratory studies on the development, longevity and fecundity of six lepidopterous pests of cotton in Arizona. US Dep. Agric. Agric. Res. Serv. Tech. Bull. Vol. 1454. 72 pp.
- Gao C, R. Yao, Z. Zhang, M. Wu, S. Zhang, and J. Su. 2013.** Susceptibility baseline and chlorantraniliprole resistance monitoring in *Chilo suppressalis* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 106: 2190–2194.
- Ghidiu, G. M., D. L. Ward, and G. S. Rogers. 2009.** Control of European corn borer in bell peppers with chlorantraniliprole applied through a drip irrigation system. *Intern. J. Veg. Sci.* 15: 193-201.
- Graves, J. B., B. R. Leonard, and C. A. White. 1995.** Efficacy of selected commercial and experimental insecticides against late season populations of beet armyworm, soybean looper, and tobacco budworm, 1994. *Arthropod Management Tests.* 20: 197-198.
- Hannig, G. T., M. Ziegler, and P. G. Marcon. 2009.** Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. *Pest Manag. Sci* 65: 969-974.
- Hardke, J. T., J. H. Temple, B. R. Leonard, and R. E. Jackson. 2011.** Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). *Florida Entomol.* 94: 272-278.
- Hardke, J. T., G. M. Lorenz III, and B. R. Leonard. 2015.** Fall armyworm (Lepidoptera: Noctuidae) ecology in Southeastern cotton. *J. Integ. Pest Mngmt.* 6(1): 10; DOI: 10.1093/jipm/pmv009.
- Hardwick, D.F. 1965.** The corn earworm complex. *Memoirs Entomol. Soc. of Am.* 40: 3-246.
- Hartstack, A. W., J. P. Hollingsworth, R. L. Ridgway, and J. R. Coppedge. 1973.** A population dynamics study of the bollworm and the tobacco budworm with light traps. *Environ. Entomol.* 2: 244-252.

- Hartstack, A. W., J. A. Witz, J. P. Hollingsworth, R. L. Ridgeway, J. D. Lopez. 1976.** MothZV-2: a computer simulation of *Heliothis zea* and *Heliothis virescens* population dynamics. User manual. US Dep. Agric. Aric. Res. Serv. S-127. Washington DC. 55 pp.
- Hartwig, E. E. 1973.** Varietal development, pp. 187-210. *In* B. E. Caldwell (ed.), Soybeans: improvement, production, and uses. Agron. Monogr., Madison, WI.
- Heatherly, L. G., A. Blaine, H. F. Hodges, R. A. Wesley, and N. Buehring. 1999.** Variety selection, planting date, row spacing, and seeding rate, pp. 41-52. *In* L. G. Heatherly and H. F. Hodges (eds.), Soybean production in the Midsouth. CRC Publishing, New York.
- Huffman, F. R., and A. J. Mueller. 1983.** Effects of beet armyworm (Lepidoptera: Noctuidae) infestation levels on soybean. *J. Econ. Entomol.* 76: 744-747.
- Hsu, E. L., and S. J. Yu. 1991.** Insecticide resistance in the corn earworm, *Heliothis zea* (Boddie). *Res. Pest Manag.* 3: 18.
- IRAC. 2015.** IRAC mode of action classification scheme. Version 8.0 [Online]. Available at <http://www.iraconline.org/documents/moa-classification/> Accessed: January 2016.
- IRAC. 2015a.** Beet armyworm. Accessed: January 2016. <http://www.iraconline.org/pests/spodoptera-exigua/>
- IRAC. 2015b.** Corn earworm. Accessed: January 2016. <http://www.iraconline.org/pests/helicoverpa-zea/>
- IRAC. 2015c.** Fall armyworm. Accessed: January 2016. <http://www.iraconline.org/pests/spodoptera-exigua/>
- Jacobson, A., R. Foster, C. Krupke, W. Hutchinson, B. Pittendrigh, and R. Weinzierl. 2009.** Resistance to pyrethroid insecticides in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Indiana and Illinois. *J. Econ. Entomol.* 102: 2289-2295.
- Johnson, M. W., R. E. Stinner, and R. L. Rabb. 1975.** Ovipositional response of *Heliothis zea* (Boddie) to its major hosts in North Carolina. *Environ. Entomol.* 4: 291-297.
- Kanga, L. H. B., F. W. Plapp, Jr., B. F. McCutchen, R. D. Bagwell, and J. D. Lopez, Jr. 1996.** Tolerance to cypermethrin and endosulfan in field populations of the bollworm (Lepidoptera: Noctuidae) from Texas. *J. Econ. Entomol.* 89: 583-589.
- Kim, Y., and N. Kim. 1997.** Cold hardiness in *Spodoptera exigua* (Lepidoptera: Noctuidae). *Environ. Entomol.* 26: 1117-1123.

- Knipling, E. F., and E. A. Stadelbacher, 1983.** The rationale of area-wide management of *Heliothis* (Lepidoptera: Noctuidae) populations. Bull. Entomol. Soc. Am. 29: 29-37.
- Kogan, M., C. G. Helm, J. Kogan, and E. Brewer. 1989.** Distribution and economic importance of *Heliothis virescens* and *Heliothis zea* in North, Central and South America and of their natural enemies and host plants. Proc. Bio. Cont. of *Heliothis*: increasing the effectiveness of natural enemies, New Delhi, India pp. 241-297.
- Kuhar, T. P., H. Doughty, E. Hitchner, and M. Cassell. 2008.** Evaluation of insecticide treatments for the control of lepidopteran pests in bell peppers in Virginia, 2007. Arthropod Mngmt. Tests 33: E7.
- Lahm, G. P., T. M. Stevenson, T. P. Selby, J. H. Freudenberger, D. Cordova, L. Flexner, C. A. Bellin, C. M. Dubas, B. K. Smith, K. A. Hughes, J. G. Hollingshaus, C. E. Clark, and E. A. Benner. 2007.** Rynaxypyr®: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator. Biorg. Med. Chem. Let. 17: 6274-6279.
- Lahm, G. P., D. Cordova, and J. D. Barry. 2009.** New and selective ryanodine receptor activators for insect control. Bioorganic & Medic. Chem. 17: 4127-4133.
- Lai, T. and J. Su. 2011.** Assessment of resistance risk in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) to chlorantraniliprole. Pest Manag. Sci. 67: 1468-1472.
- Layton, M. B. 1994.** The 1993 beet armyworm outbreak in Mississippi and future management guidelines, pp. 854-856. In Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, TN.
- Lucas, W. J., A. Groover, R. Lichtenberger, K. Furuta, S. R. Yadav, Y. Helariutta, X. Q. He, H. Fukuda, J. Kang, S. M. Brady, J. W. Patrick, J. Sperry, A. Yoshida, A. F. López-Millán, M. A. Grusak, and P. Kachroo. 2013.** The plant vascular system: evolution, development and functions. J. Integr. Plant Biol. 55: 294-388.
- Luginbill, P. 1928.** The fall armyworm. USDA Tech. Bull. No. 34
- Martinez-Carrillo, J. L. and H. T. Reynolds. 1983.** Dosage mortality studies with pyrethroids and other insecticides on the tobacco budworm (Lepidoptera: Noctuidae) from the Imperial Valley, California. J. Econ. Entomol. 80: 983-986.
- McPherson, R. M., and T. P. Moss. 1989.** Response of soybean to natural and simulated corn earworm (Lepidoptera: Noctuidae) pod injury. J. Econ. Entomol. 82: 1767-1772.

- McWilliams, J. M. 1983.** Relationship of soybean pod development to bollworm and tobacco budworm damage. *J. Econ. Entomol.* 76: 502–506.
- Mink, J. S., and R. G. Luttrell. 1989.** Mortality of fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae) eggs, larvae and adults exposed to several insecticides on cotton. *J. Entomol. Sci.* 24: 563-571.
- Mitchell, E. R. 1979.** Migration by *Spodoptera exigua* and *S. frugiperda*, North American style, pp. 386-393. *In* Movement of highly mobile insects: concepts and methodology in research. R. L. Rabb and G. G. Kennedy [Eds.]. North Carolina State Univ., Raleigh, 456 pp.
- Moulton, J. K., D. A. Pepper, and T. J. Dennehy. 2000.** Beet armyworm (*Spodoptera exigua*) resistance to spinosad. *Pest Manag. Sci.* 56: 842-848.
- Mueller, A. J., and B. W. Engroff. 1980.** Effects of infestation levels of *Heliothis zea* on soybean. *J. Econ. Entomol.* 73: 271–275.
- Musser, F. R., A. L. Catchot, Jr., J. A. Davis, D. A. Herbert, Jr., G. M. Lorenz, T. Reed, D. D. Reisig, and S. D. Stewart. 2015.** 2014 Soybean insect losses in the southern US. *Midsouth Entomol.* 8: 35-48.
- NASS. 2007.** The census of agriculture. <http://www.agcensus.usda.gov/> Accessed: Jan. 2016.
- NASS. 2014.** National soybean statistics. [http://www.nass.usda.gov/Charts\\_and\\_Maps/Field\\_Crops/soyac.asp](http://www.nass.usda.gov/Charts_and_Maps/Field_Crops/soyac.asp) Accessed: Jan. 2016.
- Nauen, R., S. Konanz, T. Hirooka, T. Nishimatsu, and H. Kodama. 2007.** Flubendiamide: a unique tool in resistance management tactics for pest Lepidoptera difficult to control. *Pflanzenschutz-Nachrichten Bayer* 60: 247-262.
- North Carolina Soybean Producers Association. 2011.** The history of soybeans. <http://www.ncsoy.org/ABOUT-SOYBEANS/History-of-Soybeans.aspx> Accessed: Jan. 2016.
- Neunzig, H. H. 1964.** The eggs and early-instar larvae of *Heliothis zea* and *Heliothis virescens* (Lepidoptera: Noctuidae). *Ann. Entomol. Soc. Amer.* 57: 98-102.
- Neunzig, H. H. 1969.** The biology of the tobacco budworm and the corn earworm in North Carolina: with particular reference to tobacco as a host. Raleigh NC.
- Ottea, J. A., and J. W. Holloway. 1998.** Target site resistance to pyrethroids in *Heliothis virescens* (F.) and *Helicoverpa zea* (Boddie). *Pestic. Biochem. Physiol.* 61: 155-167.

- Palumbo, J. C. 2008.** Systemic efficacy of Coragen applied through drip irrigation on romaine lettuce, fall 2007. *Arthropod Mngmt. Tests* 33: E24.
- Pair, S. D., J. R. Raulston, A. N. Sparks, J. K. Westbrook, and G. K. Douce. 1986.** Fall armyworm distribution and population dynamics in the southeastern states. *Florida Entomol.* 69: 468-487.
- Parsons, F. S. 1940.** Investigations on the cotton bollworm, *Heliothis armigera*, Hübner. Part III. Relationships between oviposition and the flowering curves of food-plants. *Bull. Entomol. Res.* 31: 147-77.
- Pashley, D. P. 1988.** Current status of fall armyworm host strains. *Florida Entomol.* 71: 227-234.
- Pearson, A. C. 1982.** Biology, population dynamics, and pest status of the beet armyworm (*Spodoptera exigua*) in the Imperial Valley of California. Ph.D. Dissertation, Univ. California, Riverside, 282 pp.
- Pedersen, P. 2004.** Soybean growth and development. Iowa State University Ames, IA. PM1945.
- Pitre, H. N., and D. B. Hogg. 1983.** Development of the fall armyworm on cotton, soybean and corn. *J. Ga. Entomol. Soc.* 18: 187-194.
- Pitre, H. N., J. E. Mulrooney, and D. B. Hogg. 1983.** Fall armyworm (Lepidoptera: Noctuidae) oviposition: crop preferences and egg distribution on plants. *J. Econ. Entomol.* 76: 463-466.
- Purcell, L. C., M. Salmeron, and L. Ashlock. 2014.** Soybean growth and development. University of Arkansas, Fayetteville, AR. MP197
- Qi, S., P. Lummen, R. Nauen, and J. E. Casida. 2014.** Diamide insecticide target site specificity in the *Heliothis* and *Musca* ryanodine receptors relative to toxicity. *J. Agr. And Food Chem.* 62: 4077-4082.
- Quaintance, A. L., and C. T. Brues. 1905.** The cotton bollworm. *In* A.L. Quaintance and C.T. Brues, *Bulletin / U.S. Bureau of Entomology*; no. 50. Washington, D.C.: U.S. Dept. of Agriculture, Bureau of Entomology, 1905.
- Robinson, J. V. 1999.** Biology of the fall armyworm. Accessed: Jan. 2016  
<http://overton.tamu.edu/files/2011/04/ArmywormFactSheet2008.pdf>
- Roditakis, E., E. Vasakis, M. Grispuou, M. Stavrakaki, R. Nauen, M. Gravouil, and A. Bassi. 2015.** First report of *Tuta absoluta* resistance to diamide insecticides. *J. Pest Sci.* 88: 9-16.

- Sattelle, D. B., D. Cordova, and T. R. Cheek. 2008.** Insect ryanodine receptors: molecular targets for novel pest control chemicals. *Invert. Neurosci.* 8: 107-119.
- Schuster, D. J., A. Shurtleff, and S. Kalb. 2009.** Management of armyworms and leafminers on fresh market tomatoes, fall 2007. *Arthropod Mngmt. Tests* 34: E79.
- Scott, W. O., and S. R. Aldrich. 1983.** Modern soybean production. 2nd ed. S & A Publications, Inc., Champaign, IL.
- Scott-Ward T. S., S. J. Dunbar, J. D. Windass, and A. J. Williams. 2001.** Characterization of the ryanodine receptor-Ca<sup>2+</sup> release channel from the thoracic tissues of the lepidopteran insect *Heliothis virescens*, *J. Membr. Biol.* 179: 127–141
- Smith, R. H. 1989.** Experiences with beet armyworm control in 1988, pp. 273-275. *In Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, TN.*
- Snow, J. W., and J. R. Brazzel. 1965.** Seasonal host activity of the bollworm and tobacco budworm during 1963 in northeast Mississippi. *Miss. State Coll. Agric. Exp. Stn. Bull. No. 712 Starkville, MS.*
- Sparks, A. N. 1979.** A review of the biology of the fall armyworm. *Florida Entomol.* 62: 82-87.
- Sparks, T. C. 1981.** Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. *Bull. Entomol. Soc. Amer.* 27: 186-192.
- Sparks, T. C. 2013.** Insecticide discovery: an evaluation and analysis. *Pest. Biochem. Phys.* 107: 8-17.
- Stadelbacher, E. A., and T. R. Pfrimmer. 1972.** Winter survival of the bollworm at Stoneville, Mississippi. *J. Econ. Entomol.* 65: 1030-1034.
- Stadelbacher, E. A. 1981.** Role of early-season wild and naturalized host plants in the buildup of F<sub>1</sub> generation of *Heliothis zea* and *H. virescens* in the delta of Mississippi. *Environ. Entomol.* 10: 766-770.
- Stadelbacher, E. A., H. M. Graham, V. E. Harris, J. D. Lopez, J. R. Phillips, and S. H. Roach. 1986.** *Heliothis* populations and wild host plants in southern U.S., pp. 54-74. *In* S. J. Johnson, E. G. King, and J. R. Bradley, Jr. [eds], *Theory and tactics of Heliothis population management: I- cultural and biological control.* Southern Coop. Ser. Bull. 316, Tifton, GA.
- Stadelbacher, E. A., G. L. Snodgrass, and G. W. Elzen. 1990.** Resistance to cypermethrin in first generation adult bollworm and tobacco budworm (Lepidoptera: Noctuidae) populations collected as larvae on wild geraniums, and in the second and third larval generations. *J. Econ. Entomol.* 83: 1207-1210.

- Su J., T. Lai, and J. Li. 2012.** Susceptibility of field populations of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes. *Crop Protect.* 42: 217–222.
- Sullivan, M. J., S. G. Turnipseed, and D. Robinson. 1999.** Insecticidal efficacy against a complex of fall and beet armyworms and soybean looper in South Carolina cotton, pp. 1034–1036. *In Proceedings, 1999 Beltwide Cotton Conferences, 3–7 January 1999, Orlando, FL. National Cotton Council, Memphis, TN.*
- Swenson, S. J., D. A. Prischmann-Voldseth, and F. R. Musser. 2013.** Corn earworms (Lepidoptera: Noctuidae) as pests of soybean. *J. Integ. Pest Mangmt.* 4: 1-8.
- Temple, J. H., D. R. Cook, P. L. Bommireddy, S. Micinski, W. Waltman, A. M. Stewart, B. Garber, and B. R. Leonard. 2006.** Monitoring cypermethrin susceptibility in Louisiana bollworm 2004-2005, pp. 1236-1240. *In Proc. Beltwide Cotton Conf., San Antonio, TX. 3-6 Jan. 2006. Natl. Cotton Council Am., Memphis TN.*
- Temple, J. H., P. L. Bommireddy, D. R. Cook, P. Marcon, and B. R. Leonard. 2009.** Susceptibility of selected lepidopteran pests to Rynaxypyr<sup>®</sup>, a novel insecticide. *J. Cotton Sci.* 13: 23-31
- Teixeira L. A., and J. T. Andaloro. 2013.** Diamide insecticides: global efforts to address insect resistance stewardship challenges. *Pestic. Biochem. Physiol.* 106: 76–78
- Thomas, G. D., C. M. Ignoffo, K. D. Biever, and D. B. Smith. 1974.** Influence of defoliation and depodding on yield of soybeans. *J. Econ. Entomol.* 67: 683-685.
- Thomas, G. D., C. M. Ignoffo, D. B. Smith, and C. E. Morgan. 1978.** Effects of single and sequential defoliations on yield and quality of soybean. *J. Econ. Entomol.* 71: 871-874.
- Tisdale, R. A. and T. W. Sappington. 2001.** Realized and potential fecundity, egg fertility, and longevity of laboratory-reared female beet armyworm (Lepidoptera: Noctuidae) under different adult diet regimes. *Ann. Entomol. Soc. Am.* 94: 415-419.
- Tohnishi, M., H. T. Nakao, T. Furuya, A. Seo, H. Kodama, K. Tsubata, S. Fujioka, H. Kodama, T. Hirooka, and T. Nishimatsu. 2005.** Flubendiamide, a novel insecticide highly active against lepidopterous insect pests. *J. Pestic. Sci.* 30: 354-360.
- Uchiyama T. and A. Ozawa. 2014.** Rapid development of resistance to diamide insecticides in the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae), in the tea fields of Shizuoka Prefecture, Japan. *Appl. Entomol. Zool.* 49: 1–6.

- Wu M., S. Zhang, R. Yao, S. Wu, J. Su, and C. Gao. 2014.** Susceptibility of the rice stem borer, *Chilo suppressalis* (Lepidoptera: Crambidae), to flubendiamide in China. *J. Econ. Entomol.* 107: 1250–1255.
- Wood, K. A., B. H. Wilson, and J. B. Graves. 1981.** Influence of host plant on the susceptibility of the fall armyworm to insecticides. *J. Econ. Entomol.* 74: 96-98.
- Xu X., M. B. Bhat, M. Nishi, H. Takeshima, and J. Ma. 2000.** Molecular cloning of cDNA encoding a *Drosophila* ryanodine receptor and functional studies of the carboxyl-terminal calcium release channel, *J. Biophys.* 78: 1270–1281.
- Yu, S. J. 1983.** Age variation in insecticide susceptibility and detoxification capacity of fall armyworm (Lepidoptera: Noctuidae) larvae. *J. Econ. Entomol.* 76: 219-222.
- Yu, S. J. 1992.** Detection and biochemical characterization of insecticide resistance in fall armyworm (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 85: 675-682.
- Zheng, X. L., X. P. Cong, X. P. Wang, and C. L. Lei. 2011.** A review of geographic distribution, overwintering, and migration in *Spodoptera exigua* Hübner (Lepidoptera: Noctuidae). *J. Entomol. Res. Soc.* 13: 39-48.

CHAPTER II  
SUSCEPTIBILITY OF SELECTED LEPIDOPTERAN PESTS TO THE DIAMIDES IN  
THE MID-SOUTHERN AND SOUTHEASTERN UNITED STATES

**Abstract**

Corn earworm, *Helicoverpa zea* (Boddie), and the armyworm complex including fall armyworm, *Spodoptera frugiperda* (J.E. Smith) and *Spodoptera exigua* (Hübner), are significant pests of agroecosystems in the Mid-South and Southeastern regions of the United States. These insects have developed resistance to most classes of insecticides. Insecticides in the diamide class have a novel mode of action and have become a key component in the management of agriculturally important lepidopteran pests since their introduction. In this study, field populations of *H. zea*, *S. frugiperda*, and *S. exigua* were collected in the southern region of the United States and compared to susceptible laboratory colonies of the respective species to generate baseline concentration-mortality data. LC<sub>50</sub> and LC<sub>90</sub> values were generated for flubendiamide and chlorantraniliprole using neonate larvae of each species. To achieve equivalent levels of mortality for each species, a higher concentration of flubendiamide was required compared to chlorantraniliprole. Flubendiamide LC<sub>50</sub> values for *H. zea* ranged from 16.45-30.74 ng/ml with a mean of 23.53 ng/ml, *S. frugiperda* ranged from 30.79 – 34.01 ng/ml with a mean of 32.37 ng/ml, and *S. exigua* ranged from 12.40 -18.40 ng/ml with a mean of 15.43 ng/ml. Chlorantraniliprole LC<sub>50</sub> values for *H. zea* ranged from 2.94 – 4.22 ng/ml with a

mean of 3.66 ng/ml, *S. frugiperda* ranged from 6.16 – 6.78 ng/ml with a mean of 6.18 ng/ml and *S. exigua* ranged from 6.71 13.30 ng/ml with a mean of 10.01 ng/ml.

## Introduction

The order Lepidoptera is the most damaging insect order in soybean production in the southern United States (Fitt 1989, Musser et al. 2014). Corn earworm, *Helicoverpa zea* (Boddie); beet armyworm, *Spodoptera exigua* (Hübner); and fall armyworm, *Spodoptera frugiperda* (Smith), are widely distributed polyphagous pests of numerous cultivated crops throughout the Mid-Southern and Southeastern United States. In 2014, these insects resulted in a combined US\$138,874,796 economic loss across Alabama, Arkansas, Louisiana, Mississippi, North Carolina, Tennessee, and Virginia in soybean (Musser et al. 2014). Foliar applications of synthetic insecticides are instrumental in the management of lepidopteran pests in the southern United States. The widespread use of synthetic insecticides has led to resistance and/or inconsistent control with most chemical classes, including chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, and benzoylphenylureas (Sparks 1981, Brown et al. 1998, Temple et al. 2006, Jacobson et al. 2009, Lai and Su 2011).

The diamide class of insecticides was introduced in 2008 and is the newest major class of insecticides (EPA 2008). It has a novel mode of action classified as ryanodine receptor modulators (MoA Group 28) (IRAC 2014). Ryanodine receptors (RyR) are intracellular calcium channels located in the sarcoplasmic reticulum that specialize in the rapid and massive release of calcium from intracellular stores, which is necessary for excitation contraction coupling in striated muscle (Ebbinghaus-Kintscher et al. 2006). Calcium serves as the primary physiological regulator of insect ryanodine receptors (Xu

et al. 2000, Scott-Ward et al. 2001). Diamide insecticides bind to the ryanodine receptor complex, prompting the prolonged release of intracellular calcium stores, resulting in cessation of feeding and uncoordinated muscle contraction of intoxicated insects, eventually causing mortality (Ebbinghaus-Kintscher et al. 2006, Nauen et al. 2007, Hannig et al. 2009, Roditakis et al. 2015). The diamide insecticides are characterized by their low mammalian toxicity and are effective against a large number of lepidopteran species (Sattelle et al. 2008, Tohnishi et al. 2005, Lahm et al. 2009, Teixeira and Andaloro 2013, Qi et al. 2014). Two representatives from this class of insecticides are flubendiamide (Belt Bayer CropScience, Raleigh, NC), a phthalic acid diamide and chlorantraniliprole (Prevathon DuPont Crop Protection, Newark, DE), an anthranilic diamide (Lahm et al. 2009). Although they are structurally independent, these insecticides share the same target site (Lahm et al. 2009, Teixeira and Andaloro 2013). Eight years after their introduction to the global market, these two active ingredients comprise 7% of global insecticide use (Sparks 2013). Large global market shares result from the favorable biological, ecological, and toxicological attributes of this insecticide class (Teixeira and Andaloro 2013). It is perceived that the use of this insecticide class will continue to increase globally on a wide variety of crops (Teixeira and Andaloro 2013, Roditakis et al. 2015).

Repeated field applications of the diamide insecticides has resulted in numerous reports of resistance development for several lepidopteran species (Roditakis et al. 2015). To date, cross resistance between chlorantraniliprole and flubendiamide has been documented for diamondback moth, *Plutella xylostella* (L), smaller tea tortrix, *Adoxophyes honmai* (Yasuda), and tomato borer, *Tuta absoluta* (Meyrick) (Wang and

Wu 2012, Uchiyama and Ozawa 2014, Roditakis et al. 2015). Also, resistance to chlorantraniliprole has been documented for rice stem borer, *Chilo suppressalis* (Walker), cutworm, *Spodoptera litura* (F), and *S. exigua* (Su et al. 2012, Che et al. 2013, Gao et al. 2013). Furthermore, resistance to flubendiamide has been documented in *C. suppressalis* (Walker) (Wu et al. 2014). For that reason, monitoring susceptibility levels of target pest species is important for pest management and resistance management efforts.

Furthermore, development of successful insecticide resistance management (IRM) strategies requires the establishment of baseline susceptibility levels of target pest species while resistant allele frequencies are low (French-Constant and Roush 1990, Cook et al. 2005). Baseline responses of laboratory and field strains of target pest populations to novel modes of action act as a historical reference, and are necessary to mitigate resistance development, prolonging the effectiveness of novel modes of action. The primary objective of the present study was to generate baseline dose-mortality responses of *H. zea*, *S. frugiperda*, and *S. exigua* to flubendiamide and chlorantraniliprole for future resistance monitoring efforts and development of resistance management strategies.

## **Materials and Methods**

### **Insect rearing**

The *H. zea* susceptible colony was a laboratory colony maintained at the Mississippi State University Department of Entomology, Mississippi State, MS insect rearing facility. This colony originated from non-Bt corn in 2006 and wild individuals collected from non-Bt corn were incorporated into the colony on a yearly basis. The *S. frugiperda* susceptible colony was collected from non-Bt corn whorls in 2012 and was not infused with wild individuals after collection. The *S. exigua* susceptible colony was

collected from soybean in 2013 and was not infused with wild individuals after collection. Prior to the initiation of this experiment, susceptible colonies were not exposed to insecticides. Field derived populations for this study were comprised of 15 *H. zea* colonies collected during 2013 and 2014, 2 *S. frugiperda* colonies collected in 2013, and 1 *S. exigua* colony collected in 2013 (Table 2.1). Each collection consisted of at least 300 third instar larvae. Larvae were placed in 36 mL Solo<sup>®</sup> cups (Bio-Serv<sup>®</sup>, Frenchtown, NJ) containing Stonefly Heliothis Diet (Product No. 38-0600, Ward's Natural Science, Rochester, NY) with matching lids. At pupation, approximately 50 pupae were placed in 3.79 liter cardboard containers with matching lids with the corresponding colony and generation information labeled on the outside of each bucket. Adults were fed a 10% sugar-water solution that was changed daily. For the purpose of egg collection for bioassays, the cardboard containers were lined with Reynolds<sup>®</sup> Cut-Rite<sup>®</sup> Wax Paper (Reynolds Consumer Products, Lake Forest, IL). The center of each lid was removed so that only the rim remained. Cotton cloth was placed over each bucket and kept in place by the lid to serve as an oviposition substrate. Eggs were collected daily and new cloths and wax paper were applied to every bucket. Collected egg sheets and wax paper from each colony were kept in 3.79 liter Ziploc<sup>®</sup> (S.C. Johnson & Johnson, Inc., Racine WI) bags until larvae hatched for use in bioassays. The laboratory susceptible colonies and field derived populations of each species were reared at the Mississippi State University insect rearing facility under the following conditions 25°C, 80% relative humidity, and 16:8 (L:D) photoperiod. All assays were conducted on first and/or second generation progeny of field collected colonies.

## Bioassays

Concentration-mortality bioassays were conducted with commercial formulations of flubendiamide (Belt; Bayer CropScience, Raleigh, NC) and chlorantraniliprole (Prevathon; DuPont Crop Protection, Newark, DE) to determine the susceptibility of *H. zea*, *S. frugiperda*, and *S. exigua*. Preparation of insecticide treated diet was similar to Temple et al. (2009). Dilutions of flubendiamide and chlorantraniliprole in distilled water were made from a stock solution with a concentration of 1000 ng/ml and 500 ng/ml of ai., respectively to yield eight concentrations ranging from 0 to 35 ng/ml ai for flubendiamide and 0 to 6.8 ng/ml ai for chlorantraniliprole. Aliquots from these solutions were combined with Stonefly Heliothis Diet (Product No. 38-0600, Ward's Natural Science, Rochester, NY) to yield 400 grams of insecticide treated diet for each concentration. Insecticide treated diet was stored in 0.95 L Ziploc<sup>®</sup> bags and refrigerated. All diet was used or disposed of within 7 days of preparation. Insecticide treated diet for each concentration was dispensed into 16 wells of a 128-well bioassay tray (Product No. BAW128, Frontier Agricultural Sciences, Newark, DE) in 0.5 ml aliquots. Each well was infested with one neonate (< 24 h after hatching) larva. Cells were covered with perforated, clear 16-well lids (P.E. film, Bio-Serv<sup>®</sup>, Frenchtown, NJ). Infested assay trays were labeled and placed in a rearing chamber maintained at 25°C, 80% relative humidity, and a photoperiod of 16:8 (L:D). All bioassays were replicated at least 4 times based on date of oviposition. Insect mortality ratings were taken 7 days later. Ingestion of the diamides results in feeding cessation (Nauen et al. 2007, Hannig et al. 2009). Typically the ability of larvae to right themselves after being flipped onto their dorsal surface is considered an appropriate criterion for determining mortality with intoxicated larvae.

Based on preliminary data of 4 *H. zea* colonies (data not presented) it was observed that intoxicated larvae, though severely stunted, could still right themselves when flipped onto their dorsal surface. To account for the growth inhibition of intoxicated larvae, the criterion for mortality was defined as larvae that had not molted to the second instar, weighing less than 10 mg after 7 days (Siegfried et al. 2000).

### **Data Analysis**

Data were corrected for control mortality using Abbott's formula (Abbott 1925). Corrected data were analyzed with probit analysis to calculate slope, LC<sub>50</sub>, LC<sub>90</sub>, and confidence intervals (PROC PROBIT, SAS Institute 2012). Goodness of fit tests ( $P > 0.10$ ) were evaluated to ensure the trend line fit the model. LC<sub>50</sub> and LC<sub>90</sub> values were considered different when 95 percent confidence intervals did not overlap.

### **Results and Discussion**

Significant differences in LC<sub>50</sub> and LC<sub>90</sub> values were observed among populations of *H. zea* for both chlorantraniliprole (Table 2.2) and flubendiamide (Table 2.3). Statistical differences among populations were minimal and do not appear to represent an important biological difference. Overall, mean LC<sub>50</sub> and LC<sub>90</sub> data suggest that *H. zea* were approximately 6.4-fold (23.43 vs 3.65 ng/ml) to 5.4-fold (30.51 vs 5.65 ng/ml) more tolerant to flubendiamide than chlorantraniliprole, respectively. The LC<sub>50</sub> and LC<sub>90</sub> values for *H. zea* in response to flubendiamide ranged from 16.45-30.74 ng/ml (1.86 fold) with a mean of 23.53 ng/ml and 21.22-35.33 ng/ml (1.66 fold) with a mean of 30.59 ng/ml, respectively. The LC<sub>50</sub> and LC<sub>90</sub> values for *H. zea* ranged from 2.94 to 4.22 ng/ml (1.44 fold) with a mean of 3.66 ng/ml and from 4.52 to 9.17 ng/ml (2.02 fold) with a mean of

5.68 ng/ml in response to chlorantraniliprole, respectively. Concentration-mortality values of chlorantraniliprole for *H. zea* larvae in the current studies are considerably lower than those previously reported. Temple et al. (2009) reported mean LC<sub>50</sub> values that were 15 fold greater than the results found in this study (56 vs 3.6 ng/ml). These differences are most likely due to the fact that Temple et al. (2009) tested third instar larvae compared to neonates tested in the current study.

Significant differences in susceptibility of field derived populations versus the susceptible laboratory colony of *S. frugiperda* to flubendiamide and chlorantraniliprole were observed. As with *H. zea*, these differences were not large and did not suggest an important biological difference. Overall, mean LC<sub>50</sub> and LC<sub>90</sub> data suggested that *S. frugiperda* were approximately 5-fold (32.97 vs 6.57 ng/ml) to 4.9-fold (46.19 vs 9.43 ng/ml) more tolerant to flubendiamide than chlorantraniliprole, respectively. Hardke et al. (2011) reported LC<sub>50</sub> values that suggest *S. frugiperda* was 13.67 fold more tolerant to flubendiamide compared with chlorantraniliprole. Overall, LC<sub>50</sub> and LC<sub>90</sub> values ranged from 6.16 to 6.78 ng/ml (1.1 fold) with a mean of 6.18 ng/ml and from 8.95 to 10.27 ng/ml (1.15 fold) with a mean of 9.01 ng/ml for chlorantraniliprole and from 30.79 to 34.01 ng/ml (1.11 fold) with a mean of 32.37 ng/ml and from 43.62 to 48.14 ng/ml (1.1 fold) with a mean of 45.23 ng/ml in response to flubendiamide, respectively. Furthermore, in this study, mean LC<sub>50</sub> and LC<sub>90</sub> values suggest *S. frugiperda* is 1.8 and 1.67-fold more tolerant to chlorantraniliprole and 1.4 and 1.5-fold more tolerant to flubendiamide than *H. zea*. Concentration-mortality values of chlorantraniliprole and flubendiamide for *S. frugiperda* larvae in the current studies are considerably lower than those reported by Hardke et al. (2011) when tested against third instar larvae of the same

species. Hardke et al. (2011) reported mean LC<sub>50</sub> values that were approximately 10-fold greater (68 vs 6.6 ng/ml) for chlorantraniliprole and approximately 28-fold greater (930 vs 33 ng/ml) for flubendiamide. These differences are most likely due to the fact that Hardke et al. (2011) tested third instar larvae compared to neonates tested in the current study. Without other colonies available, no definitive conclusions can be made regarding this observation. To date, there are no reports foliar applications of diamide insecticides targeting *S. frugiperda* in Mississippi soybean. However, further investigation is necessary to better understand the concentration-mortality relationship of *S. frugiperda* populations in Mississippi.

The LC<sub>50</sub> and LC<sub>90</sub> values suggest that the field derived population of *S. exigua* was more tolerant to chlorantraniliprole than *H. zea* (3.6, 3.7 fold) and *S. frugiperda* (2.0-2.0 fold) but this level of tolerance was not observed with flubendiamide. Che et al. (2013) described *S. exigua* as having a strong capability to evolve resistance to chlorantraniliprole. Because of limited availability, only one field derived population of *S. exigua* was tested against a susceptible laboratory population to chlorantraniliprole and flubendiamide. The LC<sub>50</sub> and LC<sub>90</sub> values for the field derived *S. exigua* colony compared to the susceptible laboratory colony was 1.98-fold (6.71 vs. 13.30 ng/ml) and 1.95-fold (10.59 vs. 20.70 ng/ml) higher for chlorantraniliprole, and 1.48 (12.40-18.46 ng/ml) and 1.27 (20.31-25.75 ng/ml) fold higher for flubendiamide. As with *S. frugiperda*, no definitive conclusions can be made regarding this observation without further examination of additional colonies. To date, there are no reports of foliar applications of diamide insecticides targeting *S. exigua* in Mississippi soybean, but selection could be occurring in other crops.

Baseline susceptibility to the diamide insecticide class was generated in Louisiana by Hardke et al. (2011) for *S. frugiperda* and Temple et al. (2009) for *H. zea*. However, baseline data have not been produced for these species and *S. exigua* populations for Mississippi or other states included in this study. Furthermore, differences observed between this study and previous studies can be attributed to differences in insecticide susceptibility of third instar larvae compared to neonate larvae. The long residual efficacy of the diamide insecticides may potentially expose multiple generations of the same species to the insecticide. Therefore, neonate larvae were used in this study to account for the subsequent populations that could potentially be exposed to the diamide insecticides as the growing season progresses. Nevertheless, it is critical to document the variability in the response of field populations prior to the occurrence of field control failures. Resistance is defined as “a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that pest species” (IRAC 2007). Additional collections of *S. frugiperda* and *S. exigua* are needed to increase the robustness of the data for future monitoring programs; however, this study generated baseline data that can serve as reference points for future monitoring programs associated with *H. zea* aiding in the detection of resistance alleles prior to field control failures. Future monitoring programs will aid resistance management efforts allowing the diamide insecticide class to continue to play an important role in crop protection strategies.

Table 2.1 Description of *Helicoverpa zea*, *Spodoptera frugiperda*, and *Spodoptera exigua*, field derived populations and laboratory colonies by identification code, collection host, month collected, and collection location.

Colony	Species	Collection Host	Month	Location
LAB	<i>H. zea</i>			MSSTATE, MS
AR14	<i>H. zea</i>	<i>Z. mays</i>	July	Lonoke County, AR
GA14	<i>H. zea</i>	<i>Z. mays</i>	July	Tift County, GA
LA14	<i>H. zea</i>	<i>Z. mays</i>	July	Franklin Parish, LA
MSKIL14	<i>H. zea</i>	<i>T. incarnatum</i>	May	Montgomery County, MS
MSLEL14	<i>H. zea</i>	<i>T. incarnatum</i>	May	Washington County, MS
MSNAT14	<i>H. zea</i>	<i>T. incarnatum</i>	May	Adams County, MS
MSSTARK13-1	<i>H. zea</i>	<i>T. incarnatum</i>	May	Oktibbeha County, MS
MSSTARK14-1	<i>H. zea</i>	<i>Z. mays</i>	July	Oktibbeha County, MS
MSSTONE13-1	<i>H. zea</i>	<i>Z. mays</i>	June	Washington County, MS
MSSTONE13-2	<i>H. zea</i>	<i>S. bicolor</i>	July	Washington County, MS
MSSTONE13-3	<i>H. zea</i>	<i>C. arietinum</i>	August	Washington County, MS
MSVIC14	<i>H. zea</i>	<i>T. incarnatum</i>	May	Warren County, MS
MSYAZ14	<i>H. zea</i>	<i>T. incarnatum</i>	May	Yazoo County, MS
NC14	<i>H. zea</i>	<i>Z. mays</i>	July	Washington County, NC
SC14	<i>H. zea</i>	<i>Z. mays</i>	July	Barnwell County, SC
TN14-1	<i>H. zea</i>	<i>Z. mays</i>	June	Madison County, TN
TN14-2	<i>H. zea</i>	<i>Z. mays</i>	July	Madison County, TN
LAB	<i>S. frugiperda</i>			DREC Insect Lab
MSSTARK13	<i>S. frugiperda</i>	<i>Z. mays</i>	August	Oktibbeha County, MS
MSSTONE13	<i>S. frugiperda</i>	<i>S. bicolor</i>	August	Washington County, MS
LAB	<i>S. exigua</i>			DREC Insect Lab
MSCLARK13	<i>S. exigua</i>	<i>Amaranthus sp.</i>	August	Coahoma County, MS

Table 2.2 Comparative susceptibility of *H. zea*, *S. frugiperda*, and *S. exigua* larvae to chlorantraniliprole in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet.

Colony	Species	N <sup>1</sup>	chlorantraniliprole			Slope ( $\pm$ SE)	X <sup>2</sup> (df)	P <sup>3</sup>
			LC <sub>50</sub> (95% C.L.) <sup>2</sup> (ng/ml)	LC <sub>90</sub> (95% C.L.) <sup>2</sup> (ng/ml)				
LAB	<i>H. zea</i>	991	3.58 (3.41-3.73)	5.25 (5.01-5.55)	3.35 ( $\pm$ 0.24)	6.13 (5)	0.2935	
AR14	<i>H. zea</i>	256	3.38 (2.85-3.76)	5.32 (4.79-6.23)	2.83 ( $\pm$ 0.48)	3.86 (5)	0.57	
GA14	<i>H. zea</i>	384	3.09 (2.81-3.32)	4.52 (4.20-5.00)	3.37 ( $\pm$ 0.43)	6.27 (5)	0.2808	
LA14	<i>H. zea</i>	288	3.84 (2.85-4.25)	5.61 (5.17-6.88)	3.38 ( $\pm$ 0.94)	5.25 (3)	0.1541	
MSKIL14	<i>H. zea</i>	634	4.21 (4.02-4.39)	5.86 (5.56-6.28)	3.82 ( $\pm$ 0.37)	8.57 (5)	0.1275	
MSLEL14	<i>H. zea</i>	448	3.79 (3.44-4.14)	7.61 (6.57-9.54)	1.84 ( $\pm$ 0.23)	5.73 (4)	0.2202	
MSSTARK13	<i>H. zea</i>	384	4.11 (3.50-4.77)	9.17 (7.53-12.32)	1.60 ( $\pm$ 0.21)	3.89 (5)	0.5659	
MSSTARK14	<i>H. zea</i>	512	3.62 (3.42-3.84)	4.98 (4.54-5.86)	4.02 ( $\pm$ 0.64)	1.56 (5)	0.9059	
MSSTONE13-1	<i>H. zea</i>	881	2.94 (2.75-3.11)	4.43 (4.07-5.03)	3.13 ( $\pm$ 0.40)	4.93 (4)	0.2947	
MSSTONE13-2	<i>H. zea</i>	1643	3.21 (3.05-3.36)	5.10 (4.74-5.61)	2.76 ( $\pm$ 0.23)	4.44 (5)	0.4882	
MSSTONE13-3	<i>H. zea</i>	1166	3.52 (3.36-3.68)	4.68 (4.35-5.27)	4.51 ( $\pm$ 0.64)	6.09 (5)	0.2974	
MSYAZ 14	<i>H. zea</i>	128	4.09 (3.25-4.84)	7.47 (6.15-10.72)	2.12 ( $\pm$ 0.43)	8.43 (5)	0.134	
NC14	<i>H. zea</i>	256	4.22 (3.86-4.49)	5.32 (4.98-5.91)	5.54 ( $\pm$ 1.01)	6.38 (5)	0.2702	
SC14	<i>H. zea</i>	384	4.05 (3.76-4.26)	5.06 (4.8-5.49)	5.72 ( $\pm$ 0.93)	9.07 (5)	0.1064	
TN14-1	<i>H. zea</i>	256	3.72 (3.37-3.98)	4.76 (4.44-5.32)	5.21 ( $\pm$ 0.96)	0.43 (5)	0.9946	
TN 14-2	<i>H. zea</i>	256	3.15 (2.19-3.67)	5.26 (4.65-6.53)	2.49 ( $\pm$ 0.61)	6.83 (5)	0.2336	
LAB	<i>S. frugiperda</i>	780	6.78 (6.46-7.08)	10.27 (9.72-11.00)	3.09 ( $\pm$ 0.23)	6.63 (4)	0.1566	
MSSTARK13	<i>S. frugiperda</i>	1112	6.16 (5.85-6.44)	8.95 (8.46-9.60)	3.43 ( $\pm$ 0.28)	4.86 (5)	0.4334	
MSSTONE13	<i>S. frugiperda</i>	377	6.19 (5.79-6.60)	9.06 (8.32-10.19)	3.37 ( $\pm$ 0.36)	5.35 (5)	0.3741	
LAB	<i>S. exigua</i>	913	6.71 (6.30-7.11)	10.59 (9.85-11.60)	2.81 ( $\pm$ 0.23)	1.91 (3)	0.5907	
MSCLARK13	<i>S. exigua</i>	331	13.30 (11.48-14.63)	20.70 (18.49-25.52)	2.89 ( $\pm$ 0.56)	0.69 (2)	0.7066	

<sup>1</sup>Total number of insects tested

<sup>2</sup>Confidence Limits

<sup>3</sup>Goodness of Fit test (P > 0.10).

Table 2.3 Comparative susceptibility of *H. zea*, *S. frugiperda*, and *S. exigua* larvae to flubendiamide in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet.

Colony	Species	N <sup>1</sup>	flubendiamide				P <sup>3</sup>
			LC <sub>50</sub> (95% CL) <sup>2</sup> (ng/ml)	LC <sub>90</sub> (95% CL) <sup>2</sup> (ng/ml)	Slope (±SE)	X <sup>2</sup> (df)	
LAB	<i>H. zea</i>	447	21.96 (20.36-23.21)	29.12 (27.71-30.95)	4.54 (±0.53)	5.39 (4)	0.2497
AR14	<i>H. zea</i>	256	21.88 (19.23-56.58)	29.59 (27.58-33.19)	4.25 (±0.81)	6.29 (5)	0.2789
GAI4	<i>H. zea</i>	256	27.75 (26.23-28.99)	34.79 (32.67-39.24)	5.66 (±1.06)	3.35 (5)	0.6459
LA14	<i>H. zea</i>	256	23.34 (21.85-24.66)	30.47 (28.65-33.15)	4.80 (±0.60)	7.27 (5)	0.2010
MSKIL14	<i>H. zea</i>	398	29.34 (27.58-30.48)	35.33 (34.07-37.38)	6.89 (±1.13)	0.70 (2)	0.7042
MSLEL14	<i>H. zea</i>	384	24.43 (21.82-25.90)	32.87 (30.86-37.31)	4.32 (±0.90)	9.06 (5)	0.1068
MSNAT 14	<i>H. zea</i>	192	24.04 (19.94-26.28)	30.76 (28.57-33.74)	5.22 (±1.16)	2.68 (3)	0.4439
MSSTARK13-1	<i>H. zea</i>	406	17.02 (16.19-17.91)	21.75 (20.37-23.88)	5.23 (±0.61)	1.25 (3)	0.5354
MSSTARK14-1	<i>H. zea</i>	256	16.45 (15.25-17.77)	21.22 (19.29-25.73)	5.04 (±1.03)	1.56 (5)	0.906
MSSTONE13-1	<i>H. zea</i>	947	19.72 (18.54-20.83)	32.38 (30.28-35.21)	2.58 (±0.20)	3.28 (3)	0.1938
MSSTONE13-2	<i>H. zea</i>	672	21.23 (15.30-23.73)	33.82 (30.75-43.90)	2.75 (±0.76)	4.60 (3)	0.2035
MSVIC14	<i>H. zea</i>	320	25.19 (22.51-26.86)	29.55 (27.91-31.13)	8.04 (±1.43)	1.62 (2)	0.4443
MSYAZ14	<i>H. zea</i>	560	30.74 (29.99-31.52)	37.49 (35.95-39.87)	6.46 (±0.72)	1.03 (3)	0.7948
NC14	<i>H. zea</i>	288	24.47 (23.22-25.36)	28.19 (27.23-29.60)	9.07 (±1.46)	2.60 (3)	0.4572
SC14	<i>H. zea</i>	896	25.70 (25.14-26.22)	30.58 (29.79-31.61)	7.37 (±0.60)	9.23 (5)	0.1002
TN14-1	<i>H. zea</i>	256	22.82 (20.37-24.64)	32.20 (29.66-36.70)	3.72 (±0.63)	3.85 (5)	0.5718
TN14-2	<i>H. zea</i>	256	22.30 (20.24-23.80)	28.55 (26.74-31.49)	5.19 (±0.90)	4.34 (5)	0.5009
LAB	<i>S. frugiperda</i>	780	33.63 (31.99-35.22)	48.12 (45.39-51.77)	3.57 (±0.29)	1.03 (3)	0.7938
MSSTARK13	<i>S. frugiperda</i>	975	30.73 (29.60-31.87)	43.62 (41.39-46.53)	3.66 (±0.25)	4.04 (3)	0.2568
MSSTONE13	<i>S. frugiperda</i>	1428	34.01 (31.52-34.11)	46.84 (44.78-49.40)	3.61 (±0.23)	1.39 (3)	0.7058
LAB	<i>S. exigua</i>	1197	12.40 (11.64-13.15)	20.31 (18.86-22.24)	2.60 (±0.23)	4.47 (5)	0.4838
MSCLARK13	<i>S. exigua</i>	402	18.46 (17.10-19.88)	25.75 (23.59-28.94)	3.85 (±0.42)	6.80 (5)	0.2360

<sup>1</sup>Total number of insects tested.

<sup>2</sup>Confidence Limits.

<sup>3</sup>Goodness of Fit test (P > 0.10).

## References

- Abbot, W. S. 1925.** A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Adameczyk Jr, J. J., M. R. Williams, J. T. Reed, D. W. Hubbard, and D. D. Hardee. 2003.** Spatial and temporal occurrence of beet armyworm (Lepidoptera: Noctuidae) moths in Mississippi. *Florida Entomol.* 86: 229-232.
- Brown, T. M., P. K. Bryson, D. S. Brickle, S. Pimprale, F. Arnette, M. E. Roof, J. T. Walker, and M. J. Sullivan. 1998.** Pyrethroid-resistant *Helicoverpa zea* and transgenic cotton in South Carolina. *Crop Protect.* 17: 441-445.
- Che W., T. Shi, Y. Wu, and Y. Yang. 2013.** Insecticide resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) from China. *J. Econ. Entomol.* 106: 1855–1862.
- Cook, D. R., B. R. Leonard, J. Gore, and J. H. Temple, 2005.** Baseline responses of bollworm, *Helicoverpa zea* (Boddie), and Tobacco Budworm, *Heliothis virescens* (F.), to indoxacarb and pyridalyl. *J. Agric. Urban Entomol.* 22: 100-109.
- Ebbinghaus-Kintscher U, P. Luemmen, N. Lobitz, T. Schulte, C. Funke, R. Fischer, T. Masaki, N. Yasokawa, and M. Tohnishi. 2006.** Phthalic acid diamides activate ryanodine-sensitive Ca<sup>2+</sup> release channels in insects. *Cell Calcium* 39: 21–33.
- EPA. 2008.** Pesticide Fact Sheet, Chlorantraniliprole, [http://www3.epa.gov/pesticides/chem\\_search/reg\\_actions/registration/fs\\_PC-090100\\_01-Apr-08.pdf](http://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-090100_01-Apr-08.pdf). Accessed: Jan 2016.
- Fitt, G. P. 1989.** The ecology of *Heliothis* species in relation to agroecosystems. *Ann. Rev. of Entomol.* 34: 17-52.
- French-Constant, R. H., and R. T. Roush. 1990.** Resistance detection and documentation: the relative roles of pesticidal and biochemical assays, pp 4-38. *In* R. T. Roush and B. E. Tabashnik [Eds.], *Pesticide Resistance in Arthropods*. Chapman and Hall, New York, New York and London, United Kingdom.
- Gao C, R. Yao, Z. Zhang, M. Wu, S. Zhang, and J. Su. 2013.** Susceptibility baseline and chlorantraniliprole resistance monitoring in *Chilo suppressalis* (Lepidoptera: Pyralidae). *J. Econ. Entomol.* 106: 2190–2194.
- Hannig, G. T., M. Ziegler, and P. G. Marcon. 2009.** Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. *Pest Manag. Sci* 65: 969-974.

- Hardke, J. T., J. H. Temple, B. R. Leonard, and R. E. Jackson. 2011** Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). *Florida Entomol.* 94: 272-278.
- IRAC. 2007.** Insecticide Resistance Action Committee, Resistance management for sustainable agriculture and improved public health. <http://www.irc-online.org/documents/irc-croplife-irm-booklet/?ext=pdf>. Accessed: Jan 2016.
- IRAC. 2015.** IRAC Mode of Action Classification Scheme. Version 8.0 [Online]. Available at <http://www.irc-online.org/documents/moa-classification/> Accessed: January 30, 2016.
- Jacobson, A., R. Foster, C. Krupke, W. Hutchison, B. Pittendrigh, and R. Weinzierl. 2009.** Resistance to pyrethroids insecticides in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Indiana and Illinois. *J. Econ. Entomol.* 102: 2289-2295.
- Lahm, G. P., D. Cordova, and J. D. Barry. 2009.** New and selective ryanodine receptor activators for insect control. *Bioorganic & Medic. Chem.* 17: 4127-4133.
- Lai, T., and J. Su. 2011.** Assessment of resistance risk in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) to chlorantraniliprole. *Pest Manag. Sci* 67: 1468-1472.
- Musser, F. R., A. L. Catchot, Jr., J. A. Davis, D. A. Herbert, Jr., G. M. Lorenz, T. Reed, D. D. Reisig, and S. D. Stewart. 2015.** 2014 Soybean insect losses in the southern US. *Midsouth Entomol.* 8: 35-48.
- Nauen, R., S. Konanz, T. Hirooka, T. Nishimatsu, and H. Kodama. 2007.** Flubendiamide: a unique tool in resistance management tactics for pest lepidoptera difficult to control. *Pflanzenschutz-Nachrichten Bayer* 60: 247-262.
- Qi, S., P. Lummen, R. Nauen, and J.E. Casida. 2014.** Diamide insecticide target site specificity in the *Heliothis* and *Musca* ryanodine receptors relative to toxicity. *J. Agr. food chem.* 62: 4077-4082.
- Roditakis, E., E. Vasakis, M. Grispou, M. Stavrakaki, R. Nauen, M. Gravouil, and A. Bassi. 2015.** First report of *Tuta absoluta* resistance to diamide insecticides. *J. Pest Sci.* 88: 9-16.
- Sattelle, D. B., D. Cordova, and T. R. Cheek. 2008.** Insect ryanodine receptors: molecular targets for novel pest control chemicals. *Invert. Neurosci.* 8: 107-119.
- Scott-Ward T. S., S. J. Dunbar, J. D. Windass, and A. J. Williams. 2001.** Characterization of the ryanodine receptor-Ca<sup>2+</sup> release channel from the thoracic tissues of the lepidopteran insect *Heliothis virescens*, *J. Membr. Biol.* 179: 127-141.

- Siegfried, B. D., T. Spencer, and J. Nearman. 2000.** Baseline susceptibility of the corn earworm (Lepidoptera: Noctuidae) to the Cry1Ab toxin from *Bacillus thuringiensis*. *J. Econ. Entomol.* 93: 1265-1268.
- Sparks, T. C. 1981.** Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. *Bull. Entomol. Soc. Amer.* 27: 186-192.
- Sparks, T. C. 2013.** Insecticide discovery: an evaluation and analysis. *Pest. Biochem. Physiol.* 107: 8-17.
- SAS Institute. 2012.** SAS 9.3 for windows. SAS Institute, Cary, NC.
- Su J., T. Lai, and J. Li. 2012.** Susceptibility of field populations of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) in China to chlorantraniliprole and the activities of detoxification enzymes. *Crop Protect.* 42: 217–222.
- Teixeira L. A, and J. T. Andaloro. 2013.** Diamide insecticides: global efforts to address insect resistance stewardship challenges. *Pestic. Biochem. Physiol.* 106: 76–78.
- Temple, J. H., D. R. Cook, P. L. Bommireddy, S. Micinski, W. Waltman, A. M. Stewart, B. Garber, and B. R. Leonard. 2006.** Monitoring cypermethrin susceptibility in Louisiana bollworm 2004-2005, pp. 1236-1240. *In Proc. Beltwide Cotton Conf., San Antonio, TX. 3-6 Jan. 2006. Natl. Cotton Council Am., Memphis TN.*
- Temple, J. H., P.L. Bommireddy, P. Marcon, S. Micinski, K.D. Emfinger, and B.R. Leonard. 2008.** Rynaxypyr® (DPX-E2Y45) and cypermethrin: susceptibility of selected lepidopteran insect pests, pp. 1282 - 1289. *In Proc. Belt-wide Cotton Conf., Nashville, TN. 8-11 Jan. 2008. Natl. Cotton Council Am., Memphis, TN.*
- Temple, J.H., P. L. Bommireddy, D. R. Cook, P. Marcon, and B.R. Leonard, 2009.** Susceptibility of selected lepidopteran pests to Rynaxypyr, a novel insecticide. *J. Cotton Sci.* 13: 23-31.
- Tohnishi, M., H. Nakao, T. Furuya, A. Seo, H. Kodama, K. Tsubata, S. Fujioka, H. Kodama, T. Hirooka, and T. Nishimatsu. 2005.** Flubendiamide, a novel insecticide highly active against lepidopterous insect pests. *J. Pestic. Sci.* 30: 354-360.
- Uchiyama T. and A. Ozawa. 2014.** Rapid development of resistance to diamide insecticides in the smaller tea tortrix, *Adoxophyes honmai* (Lepidoptera: Tortricidae), in the tea fields of Shizuoka Prefecture, Japan. *Appl. Entomol. Zool.* 49: 1–6.
- Wang X. and Y. Wu. 2012.** High levels of resistance to chlorantraniliprole evolved in field populations of *Plutella xylostella*. *J. Econ. Entomol.* 105: 1019–1023.

**Wu M., S. Zhang, R. Yao, S. Wu, J. Su, and C. Gao. 2014.** Susceptibility of the rice stem borer, *Chilo suppressalis* (Lepidoptera: Crambidae), to flubendiamide in China. *J. Econ. Entomol.* 107: 1250–1255.

**Xu X., M. B. Bhat, M. Nishi, H. Takeshima, and J. Ma. 2000.** Molecular cloning of cDNA encoding a drosophila ryanodine receptor and functional studies of the carboxyl-terminal calcium release channel, *J. Biophys.* 78: 1270–1281.

CHAPTER III  
RESIDUAL AND SYSTEMIC EFFICACY OF CHLORANTRANILIPROLE AND  
FLUBENDIAMIDE IN MISSISSIPPI SOYBEAN

**Abstract**

Two experiments were conducted in Starkville and Stoneville, Mississippi from 2013 to 2015 to determine the systemic and residual efficacy of chlorantraniliprole and flubendiamide on vegetative and reproductive structures of soybean. Foliar applications of chlorantraniliprole and flubendiamide were applied to soybean at the V4 and R3 growth stages to determine if they moved systemically. Ten upper-most newly emerged trifoliates that were not exposed at the time of application were collected from the V4 and R3 experiments to determine systemic efficacy. Ten leaves from the treated portion of the canopy were collected in the R3 study to determine residual efficacy. Leaves were pulled at 7 and 14 days after treatment in the V4 study, and 10, 17, 24, and 31 days after treatment in the R3 study. In the R3 study, ten pods were removed from each plot at R5.5 to determine if the insecticides moved to reproductive structures. For all assays, corn earworm, *Helicoverpa zea* (Boddie), larvae were placed on leaf material, seed, and/or seed hulls to test for presence of the insecticide. Chlorantraniliprole moved systemically and provided significantly greater control than flubendiamide in the systemic and residual study out to 31 DAT. Flubendiamide did not move systemically, but provided significant residual mortality out to 31 DAT compared with the untreated control. Neither insecticide

resulted in mortality of *H. zea* feeding on reproductive structures. These results suggest that chlorantraniliprole moves systemically to new vegetative structures but not to reproductive structures of soybean, and that flubendiamide does not move systemically.

### **Introduction**

Soybean, *Glycine max* (L) Merr., is the most valuable row crop commodity in Mississippi in terms of planted area and total commodity value. In 2014, soybean accounted for 898,402 planted ha valued at US\$1,113,200,000 in Mississippi (NASS 2014). Corn earworm, *Helicoverpa zea* (Boddie), is the most costly insect pest of soybean production in the Mid-Southern and Southeastern United States in terms of lost yield and control costs (Musser et al. 2015). During 2014, damage incurred through larval feeding by corn earworm resulted in a US\$11,009,548 economic cost in terms of lost yield and control costs in Mississippi soybean production (Musser et al. 2015).

Corn earworm is a widely distributed polyphagous pest of numerous cultivated crops (Fitt 1989, Swenson et al. 2013). The preferred crop for oviposition is corn, *Zea mays* (L). When corn senescens, corn earworm adults commonly begin to oviposit in soybean and can cause considerable economic damage (Johnson et al. 1975, Kogan et al. 1979, Musser et al. 2015, Swenson et al. 2013). Infestations generally occur during the R1 to R3 growth stages in open canopied fields (Johnson et al. 1975, Swenson et al. 2013). Larval feeding may result in defoliation, delayed pod fill, and decreased seed number per pod, ultimately resulting in yield loss (Eckel 1992a). Severity of damage from larval feeding depends on 4 factors; larval age, plant growth stage, timing of damage, and the ability of the plant to compensate for feeding (Swenson et al. 2013). All larval instars prefer to feed on blooms over leaves or pods (Mueller and Engroff 1980).

Damage per larva can be most severe in the early reproductive growth stages of soybean because more small pods and immature seeds can be consumed compared to more developed pods (McWilliams 1983).

Soybean can compensate from feeding injury incurred during early reproductive growth stages (R1-R3) (Eckel 1992b). However, the ability of soybean to compensate for larval damage is dependent on environmental conditions and damage during the early growth stages may result in delayed pod set (Eckel 1992b). The ability of a soybean plant to compensate in early growth stages is important, but the possible delay in maturity may be problematic for soybean not planted in the optimal planting window. Damage incurred during later growth stages (R4-R5) limits time for compensation, and yield losses are more directly related to pod removal and seed consumption (Thomas et al. 1974, McPherson and Moss 1989).

Foliar applications of synthetic insecticides are instrumental in the management of lepidopteran insect pests in the southern United States. Widespread foliar applications of synthetic insecticides in multiple crops has led to resistance development and/or inconsistent control with most chemical classes including, chlorinated hydrocarbons, organophosphates, carbamates, pyrethroids, and benzoylphenylureas (Sparks 1981, Brown et al. 1998, Temple et al. 2006, Jacobson et al. 2009, Lai and Su 2011). The diamide class of insecticide was introduced in 2008 and is the newest class of insecticide (EPA 2008). It has a novel mode of action and is classified as a ryanodine receptor modulator (MoA Group 28) (IRAC 2015). Two representatives from this insecticide class are chlorantraniliprole, (Prevathon<sup>®</sup>, DuPont Crop Protection, Newark, DE), an anthranilic diamide, and flubendiamide, (Belt<sup>®</sup>, Bayer CropScience, Raleigh, NC), a

phthalic acid diamide (Lahm et al. 2009). Since their introduction, these two active ingredients have been important in management of lepidopteran insect pests in multiple crops.

Chlorantraniliprole is xylem-mobile, allowing the insecticide to move upwards throughout the plant (Lahm 2007). It is often applied to the soil as seed treatments, soil drenches, or through chemigation in multiple crops such as brassicas and other vegetables (Cameron et al. 2015, Lahm et al. 2007, Schuster et al. 2009, Ghidui et al. 2009, Khuhar et al. 2008, Palumbo 2008). With those applications, the insecticide is taken up by the roots and provides effective control of lepidopteran and other insect pests on the foliage. It is currently registered in the US for use as an in-furrow spray at planting, transplant water treatment, hill drench at planting, surface band at planting, soil shank injection at planting, through drip irrigation, and by foliar application (Lahm et al., 2007; Cameron et al. 2015). Furthermore, chlorantraniliprole is also effective as a seed treatment in managing *Lissorhoptus oryzophilus* (Kuschel) infestations in rice, *Oryza sativa* (L) (Adams et al. 2015). However, chlorantraniliprole is not known to move systemically when applied as a foliar application. Furthermore, flubendiamide has greater residual efficacy compared to other insecticides as reported by Hardke et al. (2011), but it is not known to move systemically. Therefore, the objectives of this study were to determine the systemic and residual efficacy of chlorantraniliprole and flubendiamide against corn earworm through laboratory bioassays when applied as a foliar application to soybean.

### **Materials and Methods**

Multiple experiments were conducted at the R.R. Foil Plant and Soil Sciences Research Center in Starkville, MS and the Delta Research and Extension Center in

Stoneville, MS during 2013, 2014, and 2015 to evaluate the residual and systemic activity of chlorantraniliprole and flubendiamide. Activity was evaluated in lab bioassays by infesting larvae from lab colonies on to leaf tissue collected from field plots sprayed in the field at V4 and R3 growth stages. Furthermore, a greenhouse experiment was conducted during the fall of 2014 and spring of 2015 to evaluate the activity of chlorantraniliprole when applied to individual plant structures.

### **Insect Rearing**

The laboratory colonies of corn earworm used for evaluation in these experiments were obtained from non-Bt corn through multiple collections at the R.R. Foil Plant and Soil Sciences Research Center in Starkville, MS and the Delta Research and Extension Center in Stoneville, MS during 2013, 2014, and 2015. Each collection consisted of at least 300 third instar larvae. Larvae were placed in 36 mL Solo<sup>®</sup> cups (Bio-Serv<sup>®</sup>, Frenchtown, NJ) containing Stonefly Heliothis Diet (Product No. 38-0600, Ward's Natural Science, Rochester, NY) with matching lids. At pupation, approximately 50 pupae were placed in 3.79 liter cardboard containers with matching lids with the corresponding colony and generation information labeled on the outside of each bucket. Adults were fed a 10% sugar-water solution. For the purpose of egg collection for bioassays, the cardboard containers were lined with Reynolds<sup>®</sup> Cut-Rite<sup>®</sup> Waxed Paper (Reynolds Consumer Products, Lake Forest, IL). The center of each lid was removed so that only the rim remained. Cotton cloth was placed over each bucket and kept in place by the lid to serve as an oviposition substrate. Eggs were collected daily and new cloths and waxed paper were applied to every bucket. Collected egg sheets and waxed paper from each colony were kept in 3.79 liter Ziploc<sup>®</sup> (S.C. Johnson & Johnson, Inc., Racine

WI) bags until larvae hatched for use in bioassays. All insect populations were reared at the Mississippi State University insect rearing facility maintained at 25°C, 80% relative humidity, and 16:8 (L:D) photoperiod. All assays were conducted on first and/or second generation progeny of field collected *H. zea* colonies.

### **Field Plot Details**

Two experiments were conducted to determine the residual and systemic efficacy of chlorantraniliprole and flubendiamide in vegetative plant structures applied as a foliar application to soybean. The experiments were conducted using an indeterminate maturity group (MG) IV soybean variety (Asgrow 4632<sup>®</sup>, Monsanto Company, St. Louis, Mo). Plots were 4 rows by 15.24 m. Soybean were planted at 296,532 seeds/ha into raised conventional tilled beds with a 0.97 m row spacing in Starkville, MS at the R.R. Foil Plant and Soil Sciences Research Center and a 1.02 m row spacing in Stoneville, MS at the Delta Research and Extension Center. Seed were treated with a commercial premix of imidacloprid, pyraclostrobin, metalaxyl, and fluxapyroxad (Acceleron<sup>®</sup>, Monsanto Company, St. Louis, MO) to minimize the impact of early season insect pests and seedling diseases. Weed and disease pests were managed according to Mississippi State University Extension Service recommendations. Experiments were separated according to soybean growth stage at the time of application. All plots were treated with a MUDMASTER<sup>™</sup>, 4WD Multi-Purpose Sprayer, (Bowman Manufacturing, Newport, AR) equipped with a compressed air high-clearance mounted multi-boom, calibrated to deliver 94 L/ha at 4 bar through TX-6 ConeJet<sup>®</sup> VisiFlo<sup>®</sup> Hollow Cone Spray Tip nozzles (2 nozzles per row) (TeeJet<sup>®</sup> Technologies, Glendale Heights, IL).

## **Leaf Assays**

During 2013, an experiment was conducted at the R.R. Foil Plant and Soil Sciences Center in Starkville, MS, and in 2014 and 2015 at the Delta Research and Extension Center in Stoneville, MS to determine the residual and systemic efficacy of chlorantraniliprole and flubendiamide applied as a foliar application to R3 stage (Fehr and Caviness 1977) soybean. The experiment was conducted as a randomized complete block design with four replications in 2013 and 2014 and six replications in 2015. Treatments consisted of chlorantraniliprole applied at 47.25 g ai/ha, and flubendiamide, applied at 70.06 g ai/ha compared with an untreated control. Plants within each plot were flagged at the uppermost node at the time of application to differentiate between treated and non-treated foliage at each of the evaluation timings. Ten uppermost newly emerged trifoliates were removed from above the flagging at 10, 17, 24 and 31 days after treatment to determine systemic efficacy. Ten leaves from the treated portion of the plants were also removed from below the flagging at 10, 17, 24, and 31 days after treatment to determine residual efficacy. All leaves were transported to the laboratory for testing as detailed below. Leaf assays for this experiment were terminated when vegetative growth ceased.

During 2014 and 2015, an experiment was conducted at the R.R. Foil Plant and Soil Sciences Center in Starkville, MS, to determine the systemic efficacy of chlorantraniliprole applied as a foliar application to V4 stage (Fehr and Caviness 1977) soybean. The experiment was conducted as a randomized complete block design with four replications and two treatments. Treatments consisted of chlorantraniliprole applied at 47.25 g ai/ha compared with an untreated control. Ten uppermost newly emerged

trifoliates were removed at 7 and 14 days after treatment. Every attempt was made to insure that only newly emerged leaves that were not present at the time of application were selected to determine systemic efficacy. They were then transported to the laboratory for testing as detailed below.

Collected leaf material from the V4 and R3 studies were placed in 0.95 liter Ziploc<sup>®</sup> (S.C. Johnson & Johnson, Inc., Racine WI) bags labeled by plot and transported to the Mississippi State University insect rearing facility in Mississippi State, MS. In the laboratory, entire newly emerged trifoliates from the systemic study and 5 cm leaf disk from the residual study were placed in 100 x 15 mm petri dishes (Product No. 431760, Fisher Scientific, Norcross, GA), labeled by plot, containing a 1% water agar (Product No. 7060, Frontier Agricultural Sciences, Newark, DE) solution to prevent desiccation. Two corn earworm neonates obtained from the colony described above were placed onto the surface of each leaf. After infestation, a lid was placed onto the top of every petri dish and sealed with a single piece of 1.27 X 10 cm Parafilm M<sup>®</sup> All-Purpose Laboratory Film (Product No. 13-374-12, Fisher Scientific, Norcross, GA). Infested petri dishes were then placed in a rearing chamber maintained at 25°C, 80% relative humidity, and 16:8 (L:D) photoperiod. Mortality was rated after 3 days of initial exposure. Mortality was defined as larvae that failed to respond to a probe or to right themselves after being flipped onto their dorsal surface.

Mortality data were analyzed with analysis of variance (PROC GLIMMIX, SAS Institute Inc. 2012). In the V4 experiment, insecticide treatment and days after treatment were considered fixed effects in the model. Year and replication nested in year were random terms in the model. In the R3 experiment, treatment, days after treatment, and

leaf position were considered fixed effects in the model. Year, replication nested in year and replication by leaf position nested in year were random terms in the model. Degrees of freedom were calculated using the Kenward-Roger method. Means were estimated using the LSMEANS statement and adjusted according to the Tukey's HSD test and considered significant at  $\alpha = 0.05$ .

### **Pod and Seed Assays**

In 2014 and 2015, an additional laboratory experiment was conducted within plots treated at the R3 growth stage. This experiment was conducted to determine if chlorantraniliprole or flubendiamide translocated to the reproductive structures of soybean. Ten soybean pods were removed from the top 1/3 of plants in treated and untreated plots at the R5.5 growth stage (28 days after treatment) (Fehr and Caviness 1977). This portion of the plant was chosen because greater than 90% of *H. zea* oviposition occurs in the top 1/3 of the soybean canopy (Adams et al. 2015b, Dill et al. 2015).

Collected pods were placed in 0.95 liter Ziploc<sup>®</sup> bags labeled by plot and transported to the Mississippi State University insect rearing facility in Mississippi State, MS. In the laboratory, pods were separated into seed and seed hulls. To prevent mold growth that occurred in preliminary studies, the seed and seed hulls were surface sterilized with a 10% sodium hypochlorite (Clorox<sup>®</sup> Regular-Bleach<sub>1</sub>, The Clorox Company, Oakland, CA) solution by soaking for five minutes followed by rinsing with water through a 100 mesh sieve for 5 minutes. Seeds and seed hulls were then allowed to air dry on a paper towel (Brawny<sup>®</sup>, Georgia-Pacific Consumer Products, Atlanta, GA). Seeds were placed in 36 mL Solo<sup>®</sup> cups containing a 1% water agar solution to prevent

desiccation. One entire seed hull was placed in petri dishes according to the methodology previously described for leaves. In total, thirty seeds and both sides of the seed hull were used per plot per treatment. To reduce control mortality and more closely simulate what occurs in the field, larvae were reared on untreated diet for 5 days prior to infestation. One corn earworm larva was placed onto each seed totaling thirty larvae per treatment per replication. For seed hulls, one corn earworm larva was placed on the inside wall of the seed hull totaling twenty larvae per treatment per replication. After infestation, the cap was placed onto the top of every cup and petri dish lids were sealed as previously described. Infested seed and seed hulls were placed in a rearing chamber maintained at 25°C, 80% relative humidity, and 16:8 (L:D) photoperiod. Mortality was rated 3 days after exposure. Mortality was defined as larvae that failed to respond to a probe or failed to right themselves after being flipped onto their dorsal surface.

Mortality data were analyzed with analysis of variance (PROC GLIMMIX, SAS Institute Inc. 2012). In the model, insecticide treatment and reproductive structure were considered fixed effects. Year, replication nested in year, and replication by location nested in year were random terms in the model. Degrees of freedom were calculated using the Kenward-Roger method. Means were estimated using the LSMEANS statement and adjusted according to the Tukey's HSD test and considered significant at  $\alpha = 0.05$ .

### **Greenhouse Study**

An experiment was conducted to determine the route of absorption and translocation of chlorantraniliprole in soybean. This experiment was conducted in a greenhouse located at the Clay Lyle Entomology Building in Mississippi State, MS in September 2014, March 2015, and May 2015. Three soybean seed were placed into a

3.79 liter black blow molded nursery container (Product No: C408, Nursery Supplies, Kissimmee, FL) containing a 80/20 mixture of PRO-MIX<sup>®</sup> ALL PURPOSE GROWING MIX (Premier Tech Horticulture Office USA, Quakertown, PA) and soil that had not been exposed to insecticides. Each pot was fertilized with Miracle-Gro<sup>®</sup> Shake 'N<sup>®</sup> Feed All Purpose Continuous Release Plant Food (The Scotts Miracle-Gro Company, Marysville, OH) at planting. When plants reached V2 they were thinned to one plant per pot.

The experiment was initiated at the V4 growth stage. The experimental design was a randomized complete block design with five treatments and three replications. Treatments consisted of applying chlorantraniliprole as a 25% solution independently to the whole main stem, each trifoliolate, every petiole, or entire plant with a number six paint brush compared to an untreated control. Each treatment consisted of 10 plants per replication totaling 150 plants per test. Plants were watered every other day to maintain soil moisture. Special care was taken not to get water onto any plant parts when watering. After seven days, the uppermost newly emerged trifoliolate was removed from every plant and placed in 0.95 liter Ziploc<sup>®</sup> bags according to treatment and replication. Leaves were transported to the laboratory where they were tested. Testing procedures were identical to those described above in the leaf assay methodology.

Mortality data were analyzed with analysis of variance (PROC GLIMMIX, SAS Institute Inc. 2012). In the model, treatment location was considered a fixed effect. Replication was the random term in the model. Degrees of freedom were calculated using the Kenward-Roger method. Means were estimated using the LSMEANS statement and adjusted according to the Tukey's HSD test and considered significant at  $\alpha = 0.05$ .

## Results

### Leaf Assays at the V4 and R3 Applications

Chlorantraniliprole moved systemically when applied as a foliar application to soybean at the V4 growth stage. A significant interaction between treatment and days after treatment was observed for corn earworm mortality ( $F=22.72$ ;  $df= 1, 28$ ;  $P < 0.01$ ). Chlorantraniliprole resulted in greater mortality of corn earworm compared with the untreated control at seven days after treatment (Figure 3.1). At 14 days after treatment, no significant difference in mortality of corn earworm was observed between chlorantraniliprole and the untreated control.

A significant interaction between treatment, days after treatment, and leaf position was observed for corn earworm mortality on leaves at the R3 application timing ( $F=3.69$ ;  $df= 9, 222.2$ ;  $P < 0.01$ ). Chlorantraniliprole moved systemically to new vegetative growth resulting in 89 to 96% mortality of corn earworm infested on leaves not present at time of application at all evaluation times (Table 3.1). In contrast, flubendiamide did not move systemically to new vegetative growth and resulted in similar levels of mortality to the untreated control in upper leaves. Mortality of corn earworm on leaves present at time of application was similar between chlorantraniliprole and flubendiamide at 10 and 17 days after treatment (Table 3.1). Both insecticides provided significantly greater mortality of corn earworm than the untreated control on lower leaves at 10 and 17 days after treatment. At 24 days after treatment, chlorantraniliprole provided significantly greater mortality on lower leaves than flubendiamide providing 19% greater residual mortality of corn earworm compared with flubendiamide and 90% greater residual mortality compared to the untreated control (Table 3.1). Furthermore, the residual mortality of

chlorantraniliprole at 24 days after treatment was not significantly different than chlorantraniliprole at 10 and 17 days after treatment (Table 3.1). Flubendiamide provided significantly greater mortality of corn earworm compared with the untreated control on lower leaves throughout the experiment. The mortality of corn earworm on lower leaves treated with chlorantraniliprole did not vary throughout the experiment, and was not significantly different compared with the 10 and 17 day efficacy ratings of flubendiamide. However, mortality of corn earworm on lower leaves treated with flubendiamide declined significantly at 31 days after treatment, providing approximately 30% less mortality compared with chlorantraniliprole at 31 days and approximately 15% less mortality compared with flubendiamide at 24 days after treatment (Table 3.1).

### **Pod and Seed Assays at the R3 Application**

No significant interaction between insecticide treatment and fruiting structure was observed for corn earworm mortality when chlorantraniliprole or flubendiamide were applied as a foliar application at the R3 growth stage and measured in mortality of corn earworm from feeding on R5.5 seed and pod walls ( $F = 0.94$ ;  $df = 2$ , 20.13;  $P = 0.41$ ). Furthermore, there was no significant effect observed for insecticide treatment ( $F = 0.42$ ;  $df = 2$ , 18.83;  $P = 0.67$ ) or reproductive structure ( $F = 4.11$ ;  $df = 1$ , 5.56;  $P = 0.09$ ) (Figure 3.2).

### **Greenhouse Study**

A significant effect was observed for treatment location when chlorantraniliprole was applied to vegetative structures in the greenhouse at V4 ( $F = 59.88$ ;  $df = 4$ , 50;  $P < 0.01$ ). Overall, the application of chlorantraniliprole to the entire plant provided

significantly greater mortality of corn earworm compared to applying chlorantraniliprole individually to the stem, leaf, or petiole (Figure 3.3). Chlorantraniliprole applied to the whole plant provided approximately 22, 42, 45, and 48% greater mortality compared to the stem, leaf, petiole and the untreated control, respectively. Chlorantraniliprole applied to the stem provided significantly greater mortality of corn earworm than application to the leaf, petiole or the untreated control. Furthermore, application to the stem provided approximately 20, 23, and 26% greater mortality than application to the leaf, petiole, and the untreated control, respectively. No significant differences in mortality were observed for application to the leaf and petiole compared with the untreated control.

### **Discussion**

The systemic efficacy of chlorantraniliprole against lepidopteran pest species when applied to the root zone has been well documented (Lahm et al. 2007, Schuster et al. 2009, Ghidui et al. 2009; 2012, Khuhar et al. 2008, Palumbo 2008). Prior to this study, there had been no reports of systemic efficacy of chlorantraniliprole when applied as a foliar application. In this paper, it is reported that chlorantraniliprole was observed to move systemically to the vegetative structures of soybean.

Ghidui et al. (2009) reported that two applications of chlorantraniliprole through drip irrigation provided season long control of European corn borer, *Ostrinia nubilalis* (Hübner), in bell peppers, *Capsicum annuum* (L), and was as effective as up to nine foliar applications of a standard insecticide program. The systemic efficacy of foliar applied chlorantraniliprole was variable in the current study, and appeared to be dependent on plant size at the time of application. The differences observed in systemic efficacy between the V4 application and the R3 application could be attributed to rapid node

development occurring from the V4 to the R2 growth stage (Pedersen 2004). When applied at V4, it appeared that the vegetative surface area was not great enough at the time of application to intercept an adequate amount of chlorantraniliprole to provide any mortality beyond the 7 day rating. Although mortality from chlorantraniliprole at the 7 day rating was greater than the untreated control, it was not adequate to provide acceptable control in a field situation with substantial pressure. In contrast, soybean at R3 has developed close to its total number of nodes. The size of the plant at the time of application was sufficient to intercept enough chlorantraniliprole to provide systemic control until no new terminal growth was present. Furthermore, based on the results of the greenhouse portion of this study, it appears that absorption occurs primarily in the stem. Application to the leaf and petiole alone did not result in significant levels of mortality, but the application to the entire plant appears to have an additive effect and a greater level of efficacy was observed.

Chlorantraniliprole is xylem mobile and moves throughout the green tissue of plants (Lahm et al. 2007). Furthermore, because larval mortality from feeding on reproductive structures in chlorantraniliprole treated plots was not different from untreated plots, it appears that chlorantraniliprole is not phloem mobile. While, the primary function of xylem is to transport water and minerals from the roots to aerial portions of the plant (Lucas et al. 2013). Phloem primarily functions as a food and nutrient transport from leaves to storage organs (source to sink) (Lucas et al. 2013). Vijayasree et al. (2013) found that chlorantraniliprole residues were undetectable and had completely dissipated from cowpea fruits 10 days after treatment. This supports the findings that larval feeding on reproductive structures resulted in no larval mortality.

Large monocultures with staggered planting dates are a standard practice in current agriculture systems. The biological and ecological characteristics of the corn earworm allow this insect pest to thrive in the current production landscape (Stinner et al. 1982, Fitt 1989). Chlorantraniliprole and flubendiamide produced long residual mortality of corn earworm when applied at the R3 growth stage and will continue to play an important role in lepidopteran insect pest management. The systemic efficacy of chlorantraniliprole, though variable, may provide greater benefits for overall management of corn earworm in soybean than flubendiamide. However, this will depend on plant size at time of application and the duration of infestation. Nevertheless, when soybeans are infested at R1-R3 the systemic efficacy of chlorantraniliprole may prove valuable in protection of crop yields. Flubendiamide provided good residual mortality on treated leaf tissue. Infestations at growth stage R4-R5 are common in some areas. At R4-R5, soybean has produced the majority of its leaf surface area (Pedersen 2004). Further, accumulation of biomass will be limited and the residual efficacy of flubendiamide should persist for the remainder of the growing season. In conclusion, both chlorantraniliprole and flubendiamide are remarkable products and are valuable tools for lepidopteran insect pest management. Each insecticide provides exceptional control of corn earworm. Understanding the population dynamics, growth stage of the plant, and time of year will be beneficial in making an application decision.

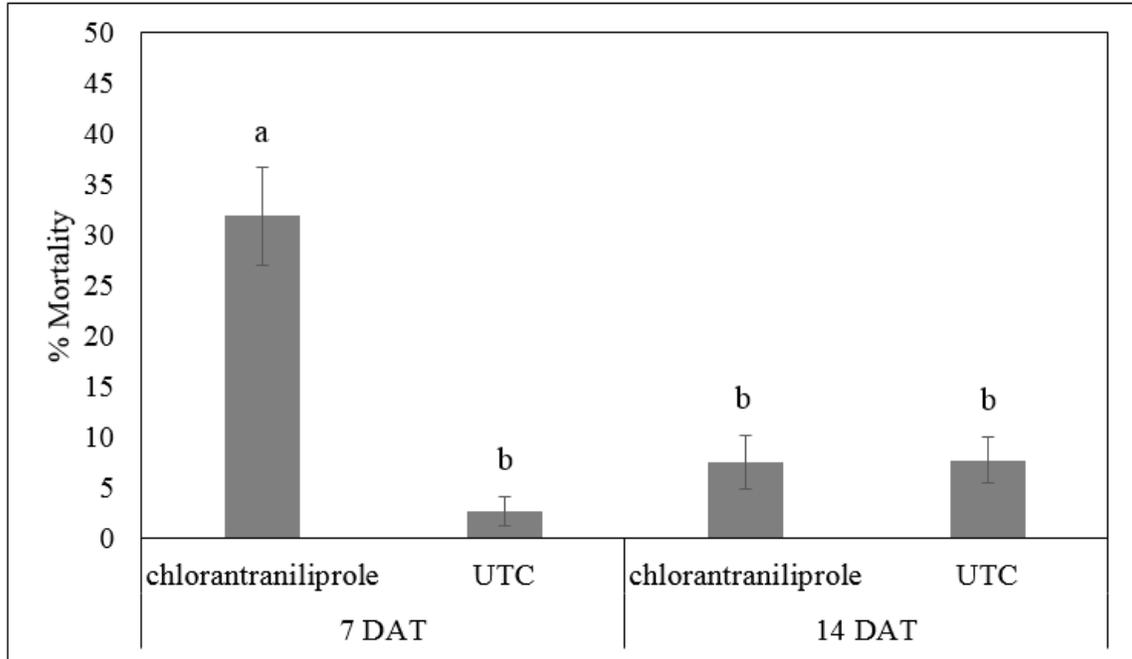


Figure 3.1 Mean (SEM) levels of mortality of *H. zea* exposed to leaves that developed after application of chlorantraniliprole at the V4 growth stage during 2013-2015.

Bars sharing the same letter grouping are not significantly different (P<0.05).

Table 3.1 Mean (SEM) levels of mortality of *H. zea* exposed to *G. max* leaves that developed after application and leaves present at time of application when treated with chlorantraniliprole or flubendiamide at the R3 growth stage during 2013-2015.

Treatment	Leaf Position	Mean $\pm$ S.E. <sup>1,2</sup>				
		10 DAT <sup>3</sup>	17 DAT <sup>3</sup>	24 DAT <sup>3</sup>	31 DAT <sup>3</sup>	Mean
<b>chlorantraniliprole</b>	Upper	96.02 $\pm$ 1.21 a	89.11 $\pm$ 2.52 ab	92.88 $\pm$ 2.08 a	92.46 $\pm$ 1.80 a	92.62 $\pm$ 1.90
<b>flubendiamide</b>	Upper	15.43 $\pm$ 3.32 de	16.34 $\pm$ 2.72 de	11.82 $\pm$ 2.30 de	12.83 $\pm$ 2.57 de	14.11 $\pm$ 2.73
<b>untreated control</b>	Upper	6.79 $\pm$ 1.50 e	10.96 $\pm$ 2.10 de	7.49 $\pm$ 1.85 e	6.08 $\pm$ 1.36 e	7.83 $\pm$ 1.70
<b>chlorantraniliprole</b>	Lower	98.47 $\pm$ 0.78 a	95.00 $\pm$ 2.11 a	98.21 $\pm$ 0.86 a	94.51 $\pm$ 1.58 a	96.55 $\pm$ 1.33
<b>flubendiamide</b>	Lower	96.67 $\pm$ 1.67 a	89.91 $\pm$ 4.28 ab	79.56 $\pm$ 4.88 b	64.42 $\pm$ 5.67 c	82.64 $\pm$ 4.13
<b>untreated control</b>	Lower	10.50 $\pm$ 1.89 de	10.17 $\pm$ 1.91 de	8.29 $\pm$ 1.81 de	8.86 $\pm$ 2.21 de	9.45 $\pm$ 1.96

<sup>1</sup> Means followed by the same letter are not significantly different, Tukey's HSD ( $\alpha = 0.05$ ).

<sup>2</sup> Means and standard error are expressed as percentage control of *H. zea*.

<sup>3</sup> DAT=Days after Treatment

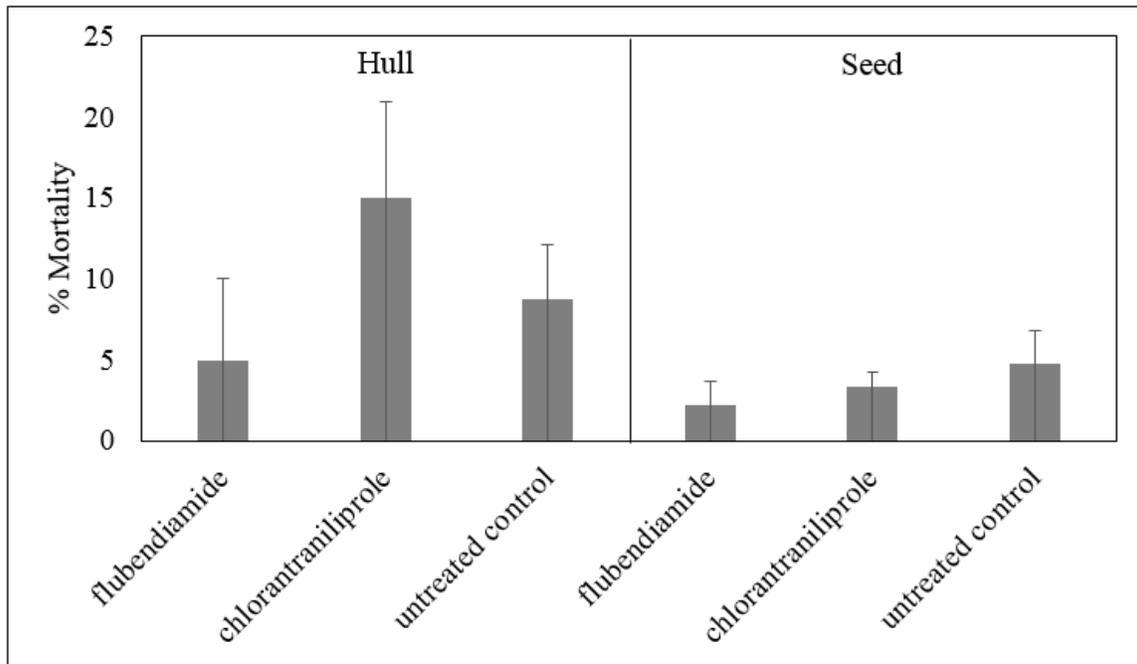


Figure 3.2 Mean (SEM) levels of mortality of *H. zea* larvae exposed to *G. max* reproductive structures sprayed with chlorantraniliprole or flubendiamide at the R3 growth stage during 2014-2015.

Bars sharing the same letter grouping are not significantly different ( $P < 0.05$ ).

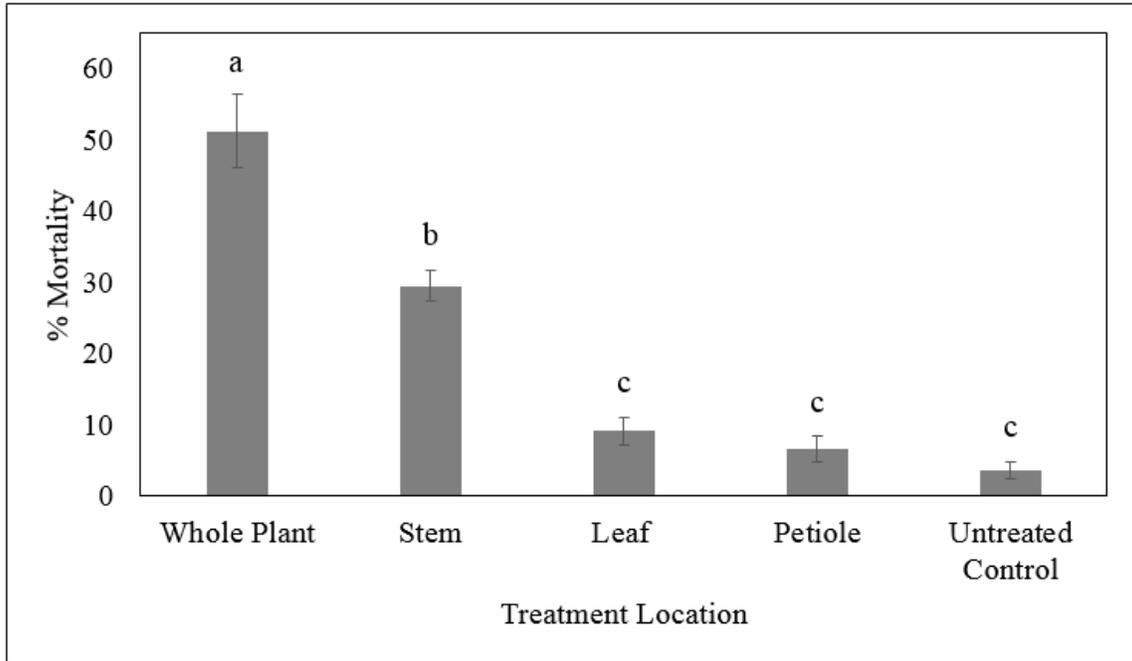


Figure 3.3 Mean (SEM) levels of mortality of *H. zea* larvae exposed to *G. max* leaf material in laboratory assays with chlorantraniliprole applied to specific vegetative structures at V4 growth stage in a controlled environment during 2014-2015.

Bars sharing the same letter grouping are not significantly different ( $P < 0.05$ ).

## Literature Cited

- Adams, A., J. Gore, F. Musser, D. Cook, A. Catchot, T. Walker, and C. Dobbins. 2015a.** Efficacy of selected insecticides applied to hybrid rice seed. *J. Econ. Entomol.* doi: 10.1093/jee/tov310.
- Adams, B. P. 2015b.** An evaluation of corn earworm damage and thresholds in soybean. Mississippi State University, Ph.D. Dissertation.
- Brown, T. M., P. K. Bryson, D. S. Brickle, S. Pimprale, F. Arnette, M. E. Roof, J. T. Walker, and M. J. Sullivan. 1998.** Pyrethroid-resistant *Helicoverpa zea* and transgenic cotton in South Carolina. *Crop Protect.* 17: 441-445.
- Cameron R. A., C. J. Williams, H. E. Portillo, P. C. Marcon, and L. A. Teixeira. 2015.** Systemic application of chlorantraniliprole to cabbage transplants for control of foliar-feeding lepidopteran pests. *Crop Protect.* 67: 13-19.
- Dill, T. D. 2015.** Adult emergence and egg distribution of the Heliiothine complex and their impact on Bt resistance management in agricultural landscapes. Mississippi State University, Thesis.
- Eckel, C. S., J. R. Bradley, Jr., and J. W. Van Duyn. 1992a.** Soybean growth and development alterations caused by *Helicoverpa zea* (Boddie) feeding. *Agron. J.* 84: 813-820.
- Eckel, C. S., J. R. Bradley, Jr., and J. W. Van Duyn. 1992b.** Reductions in soybean yield and quality from corn earworm flower feeding. *Agron. J.* 84: 402-409.
- EPA. 2008.** Pesticide Fact Sheet, Chlorantraniliprole, [http://www3.epa.gov/pesticides/chem\\_search/reg\\_actions/registration/fs\\_PC-090100\\_01-Apr-08.pdf](http://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-090100_01-Apr-08.pdf). Accessed: Jan 2016.
- Fehr, W. R., and C. E. Caviness. 1977.** Stages of soybean development, Iowa State University, SR80.
- Fitt, G. P. 1989.** The ecology of Heliiothis species in relation to agroecosystems. *Ann. Rev. of Entomol.* 34: 17-52.
- Ghidiu, G. M., D. L. Ward, and G. S. Rogers. 2009.** Control of European corn borer in bell peppers with chlorantraniliprole applied through a drip irrigation system. *Intern. J. Veg. Sci.* 15: 193-201.
- Ghidiu, G. M., T. Kuhar, J. Palumbo, and D. Schuster. 2012.** Drip chemigation of insecticides as a pest management tool in vegetable production. *J. Integ. Pest Mngmt.* 3: E1-E5.

- Hardke, J. T., J. H. Temple, B. R. Leonard, and R. E. Jackson. 2011.** Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). *Florida Entomol.* 94: 272-278.
- IRAC. 2015.** IRAC Mode of Action Classification Scheme. Version 8.0 [Online]. Available at <http://www.irc-online.org/documents/moa-classification/> Accessed: January 2016.
- Jacobson, A., R. Foster, C. Krupke, W. Hutchison, B. Pittendrigh, and R. Weinzierl. 2009.** Resistance to pyrethroids insecticides in *Helicoverpa zea* (Lepidoptera: Noctuidae) in Indiana and Illinois. *J. Econ. Entomol.* 102: 2289-2295.
- Johnson, M. W., R. E. Stinner, and R. L. Rabb. 1975.** Ovipositional response of *Heliothis zea* (Boddie) to its major hosts in North Carolina. *Environ. Entomol.* 4: 291-297.
- Kogan, M. 1979.** Insect problems of soybean in the United States., pp. 303-325. *In* F. T. Corbin (ed.), *World Soybean Research Conference II*. Westview, Boulder CO.
- Kuhar, T. P., H. Doughty, E. Hitchner, and M. Cassell. 2008.** Evaluation of insecticide treatments for the control of lepidopteran pests in bell peppers in Virginia, 2007. *Arthropod Management Tests*. Tests 33: E7.
- Lahm, G. P., D. Cordova, and J. D. Barry. 2009.** New and selective ryanodine receptor activators for insect control. *Bioorganic & Medic. Chem.* 17: 4127-4133.
- Lahm, G. P., T. M. Stevenson, T. P. Selby, J. H. Freudenberger, D. Cordova, L. Flexner, C. A. Bellin, C. M. Dubas, B. K. Smith, K. A. Hughes, J. G. Hollingshaus, C. E. Clark, and E. A. Benner. 2007.** Rynaxypr<sup>®</sup>: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator. *Biorg. Med. Chem. Let.* 17: 6274-6279.
- Lai, T., and J. Su. 2011.** Assessment of resistance risk in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) to chlorantraniliprole. *Pest Manag. Sci.* 67: 1468-1472.
- Lucas, W. J., A. Groover, R. Lichtenberger, K. Furuta, S. R. Yadav, Y. Helariutta, X. Q. He, H. Fukuda, J. Kang, S. M. Brady, J. W. Patrick, J. Sperry, A. Yoshida, A. F. López-Millán, M. A. Grusak, and P. Kachroo. 2013.** The plant vascular system: evolution, development and functions. *J. Integr. Plant Biol.* 55: 294-388.
- McPherson, R. M., and T. P. Moss. 1989.** Response of soybean to natural and simulated corn earworm (Lepidoptera: Noctuidae) pod injury. *J. Econ. Entomol.* 82: 1767-1772.
- McWilliams, J. M. 1983.** Relationship of soybean pod development to bollworm and tobacco budworm damage. *J. Econ. Entomol.* 76: 502-506.

- Mueller, A. J., and B. W. Engroff.** 1980. Effects of infestation levels of *Heliothis zea* on soybean. *J. Econ. Entomol.* 73: 271-275.
- Musser, F. R., A. L. Catchot, Jr., J. A. Davis, D. A. Herbert, Jr., G. M. Lorenz, T. Reed, D. D. Reisig, and S. D. Stewart.** 2015. 2014 Soybean insect losses in the southern US. *Midsouth Entomol.* 8:35-48.
- NASS.** 2014. National soybean statistics.  
[http://www.nass.usda.gov/Charts and Maps/Field Crops/sovac.asp](http://www.nass.usda.gov/Charts_and_Maps/Field_Crops/sovac.asp)
- Palumbo, J. C.** 2008. Systemic efficacy of Coragen applied through drip irrigation on romaine lettuce, fall 2007. *Arthropod Mngmt. Tests* 33: E24.
- Pedersen, P.** 2004. Soybean Growth and Development. Iowa State University Ames, IA. PM1945.
- SAS Institute.** 2012. SAS 9.3 for windows. SAS Institute, Cary, NC.
- Schuster, D. J., A. Shurtleff, and S. Kalb.** 2009. Management of armyworms and leafminers on fresh market tomatoes, fall 2007. *Arthropod Mngmt. Tests* 34: E79.
- Sparks, T. C.** 1981. Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. *Bull. Entomol. Soc. Amer.* 27: 186-192.
- Stinner, R. E., J. Regniere, and K. Wilson.** 1982. Differential effects of agroecosystem structure on dynamics of three soybean herbivores. *Environ. Entomol.* 11: 538-543.
- Swenson, S. J., D. A. Prischmann-Voldseth, and F. R. Musser.** 2013. Corn earworms (Lepidoptera: Noctuidae) as pests of soybean. *J. Integ. Pest Mangmt.* 4: 1-8.
- Temple, J. H., D. R. Cook, P. L. Bommireddy, S. Micinski, W. Waltman, A. M. Stewart, B. Garber, and B. R. Leonard.** 2006. Monitoring cypermethrin susceptibility in Louisiana bollworm 2004-2005, pp. 1236-1240. *In Proc. Beltwide Cotton Conf., San Antonio, TX. 3-6 Jan. 2006. Natl. Cotton Counc. Am., Memphis TN.*
- Thomas, G. D., C. M. Ignoffo, K. D. Biever, and D. B. Smith.** 1974. Influence of defoliation and depodding on yield of soybean. *J. Econ. Entomol.* 67: 683-685.
- Vijayasree V., H. Bai, S. N. Beevi, T. B. Mathew, V. Kumar, T. George, and G. Xavier.** 2013. Persistence and effects of processing on reduction of chlorantraniliprole residues on cowpea fruits. *Bull. Environ. Contam. Toxicol.* 90:494-498.

CHAPTER IV  
LABORATORY SELECTION FOR BEET ARMYWORM (LEPIDOPTERA:  
NOCTUIDAE) RESISTANCE TO FLUBENDIAMIDE

**Abstract**

Beet armyworm, *Spodoptera exigua* (Hübner), were exposed to flubendiamide to determine the risk of resistance development to the diamide insecticide class. A field collected beet armyworm colony was separated into three cohorts that were independently selected with three concentrations (0.016, 0.020, and 0.025 ppm ai) of flubendiamide incorporated into meridic diet. These concentrations were chosen based on the LC<sub>30</sub>, LC<sub>60</sub> and LC<sub>90</sub> values of the original colony. All of the colonies were significantly less susceptible to flubendiamide compared with the original colony. However, resistance ratios never increased above 2.11-fold. The highest LC<sub>50</sub> observed for each colony was 0.033, 0.033, and 0.039 ppm for colonies exposed at the LC<sub>30</sub>, LC<sub>60</sub>, and LC<sub>90</sub>, respectively. The highest resistance ratios occurred after 18 generations for the LC<sub>30</sub> colony, 19 generations for the LC<sub>60</sub> colony, and 13 and 15 generations for the LC<sub>90</sub> colony. After reaching their highest point of resistance the colonies began to decline in egg production and larval survivability and did not recover. After 22 generations the selected colonies were terminated. The results of this work suggest that the potential for resistance development of beet armyworm to flubendiamide is unclear.

## Introduction

Beet armyworm, *Spodoptera exigua* (Hübner), is an occasional yet potentially severe pest of cotton and soybean in Mississippi (Layton 1994, Gore and Adamczyk 2004, Cook et al. 2004). Layton (1994) described *S. exigua* as being inherently tolerant to most classes of insecticides. The majority of resistance development research for beet armyworm has been on the organochlorine, organophosphate, carbamate, and pyrethroid insecticide classes (Gore and Adamczyk 2004). Moulton et al. (2000) found that a population of beet armyworm collected in Arizona possessed the ability to develop resistance to spinosad. Similarly, Gore and Adamczyk (2004) were able to develop up to 9.7-fold resistance to methoxyfenozide through selection of a population of beet armyworm originating from Mississippi.

In 2003, 75% of the insecticide market acted on only four target sites (Casida 2009). The development and use of new insecticide technologies is important for insecticide resistance management. In 2008, the diamide class of insecticides was introduced (EPA 2008). With a novel mode of action classified as ryanodine receptor modulators (MoA Group 28) (IRAC 2015). The diamide insecticides are characterized by low mammalian toxicity and are effective against a large number of lepidopteran species (Ebbinghaus-Kintscher et al. 2006). Flubendiamide (Belt Bayer CropScience, Raleigh, NC), a phthalic acid diamide, has been important for Mississippi soybean production. To date, there have been no reports of cross resistance with previous classes of insecticides.

Hardke et al. (2011) reported that flubendiamide had longer residual efficacy compared with other insecticides. The use of flubendiamide has been widely adopted since its introduction. It is perceived that the use of this insecticide will continue to

increase globally on a wide variety of crops (Roditakis et al. 2015). Repeated field applications of flubendiamide has resulted in numerous reports of resistance development for several lepidopteran species (Roditakis et al. 2015).

The necessity for new insecticide technologies to be effective, selective, and safe has resulted in the introduction of new insecticides that are more potent and have a higher degree of organismal specificity (Casida 2009). However, all of the investment in developing a potent and safe insecticide can be lost if insect resistance management strategies are not used. Insecticide resistance management is growing increasingly more difficult by the optimization of target site potency and low doses (Casida 2009). This could lead to more rapid detoxification by the pest of the exceedingly small amount of pesticide to which insects are exposed (Casida 2009).

Long residual efficacy can expose multiple generations of insect pests to the insecticide. Because of the long residual efficacy of flubendiamide, and its widespread use, it is important to understand the risk of resistance development with this insecticide. Therefore, it is necessary to understand the impact of selection pressure on the risk of resistance development. The objectives of this study were to expose beet armyworm to multiple concentrations of flubendiamide to determine the impact of selection pressure on the risk of resistance development.

### **Materials and Methods**

A colony of beet armyworm was established from larvae collected on soybean during the summer of 2013. Collected larvae were placed in 36 mL Solo<sup>®</sup> cups (Bio-Serv<sup>®</sup>, Frenchtown, NJ) containing Stonefly Heliothis Diet (Product No. 38-0600, Ward's Natural Science, Rochester, NY) with matching lids. At pupation, approximately

50 pupae were placed in 3.79 liter cardboard containers with matching lids with the corresponding colony and generation information labeled on the outside of each bucket. For the purpose of egg collection, the cardboard containers were lined with Reynolds® Cut-Rite® Wax Paper (Reynolds Consumer Products, Lake Forest, IL). The center of each lid was removed so that only the rim remained. Wax paper was placed over each bucket and kept in place by the lid to serve as an oviposition substrate. Eggs were collected three times per week (Monday, Wednesday, and Friday) and wax paper was replaced in every bucket. Wax paper with egg masses were kept in 3.79 liter Ziploc® (S.C. Johnson & Johnson, Inc., Racine WI) bags until larvae hatched for use in bioassays or to go back into the colony. Larvae that were used to maintain the colony were placed into 236 ml cardboard containers containing approximately 20 grams of untreated diet. Larvae were kept on the diet for seven days and then removed and placed in 36 mL cups containing untreated diet until pupation. The colony was reared at the Mississippi State University insect rearing facility at 25°C, 80% relative humidity, and 16:8 (L:D) photoperiod. This colony was maintained in the laboratory for five generations prior to the initiation of this experiment.

A concentration-mortality bioassay was conducted with flubendiamide (Belt; Bayer CropScience, Raleigh, NC) to determine the susceptibility of the colony before selection. Preparation of insecticide treated diet was similar to Temple et al. (2009). Dilutions of flubendiamide in distilled water were made from a stock solution with a concentration of 1 ppm to yield eight concentrations of insecticide treated diet ranging from 0 to 0.0625 ppm ai. Stock solution was created by combining 0.104 ml ai of formulated Belt in 499.9 ml of water to yield 500 ml of 100 ppm stock solution. Then 5

ml of the 100 ppm stock solution were added to 495 ml of distilled water to create 500 ml of a 1 ppm ai stock solution. Insecticide treated diet was then created by combining 115.2 g of diet, 0.36 ml of formalin and 0.6 ml of acetic acid with the appropriate ratio of distilled water and stock solution to yield 480 g of insecticide treated diet. Insecticide treated diet was stored in 0.95 L Ziploc<sup>®</sup> bags and refrigerated. All diet was used or disposed of within 7 days of preparation. Insecticide treated diet for each concentration was dispensed into 16 wells of a 128-well bioassay tray (Product No. BAW128, Frontier Agricultural Sciences, Newark, DE) in 0.5 ml aliquots. Each well was infested with one neonate (< 24 h after hatching) larva. Cells were covered with perforated, clear 16-well lids (P.E. film, Bio-Serv<sup>®</sup>, Frenchtown, NJ). Infested assay trays were labeled and placed in a rearing chamber maintained at 25°C, 80% relative humidity, and a photoperiod of 16:8 (L:D). All bioassays were replicated at least 4 times based on date of oviposition. Insect mortality ratings were taken 7 days later. Ingestion of the diamides results in feeding cessation (Nauen et al. 2007, Hannig et al. 2009). Typically the ability of larvae to right themselves after being flipped onto their dorsal surface is considered an appropriate criterion for determining mortality with intoxicated larvae. However, based on preliminary assays with *H. zea* (data not presented) it was observed that intoxicated larvae, though severely stunted, could still right themselves when flipped onto their dorsal surface. To account for the growth inhibition of intoxicated larvae, the criterion for mortality was defined as larvae that had not molted to the second instar, weighing less than 10 mg after 7 days (Siegfried et al. 2000). Data were corrected for control mortality using Abbott's formula (Abbott 1925). Corrected data were analyzed with probit analysis to calculate slope, LC<sub>50</sub>, LC<sub>90</sub>, and confidence intervals (PROC PROBIT, SAS Institute

2012). Goodness of fit tests ( $P > 0.10$ ) were evaluated to ensure the trend line fit the model.

Selection experiments were initiated in January of 2014 and were similar to Gore and Adamczyk (2004). A colony was divided into 4 cohorts that were independently selected for resistance to flubendiamide with different levels of selection pressure (none, low, moderate, and high). Selection concentrations were 0.016, 0.020, and 0.025 ppm ai of insecticide treated diet, which corresponded closely with the  $LC_{30}$ ,  $LC_{60}$ , and  $LC_{90}$  values of the original colony, respectively.

Larvae were exposed to the insecticide by incorporating flubendiamide into meridic diet as previously described. To select a greater number of individuals, each dose of insecticide treated diet was dispensed in 20 ml aliquots into 236 ml cardboard cups. Approximately 200 neonates were placed into each cup and allowed to feed for 7 days. After 7 days, larvae weighing greater than 10 mg were transferred individually onto untreated diet in 29.5 ml cups where they were allowed to complete development. After pupation, beet armyworms from each independent colony were maintained as previously described. Offspring from each colony were subjected to a concentration-mortality bioassay to monitor for changes in susceptibility.

Data were corrected for control mortality using Abbott's formula (Abbott 1925). Corrected data were analyzed with probit analysis to calculate slope,  $LC_{50}$ ,  $LC_{90}$ , and confidence intervals (PROC PROBIT, SAS Institute 2012). Goodness of fit tests ( $P > 0.10$ ) were evaluated to ensure the trend line fit the model.  $LC_{50}$  and  $LC_{90}$  values were considered different when 95 percent confidence intervals did not overlap. Resistance

ratios were calculated by dividing the LC<sub>50</sub> and LC<sub>90</sub> values of the selected colony for each generation by the LC<sub>50</sub> and LC<sub>90</sub> values of the unselected colony.

### **Results and Discussion**

Lai and Su (2011) and Che et al. (2013) reported that beet armyworm have the capability to evolve resistance to chlorantraniliprole. The LC<sub>50</sub> value (95% fiducial limits) for the original colony was 0.018 (0.017-0.020) ppm (Table 4.1). The colony exposed to low selection pressure developed 1.77-fold level of resistance with an LC<sub>50</sub> value (95% fiducial limits) of 0.033 (0.029-0.037) ppm after 18 generations (Table 4.2). The colony exposed to moderate selection pressure developed 1.79-fold level of resistance with an LC<sub>50</sub> value (95% fiducial limits) of 0.033 (0.030-0.036) ppm after 19 generations (Table 4.3). The colony exposed to high selection pressure developed 2.11-fold level of resistance with an LC<sub>50</sub> value (95% fiducial limits) of 0.039 (0.031-0.064) ppm after 13 and 0.039 (0.033-0.059) ppm after 15 generations (Table 4.4). The LC<sub>50</sub> values of all selection colonies were significantly different from the original colony (Table 4.1-4.4). However, based on fiducial limits, the LC<sub>50</sub> values of the selected colonies were not different from each other (Table 4.1-4.3). Gore and Adamczyk (2004) were able to select for 9.7 and 9.4-fold levels of resistance of beet armyworm exposed to methoxyfenozide within 7 generations. Lai and Su (2011) were able to select for a 12-fold level of resistance for beet armyworm exposed to chlorantraniliprole after 22 generations in the laboratory. Che et al. (2013) found that beet armyworm that were resistant to chlorantraniliprole had higher pupal weights than those that were not resistant to chlorantraniliprole.

Concentration-mortality bioassays were used to detect changes in susceptibility of the four populations. This study was intended to select for resistance and then to determine the mechanism of resistance and the heritability of the resistance mechanism. However, resistance ratios never surpassed 2.11-fold. The colony exposed to high selection pressure showed the greatest promise for resistance development but declined after reaching its highest level of resistance. Furthermore, after 18 generations for the colony exposed to high selection pressure and 19 generations for the colonies exposed to the low and moderate selection pressure, egg production and larval survivability declined to the point where no further studies could be conducted. After 22 generations the selected colonies were weak and the experiment was terminated. However, the unselected colony never declined in egg production or larval survivability.

Future experiments assessing the risk of resistance development should include additional measures (pupal weight, larval development time, or egg mass number etc.) to try and detect what is occurring in populations exposed to flubendiamide. Lai and Su (2011) had success selecting with the  $LC_{70}$  of the original colony that was derived from a field collection of only 150 larvae. For efficiency, only one colony should be selected for resistance. Partitioning the original colony into 3 separate selection cohorts could have reduced the number of heterozygote resistant individuals and limited the possibility of selecting for resistance. Furthermore, using concentration-mortality assays to assess resistance development is tedious and requires a great amount of labor. The use of one colony would reduce the amount of effort, labor costs, and materials involved. Beet armyworm is a sporadic pest of row crops in Mississippi and at this time there have been no reports of diamide insecticide applications targeting this pest.

Table 4.1 Susceptibility of *S. exigua* reared on non-treated diet measured in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet through 18 generations

Original Colony									
Generation	n <sup>1</sup>	LC50 (95% C.L.) <sup>2</sup> ppm	RR <sup>4</sup>	LC90 (95% C.L.) <sup>2</sup> ppm	RR	Slope ± SE	X <sup>2</sup>	df	P <sup>3</sup>
0	351	0.018 (0.017-0.020)	.	0.026 (0.024-0.029)	.	3.88 ± 0.41	2.74	5	0.7403
3	329	0.017 (0.013-0.014)	0.898	0.022 (0.020-0.027)	0.864	4.39 ± 1.10	8.87	5	0.1142
6	430	0.012 (0.011-0.014)	0.649	0.018 (0.016-0.022)	0.707	3.08 ± 0.43	1.78	4	0.7755
11	768	0.014 (0.012-0.015)	0.751	0.018 (0.016-0.031)	0.682	5.47 ± 2.02	2.60	5	0.7618
12	224	0.014 (0.011-0.017)	0.778	0.026 (0.022-0.031)	0.997	2.22 ± 0.37	4.10	4	0.3923
13	256	0.015 (0.014-0.016)	0.827	0.018 (0.017-0.020)	0.695	8.22 ± 1.97	0.48	5	0.993
15	448	0.022 (0.021-0.023)	1.191	0.027 (0.025-0.028)	1.034	6.77 ± 0.85	5.25	4	0.2629
16	512	0.021 (0.019-0.022)	1.135	0.026 (0.025-0.028)	1.024	5.62 ± 0.95	2.72	5	0.7435
18	448	0.022 (0.021-0.023)	1.187	0.026 (0.025-0.028)	1.015	7.35 ± 1.01	0.50	4	0.9739

<sup>1</sup>Total number of insects tested

<sup>2</sup>Confidence Limits

<sup>3</sup>Goodness of Fit test (P > 0.10).

<sup>4</sup>Resistance Ratios

Table 4.2 Susceptibility of *S. exigua* exposed to flubendiamide at a concentration of 0.016 ppm measured in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet through 19 generations.

<b>LC30</b>									
<b>Selection</b>	<b>n<sup>1</sup></b>	<b>LC50 (95% C.L.)<sup>2</sup> ppm</b>	<b>RR<sup>4</sup></b>	<b>LC90 (95% C.L.)<sup>2</sup> ppm</b>	<b>RR</b>	<b>Slope ± SE</b>	<b>X<sup>2</sup></b>	<b>df</b>	<b>P<sup>3</sup></b>
0	351	0.018 (0.017-0.020)	.	0.026 (0.024-0.029)	.	3.88 ± 0.41	2.74	5	0.7403
6	505	0.018 (0.006-0.021)	1.00	0.023 (0.018-0.025)	0.88	6.44 ± 2.72	0.26	5	0.9983
10	512	0.015 (0.012-0.017)	0.81	0.020 (0.018-0.025)	0.79	4.23 ± 1.03	0.80	5	0.9768
12	256	0.021 (0.018-0.023)	1.13	0.029 (0.027-0.034)	1.15	3.68 ± 0.70	8.36	5	0.1375
13	384	0.021 (0.018-0.023)	1.13	0.032 (0.030-0.036)	1.25	3.00 ± 0.43	4.95	3	0.1756
15	256	0.025 (0.023-0.026)	1.34	0.030 (0.029-0.034)	1.18	6.11 ± 1.12	2.43	5	0.7872
16	512	0.021 (0.019-0.023)	1.15	0.031 (0.029-0.034)	1.20	3.42 ± 0.52	2.29	5	0.8076
17	512	0.031 (0.029-0.033)	1.70	0.041 (0.038-0.050)	1.61	4.73 ± 1.04	1.48	5	0.9153
18	512	0.033 (0.029-0.037)	1.77	0.054 (0.045-0.095)	2.11	2.54 ± 0.67	4.91	5	0.4268
19	512	0.028 (0.026-0.029)	1.50	0.033 (0.031-0.035)	1.28	7.47 ± 1.50	2.89	5	0.7176

<sup>1</sup>Total number of insects tested

<sup>2</sup>Confidence Limits

<sup>3</sup>Goodness of Fit test (P > 0.10).

<sup>4</sup>Resistance Ratios

Table 4.3 Susceptibility of *S. exigua* exposed to flubendiamide at a concentration of 0.020 ppm measured in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet through 19 generations.

LC60									
Selection	n <sup>1</sup>	LC50 (95% C.L.) <sup>2</sup> ppm	RR <sup>4</sup>	LC90 (95% C.L.) <sup>2</sup> ppm	RR	Slope ± SE	X <sup>2</sup>	df	P <sup>3</sup>
0	351	0.018 (0.017-0.020)	.	0.026 (0.024-0.029)	.	3.88 ± 0.41	2.74	5	0.7403
3	193	0.016 (0.012-0.018)	0.87	0.023 (0.020-0.029)	0.90	3.51 ± 0.86	1.24	4	0.8714
6	442	0.017 (0.012-0.019)	0.93	0.025 (0.022-0.029)	0.96	3.56 ± 0.92	5.09	4	0.2784
10	384	0.024 (0.017-0.028)	1.28	0.038 (0.034-0.044)	1.47	2.71 ± 0.60	6.03	3	0.11
11	672	0.020 (0.016-0.024)	1.06	0.026 (0.024-0.025)	1.01	4.51 ± 1.08	5.57	4	0.2337
13	512	0.023 (0.022-0.025)	1.27	0.031 (0.030-0.034)	1.21	4.45 ± 0.51	4.79	5	0.442
15	512	0.028 (0.027-0.029)	1.52	0.036 (0.034-0.039)	1.41	4.96 ± 0.61	5.33	5	0.3772
16	256	0.026 (0.019-0.029)	1.43	0.041 (0.038-0.050)	1.41	4.09 ± 1.33	7.17	5	0.2082
17	512	0.031 (0.029-0.033)	1.70	0.031 (0.029-0.034)	1.61	4.72 ± 1.04	1.48	5	0.9153
18	512	0.023 (0.021-0.025)	1.27	0.031 (0.029-0.034)	1.19	4.83 ± 0.92	6.27	5	0.2811
19	512	0.033 (0.030-0.036)	1.79	0.052 (0.041-0.067)	2.02	2.87 ± 0.67	5.11	5	0.4028

<sup>1</sup>Total number of insects tested

<sup>2</sup>Confidence Limits

<sup>3</sup>Goodness of Fit test ( $P > 0.10$ ).

<sup>4</sup>Resistance Ratios

Table 4.4 Susceptibility of *S. exigua* exposed to flubendiamide at a concentration of 0.025 ppm measured in dose-mortality curves generated with concentration-mortality bioassays with insecticide treated diet through 18 generations.

LC90									
Selection	n <sup>1</sup>	LC50 (95% C.L.) <sup>2</sup> ppm	RR <sup>4</sup>	LC90 (95% C.L.) <sup>2</sup> ppm	RR	Slope ± SE	X <sup>2</sup>	df	P <sup>3</sup>
0	351	0.018 (0.017-0.020)	.	0.026 (0.024-0.029)	.	3.88 ± 0.4107	2.74	5	0.7403
3	243	0.017 (0.014-0.020)	0.94	0.023 (0.020-0.028)	0.88	4.75 ± 1.15	0.80	5	0.977
6	512	0.020 (0.018-0.022)	1.09	0.031 (0.028-0.036)	1.21	2.91 ± 0.40	2.06	5	0.8405
10	384	0.021 (0.016-0.025)	1.16	0.033 (0.029-0.038)	1.27	3.03 ± 0.59	1.59	3	0.6607
12	448	0.026 (0.025-0.028)	1.43	0.035 (0.033-0.038)	1.36	4.64 ± 0.74	3.81	4	0.4328
13	384	0.039 (0.031-0.064)	2.11	0.111 (0.066-1.453)	4.32	1.20 ± 0.41	4.29	3	0.2321
15	384	0.039 (0.033-0.059)	2.11	0.051 (0.044-0.089)	2.92	1.96 ± 0.50	0.40	3	0.9404
16	512	0.037 (0.035-0.043)	2.01	0.041 (0.038-0.050)	1.97	4.12 ± 1.18	3.17	5	0.6735
18	672	0.026 (0.024-0.028)	1.42	0.046 (0.042-0.055)	1.81	2.23 ± 0.28	2.45	4	0.6529

<sup>1</sup>Total number of insects tested

<sup>2</sup>Confidence Limits

<sup>3</sup>Goodness of Fit test (P > 0.10).

<sup>4</sup>Resistance Ratios

## Literature Cited

- Abbot, W. S. 1925.** A method for computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265-267.
- Casida, J. E. 2009.** Pest toxicology: the primary mechanisms of pesticide action. *Chem. Res. Toxicol.* 22: 609-619.
- Che W., T. Shi, Y. Wu, and Y. Yang. 2013.** Insecticide resistance status of field populations of *Spodoptera exigua* (Lepidoptera: Noctuidae) from China. *J. Econ. Entomol.* 106: 1855–1862.
- Cook, D. R., B. R. Leonard, and J. Gore. 2004.** Field and laboratory performance of novel insecticides against armyworms (Lepidoptera: Noctuidae). *Florida Entomol.* 87: 433-439.
- Ebbinghaus-Kintscher U, P. Luemmen, N. Lobitz, T. Schulte, C. Funke, R. Fischer, T. Masaki, N. Yasokawa, and M. Tohnishi. 2006.** Phthalic acid diamides activate ryanodine-sensitive Ca<sup>2+</sup> release channels in insects. *Cell Calcium* 39: 21–33.
- Gore, J. and J. Adamczyk Jr. 2004.** Laboratory selection for beet armyworm (Lepidoptera: Noctuidae) resistance to methoxyfenozide. *Florida Entomol.* 87: 450-453.
- Hannig, G.T., M. Ziegler, and P. G. Marcon. 2009.** Feeding cessation effects of chlorantraniliprole, a new anthranilic diamide insecticide, in comparison with several insecticides in distinct chemical classes and mode-of-action groups. *Pest Manag. Sci* 65:969-974.
- Hardke, J. T., J. H. Temple, B. R. Leonard, and R. E. Jackson. 2011** Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). *Florida Entomol.* 94: 272-278.
- IRAC. 2015.** IRAC mode of action classification scheme. Version 8.0 [Online]. Available at <http://www.irac-online.org/documents/moa-classification/> Accessed: January 2016.
- Layton, M. B. 1994.** The 1993 beet armyworm outbreak in Mississippi and future management guidelines, pp. 854-856. *In Proc. Beltwide Cotton Conf., National Cotton Council, Memphis, Tennessee.*
- Lai, T., and J. Su. 2011.** Assessment of resistance risk in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) to chlorantraniliprole. *Pest Manag. Sci* 67: 1468-1472.
- Moulton, J. K., D. A. Pepper, and T. J. Dennehy. 2000.** Beet armyworm (*Spodoptera exigua*) resistance to spinosad. *Pest Manag. Sci.* 56: 842-848.

- Nauen, R., S. Konanz, T. Hirooka, T. Nishimatsu, and H. Kodama. 2007.**  
Flubendiamide: a unique tool in resistance management tactics for pest  
Lepidoptera difficult to control. *Pflanzenschutz-Nachrichten Bayer* 60: 247-262.
- Roditakis, E., E. Vasakis, M. Grispou, M. Stavrakaki, R. Nauen, M. Gravouil, and  
A. Bassi. 2015.** First report of *Tuta absoluta* resistance to diamide insecticides. *J.  
Pest Sci.* 88: 9-16.
- SAS Institute. 2012.** SAS 9.3 for windows. SAS Institute, Cary, NC.
- Siegfried, B. D., T. Spencer, and J. Nearman. 2000.** Baseline susceptibility of the corn  
earworm (Lepidoptera: Noctuidae) to the Cry1Ab toxin from *Bacillus*  
*thuringiensis*. *J. Econ. Entomol.* 93: 1265-1268.
- Temple, J. H., P. L. Bommireddy, D. R. Cook, P. Marcon, and B. R. Leonard. 2009.**  
Susceptibility of selected lepidopteran pests to Rynaxypyr<sup>®</sup>, a novel insecticide. *J.  
Cotton Sci.* 13: 23-31

## CHAPTER V

### SUMMARY AND CONCLUSION

Since its introduction in 2008, the diamide insecticide class has been an important tool for lepidopteran insect pest management. Chlorantraniliprole and flubendiamide both exhibit long residual control of *Helicoverpa zea*. Chlorantraniliprole moves systemically when applied as a foliar application. However, systemic efficacy was variable and dependent on plant size and growth stage at the time of application. The residual efficacy of chlorantraniliprole was better than flubendiamide. Where the residual efficacy of flubendiamide declined over time, the residual efficacy of chlorantraniliprole did not decline over 31 days when applied at the R3 growth stage. In cotton production, the incorporation of *Bt* proteins reduces the reliance on foliar insecticides for lepidopteran control and perhaps minimizes the diamide selection pressure compared to what it would be if growers planted a large percentage of non-Bt cotton. Corn and soybean production acreage in the Mid-South has increased while cotton production has declined. Corn and soybean share a source sink relationship with corn earworm. The increased production of corn and soybean has resulted in the number of soybean acres treated for corn earworm in Mississippi to increase.

Soybean planted later in the season typically have higher levels of infestation of lepidopteran insect pests and consequently require more insecticide applications. This is because later planting dates are in the R1-R3 growth stages when corn earworm emerge

from senesced corn, while soybeans planted earlier have reached the R4-R5 growth stage and are no longer attractive. Overall, researchers have not been able to demonstrate yield losses occurring from corn earworm damage during the R1-R3 growth stages. However, this feeding may result in delayed maturity which can be an issue for later planted soybeans, because it may limit the time for compensation and result in delayed harvest. Still, it is important to follow the economic threshold recommendations because unnecessary insecticide applications can result in an economic loss.

The ecology of the corn earworm combined with the long residual efficacy of the diamide insecticides could potentially lead to insecticide resistance development if additional modes of action are not used. The decline of cotton production and the increase of soybean production can limit the number of modes of action in a given landscape, because flubendiamide and chlorantraniliprole are the primary insecticides used in Mississippi for control of corn earworm and other lepidopteran pests in soybean. Because of that, benchmarks of susceptibility data for future resistance monitoring efforts are needed.

Overall, this study provided a benchmark of information for *H. zea* susceptibility to the diamide insecticides. No instances of resistance development were observed with chlorantraniliprole or flubendiamide and variation in response among populations and locations to chlorantraniliprole and flubendiamide were small. It will be important to monitor susceptibility levels because the slopes of the lines across populations were steep. This means that a small increase in the dose will result in a large increase in mortality. However, the long residual efficacy of the diamide insecticides may potentially expose multiple generations of the same species to the insecticide. As the insecticide

toxicity declines slowly overtime, multiple generations of the same species would be exposed to doses that could be favorable for selection of heterozygote resistant alleles. This understanding will be beneficial for future resistance monitoring efforts by providing a point of reference to determine if resistance is in fact occurring. Resistance monitoring will be key to determine if there is a heritable change in the response to an insecticide.

We were unable to determine the impact of selection pressure of flubendiamide on resistance development of beet armyworm. Future work should be done to better understand this relationship. However, gaining a better understanding of how the diamide insecticides perform when applied as a foliar application and developing baseline susceptibility levels, will allow users of these insecticides to make the appropriate recommendations and applications to delay insecticide resistance development. At this point, the diamide insecticide class is a valuable tool for insect pest management and the use of this insecticide class will continue to increase. Following economic thresholds, minimizing wild hosts, and rotation of insecticide classes in an insecticide resistance management program will be key to prolong the life of this insecticide class.