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# Evaluating the efficacy of methoxyfenozide on Louisiana, Texas and the mid-southern soybean looper populations

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EVALUATING THE EFFICACY OF METHOXYFENOZIDE ON LOUISIANA, TEXAS AND  
THE MID-SOUTHERN SOYBEAN LOOPER POPULATIONS

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Science

In

The Department of Entomology

by  
Sebe Anthony Brown  
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## ABSTRACT

The soybean looper, *Chrysodeixis includens* (Walker), is a defoliating insect pest of soybean in the Mid-South and Texas. In 2008, Louisiana producers reported unsatisfactory control of soybean loopers in soybean with methoxyfenozide. In 2009 and 2010, field collections from Louisiana, Missouri, Mississippi and Texas were exposed to discriminating concentrations (0.020 to 5.000 ai  $\mu\text{g/ml}$ ) of methoxyfenozide in diet incorporation bioassays. All field colonies were compared to a reference strain  $\text{LC}_{50}$  of 0.007  $\mu\text{g/ml}$  for 2009 and 0.008  $\mu\text{g/ml}$  for 2010. Louisiana populations exhibited  $\text{LC}_{50}$ 's of 0.079  $\mu\text{g/ml}$  and 0.122  $\mu\text{g/ml}$  for 2009 and 2010, respectively, which were the highest values among all field collections during three years. The Missouri collections demonstrated the lowest  $\text{LC}_{50}$ 's with 0.025  $\mu\text{g/ml}$  in 2009 and 0.011  $\mu\text{g/ml}$  in 2010. In general, results of these discriminating concentration tests indicated that all field collections showed elevated  $\text{LC}_{50}$ 's compared to the reference colony.

Additional field experiments were conducted to evaluate the efficacy of foliar insecticides against Louisiana soybean loopers. Significant differences among treatments for defoliation area were determined between lepidopteran specific and broad spectrum insecticides. For the 2009-10 experiment chlorantraniliprole had the lowest defoliation 3,7,14,21 and 28 days after treatment (DAT) (0.20, 0.20, 0.50, 0.57, 0.97  $\text{cm}^2$ ) while lambda-cyhalothrin had the highest (1.12, 2.38, 2.06, 2.79, 2.25  $\text{cm}^2$ ). In 2011, defoliation and days to 100% mortality were evaluated at two locations for R3 and R5 development stages. Chlorantraniliprole provided the lowest days to mortality (2.3) at R3 and (1.0) at R5 while oxadiazine resulted in the highest (5.7 and 6.0).



## CHAPTER 1

### INTRODUCTION AND REVIEW OF LITERATURE

Soybean, *Glycine max* (L.) Merr is one of Louisiana's major agronomic crops. In 2010 Louisiana producers planted 1,030,000 acres of soybeans yielding 41,820,000 bushels (USDA NASS 2011). Soybeans also provide a significant contribution to Louisiana and the Mid-South's agricultural economy through their use as a rotational and double crop (Heatherly and Hodges 1999). One quality contributing to soybeans success in Louisiana is the crop's adaptability to varying soil and climactic conditions (Morrison and McCormick 1996).

Soybeans grown in states along the gulf coast have the greatest amount of insect pressure (Way 1994). Economically important insects that plague Louisiana and Mid-South soybeans are primarily composed of the pod and stem feeding guild and a complex of lepidopteran defoliators (Pedigo and Buntin 1993). The most damaging defoliating insects are the soybean looper *Chrysodeixis includens* (Walker), velvetbean caterpillar *Anticarsia gemmatalis* (Hübner) and green cloverworm *Plathypena scabra* (Fabricius). In 2010, in Mississippi, soybean loopers infested 1.7 million acres of soybeans and caused a 19% total loss plus cost of control to producers (Musser et al. 2011).

The soybean looper is the prominent Plusiinae noctuid species in Louisiana and the Mid-South. This pest is highly polyphagous and infests 31 known hosts, with soybeans being the optimum host for feeding and oviposition (Herzog et al. 1980). Adult soybean loopers are believed to migrate from overwintering reservoirs in the extreme portions of southern Texas and Florida and the Caribbean to soybean production areas around the United States (Herzog et al 1980, Boethel et al. 1992).

The soybean looper is in the family Noctuidae al order Lepidoptera (Triplehorn and Johnson 2005). Adults are black to mottled grey and have a wing span of 3.17 to 3.81cm (Smith

1994). The hindwings are typically lighter in color than the forewings and a white figure eight is visible near the middle of the fore wings (Smith 1994). Larvae are light green with white longitudinal stripes down the sides and back. Immature loopers have three pairs of abdominal prolegs and the body gradually tapers toward the head (Shour and Sparks 1981). Other species of Plusiinae caterpillars also attack soybeans in the Mid-South.

The cabbage looper, *Trichoplusia ni* (Hübner) and the grey looper moth, *Rachiplusia ou* (Guenée 1852) are found in mixed populations in soybeans (Canerday and Arant 1967). Both are similar in appearance to the soybean looper and the three species can be misidentified. All three species have three pairs of vestigial prolegs located on abdominal segments 3 and 4; however, the soybean looper has dark thoracic legs and the cabbage and grey looper do not (Shorey et al. 1962, Canerday and Arant 1967).

### **Soybean Looper Biology**

The soybean looper overwinters in Central America and the southern most regions of the United States (Boethel et al. 1992, Herzog 1980). Soybean loopers have no diapause and can reproduce year round in semi-tropical climates (Boethel et al. 1992). These insects produce between 3 to 4 generations in one soybean growing season (Funderburk et al. 1999). The adults are strong fliers and migration between cotton and soybean is common in the Mid-South (Jensen et al. 1974). Adult moths require food sources that support egg production and longevity. Jensen et al. (1974) demonstrated that female soybean loopers oviposited significantly more eggs (515 eggs) when supplied with a sucrose solution than with no food (0 eggs). Cotton nectaries have been shown to supply the adequate carbohydrate source required by female soybean loopers (Burleigh 1972, Jensen et al. 1974). Female moths are the most fecund between 17 and 32°C (Mason and Mack 1984). Oviposition temperature closely follows optimum egg production

temperatures with the greatest number of eggs being oviposited at 29°C (26.8 eggs/day) (Mason and Mack 1984). Eggs are morphologically similar to other Noctuidae species and are laid singly on the underside of leaves (Smith 1994).

Larval duration for the first stadium under laboratory conditions was three to four days, whereas second through fourth stadia required only two to three days and fifth and sixth stadia five to six days (Shour and Sparks 1981). Optimal temperature for larval development to six instars is 27°C (Shour and Sparks 1981). Trichilo and Mack (1989) found that larval feeding intensifies with increasing temperatures. Larval feeding failed to peak at temperatures tested by Trichilo and Mack (1989), indicating soybean looper larvae consume more foliage in hot weather. However, no temperature dependent upper and lower development thresholds have been determined. Larvae have chewing mouthparts and feed on the foliage of 31 identified hosts (Herzog 1980). Pupation occurs in silken cocoons attached to the underside of leaves and usually lasts between 7 to 9 days (Shour and Sparks 1981). The entire life cycle is completed in 30 days.

### **Soybean Looper Migration**

This pest is only known to overwinter in southern Florida and Texas in the continental United States (Herzog 1980). Soybean loopers are strong fliers and migration from states where overwintering occurs to soybean production areas of the Mid-South is common. Invasions of soybean-producing areas are thought to originate from these reservoirs or from areas in Central or South America and the Caribbean (Herzog 1980). Soybean looper adults were captured using pheromone-baited black light traps during the winter months at seven different locations in southern Florida by Mitchell et al. (1975). They concluded that the soybean looper can survive winters in southern Florida where winter temperatures do not exceed 60°F. Tingle and Mitchell (1977) also conducted pheromone-baited black light traps in North Central Florida (near

Hastings); their results indicated that soybean looper adults have enough hosts and warm temperatures to successfully overwinter in the region.

Similarly, Harding (1976) reported collecting soybean looper larvae during all 12 months in the Lower Rio Grande Valley of Texas, indicating this insect's ability to reproduce during the winter months in southern Texas. Chapin and Callahan (1967) collected adult soybean loopers in Louisiana from March to December; these findings suggest this insect is not active during the winter months in Louisiana (Herzog 1980). With the annual migration to soybean-producing regions of the southern United States, late season outbreaks and population influxes of soybean looper force management by producers to avoid late season losses.

### **Soybean Looper Damage to Soybeans**

Soybean loopers damage soybeans in late season (July – September) in the Mid-South (Herzog 1980). A single immature soybean looper is able to consume 114 cm<sup>2</sup> of foliage area during larval development (Boldt et al. 1975, Reid and Greene 1973) Most of the consumption occurs in the final 3 stadia, when the larvae indiscriminately consume every part of the leaf. (Boldt et al. 1975, Reid and Greene 1973). The soybean looper's preferred feeding area is the lower two thirds of the crop canopy (Herzog 1980). These insects consume foliage from the inside of the canopy and work outwards, eventually defoliating the entire plant (Smith 1994). Injury by the soybean looper is best tolerated when soybean plants are R3 or prior (Fehr et al. 1971, Turnipseed and Kogan 1987). Prior to R3, soybeans can tolerate up to 35% defoliation and see no significant drop in yield (Fehr et al.1971, Turnipseed and Kogan 1987). After R3, yield is significantly lowered when soybeans are defoliated. Hinson et al. (1978) demonstrated yield losses of 8, 21, and 31% when plants were defoliated 3, 17, and 42 days after flowering. Soybean loopers are not considered a pest after the plant reaches R7 (Higley 1992).

## **Economic Injury Levels and Economic Thresholds of Soybean Looper**

Damage from soybean loopers can result in severe defoliation and dramatic yield loss. Soybean looper populations must be controlled if the numbers reach economic threshold to prevent yield loss. In 2009, soybean loopers accounted for a 565,575 bushels yield loss in Mississippi. (Musser and Catchot 2009). Soybean looper larval thresholds in most Mid-South soybean producing states are 8 larvae per row foot for caterpillars one-half inch or longer or when 35% defoliation has occurred during vegetative stages (V stages) to bloom (R1) (Kogan 1976, Herzog 1980). During pod-filling stages, R2 to R5, larval thresholds are 4 larvae per row foot for caterpillars one-half inch or longer or when 20% defoliation has occurred (Kogan 1976). However, in Florida, the recommended treatment level is 4 larvae per row foot during R2-R5 stages and 10 larvae per row foot in vegetative through bloom stages (Strayer and Greene 1974, Herzog 1980). Prior to flowering, soybeans can withstand defoliation up to 35% before significant losses are incurred and insecticide applications should be made based on number of soybean looper larvae present and defoliation percentage (Fehr et al. 1971). The economic threshold levels and economic injury levels for soybean loopers was determined using the amount of foliage consumed per larval instar as well as the amount of leaf area required by the soybean plant for optimal growth and pod development (Kogan 1976).

## **Integrated Pest Management of Soybean Loopers**

Integrated pest management strategies for controlling soybean loopers include the use of natural enemies and adherence to economic injury levels and economic thresholds. Natural enemies provide a degree of control in fields not treated with broad spectrum insecticides. Richman et al. (1980) initiated cage studies with 16 species of soybean looper natural enemies commonly found in soybean fields. All life stages of soybean looper were tested with the

exception of adults. The highest egg predation rates were attributed to nabids, green lacewings and lygaeids (Richman et al. 1980). Carabid beetles, reduviids, and predacious pentatomids were found to control small to medium sized larvae (Richman et al. 1980). Similarly, Harding (1976) collected 29 species of parasitoids from *C. includens* and *Trichoplusia. ni* in the Lower Rio Grande Valley of Texas. Total parasitism of soybean loopers was found to be 58-71% over a four month period (Harding 1976). Fungal and viral pathogens, in conjunction with natural enemies, can give producers adequate control of soybean loopers. However, several biotic factors must be present for the proliferation of these natural control agents (Funderburk et al. 1999). Burleigh (1972) found two species of fungal pathogens *N. rileyi* and *Massospora* spp. controlling soybean looper populations at high levels in cotton-soybean agro-ecosystems. Beach and Todd (1986) collected *E. grammae*, *N.rileyi*, and nuclear polyhedrosis virus at low levels in South Georgia soybean fields. Mortality from simultaneous combinations of parasitism and disease provided control levels of 77.6 (1982), 73.2 (1983) and 93.9% (1984) (Beach and Todd 1986). However, despite the high level of suppression attained by natural control agents, populations of soybean loopers exceed economic thresholds in mid-southern states forcing producers to apply insecticides.

Chemical control strategies remain the main tool in the suppression of soybean loopers. In the past, soybean loopers were controlled using broad spectrum insecticides such as organochlorines, organophosphates, pyrethroids, and carbamates (Boethel et al. 1992). Overuse and reliance on these insecticides led to many documented cases of resistance to virtually all classes of insecticides. Today, insecticide applications are mainly limited to lepidopteran-specific compounds and newer chemistries of insecticides, such as the diamides. Presently the insecticides recommended for control of soybean loopers are thiodicarb (carbamate), methomyl

(carbamate), spinosad (spinosyn), indoxacarb (oxadiazine), methoxyfenozide (insect growth regulator) and flubendiamide (diamide). With available insecticidal control options for the soybean looper becoming more limited, preserving natural enemies, effectively timing insecticide applications, and spraying when the economic threshold is met will help slow the development of resistance by the soybean looper.

Given the limited integrated pest management recommendations for controlling soybean loopers, chemical control is likely to continue to be the producer's most effective tool in controlling damage by this pest. However, as history has shown, heavy reliance on chemical control pressures the formation of resistance. To provide greater insecticidal efficacy in the field, it is necessary to locate and detect instances of insecticide resistance to increase our ability to forecast possible resistant populations of soybean loopers.

### **History and Mechanisms of Insecticide Resistance in Soybean Looper**

Insecticide resistance is "the developed ability in a strain of insects to tolerate doses of toxicant which would prove lethal to the majority of individuals in a normal population of the same species" (Anonymous 1957). Insecticide resistance in soybean loopers is a problem that has been faced by producers in the southeastern United States since the 1960's. Producers were able to achieve only marginal control with broad spectrum insecticides for over 10 years (Boethel et al. 1992). Resistance to DDT, cyclodienes, carbamates and organophosphates was thought to have occurred prior to 1970 and for a two year period (1970-72), no insecticides registered in Louisiana adequately controlled soybean loopers (Boethel et al. 1992). Substantial field control problems with methomyl were experienced along the east coast in the early 1970s. This prompted Newsom et al. (1980) to conclude that insecticide pressure from treated soybeans had been too small to generate such large resistance levels (Boethel et al. 1992). The question of how soybean loopers developed such rapid resistance was potentially explained by inconclusive

evidence that some hosts such as tomatoes and chrysanthemums, were sprayed with methomyl up to 100 times per year (Boethel et al. 1992). Although this evidence was not substantiated, it suggests that insect control practices in one state can influence resistance problems in other states and commodities (Boethel et al. 1992).

With the use of permethrin in 1982, producers regained the ability to adequately and economically control this pest (Thomas and Boethel 1995). However, five years after the adoption of permethrin, resistance by the soybean looper was documented in Louisiana and Mississippi (Felland et al. 1990, Leonard et al. 1990). Permethrin resistance followed the trend observed with methomyl resistance 10 years earlier. The highest levels of resistance were experienced in states adjacent to Florida or situated along the east coast (Boethel et al. 1992). Boethel et al. (1992) concluded that selection pressure of permethrin on soybean loopers in southern growing regions would not have had a substantial influence on the formation of resistance. A distinguishing characteristic of field failures with permethrin on soybeans was their proximity to cotton fields. Felland et al. (1990) found greater levels of resistance in Mississippi soybean looper populations collected from areas with large acreages of cotton and soybeans. Jensen et al. (1974) found that soybean looper adults were significantly more fecund, lived significantly longer, and mated more frequently when supplied a diet of cotton nectaries compared to water or not fed. These findings are a possible explanation of how cotton may have affected the severity and virulence of soybean looper infestations in soybean-cotton agro-ecosystems. They also support the theory of higher permethrin resistance in soybean looper populations where soybeans are planted in close proximity to cotton. Cotton which required intensive chemical management and frequent applications of permethrin for insect control could have selected for resistant soybean looper individuals (Felland et al. 1990).



## **Soybean Looper Insecticide Resistance Mechanisms**

The rapid development of resistance by the soybean looper to almost every class of insecticides triggered toxicological studies to discover the mechanisms of resistance and help generate new information used in resistance management. Martin and Brown (1984) found that a field-collected Louisiana soybean looper population was able to detoxify and excrete methamidophos prior to activation of the compound *in vivo*. Similarly Dowd and Sparks (1988), Rose et al. (1990), Thomas and Boethel (1995), and Thomas et al. (1996) found that several enzymes including glutathione transferases, monooxygenases, and hydrolases may be responsible for the reduced susceptibility of soybean looper to pyrethroid insecticides. All authors noted that reduced target site sensitivity is another factor that may affect the efficacy of pyrethroids for soybean looper control. Glutathione transferases, monooxygenases, and various hydrolases have been demonstrated to detoxify numerous insecticides in pest insects. Thomas and Boethel (1995) found that dominant permethrin resistance in soybean loopers may provide a mechanism for susceptible alleles to remain in soybean looper populations through heterozygotes. Heterozygotes decrease insecticide efficacy and lead to eventual buildup of resistance (Thomas and Boethel 1995). Failure to practice integrated pest management on localized scales in the United States and elsewhere and an intense reliance on broad spectrum insecticides continue to influence resistance development (Boethel et al. 1992).

### **Intrepid 2F Insecticide**

Intrepid 2F<sup>®</sup>, possessing the active ingredient (AI) methoxyfenozide, is a dibenzoylhydrazine (DBH) non-steroidal ecdysteroid agonist developed by Rohm and Haas (Mosallanejad and Smaghe 2009). This class of insecticide is termed an insect growth regulator (IGR) or molting accelerator compound (MAC). Methoxyfenozide's mode of action acts as an

agonist of 20-hydroxyecdysone receptors in lepidopteran pests (Wing et al. 1988, Moulton et al. 2002). ). The Environmental Protection Agency defines mode of action as “how a particular chemical or chemical group acts to kill or disable insects, noxious plants, or fungi through specific interaction (activity) with a target within the plant or animal” (EPA 2010). 20-hydroxyecdysone is a steroid insect molting hormone that plays a critical role in growth regulation of insects (Dhadialla and Jansson 1999). Methoxyfenozide was first sold in the United States in 1999, and received full registration for use on cotton in 2000 (Moulton et al. 2002). The principle avenue of intoxication is through ingestion, and acute poisoning induces termination of feeding followed by death due to premature larval molt (Wing et al. 1988, Rohm and Haas Company 1989, Moulton et al. 2002). Symptoms of acute poisoning include retarded larval growth, fused mandibles, cuticular blackening and loss of hemolymph (Moulton et al. 2002).

### **Fitness Costs of Insect Growth Regulator Intoxication**

Reduced fitness of insects exposed to insect growth regulators has been documented in economically important species of lepidopteran pests. Reay-Jones et al. (2005) demonstrated longer development times in *Diatraea saccharalis* (F.) (Lepidoptera: Crambidae) populations exposed to discriminating concentrations of tebufenozide. The authors concluded that extended development times for *D. saccharalis* populations may have an impact on area wide pest populations (Reay-Jones et al. 2005). Akbar et al. (2008) selected for tebufenozide resistance over 12 generations of *D. saccharalis* populations from two locations in Louisiana. The authors found that populations exposed to an equitoxic (LC<sub>20</sub>) level of tebufenozide resulted in lower pupal weights, increase days to pupation, reduced egg viability and number of eggs oviposited (Akbar et al. 2008). Similarly, Pineda et al (2007) demonstrated reduced fecundity, reduced larval weights and adverse reproductive function of *Spodoptera littoralis* (Boisduval)

(Lepidoptera: Noctuidae) when insects were exposed to methoxyfenozide. Reductions in fecundity were also seen in *Cydia pomonella* (Linnaeus) and *Argyrotaenia velutinana* (Walker) (Lepidoptera: Tortricidae) adults that were continuously exposed to methoxyfenozide treated substrate (Pineda et al. 2007). The selective control of lepidopteran insects along with the potential for area wide control makes the use and preservation of insect growth regulators essential to integrated pest management programs.

### **Noctuidae Resistance to Methoxyfenozide**

Resistance to methoxyfenozide by other noctuid pests has been confirmed in beet armyworm *Spodoptera exigua* (Hübner) and the cotton leafworm *Spodoptera littoralis* (Boisduval) (Moulton et al. 2002, Gore and Adamczyk 2004, Swevers et al. 2008, Mosallanejad and Smagghe 2009). Moulton et al. (2002) discovered 9.7 and 7.3 fold resistance to methoxyfenozide at the LC<sub>50</sub> and LC<sub>90</sub> levels in a population of beet armyworm collected from Thailand. This high level of methoxyfenozide resistance is due in part from Thai producer's sole dependence on insecticides for insect control and the process of overhead drench irrigation (Moulton et al. 2002). Gore and Adamczyk (2004) demonstrated that methoxyfenozide resistance is heritable in beet armyworm; however, the mode of inheritance was not determined. Swevers et al. (2008) concluded that methoxyfenozide resistance may not be based on decreased absorption or increased cellular metabolism, but on a mutation at the ecdysteroid signaling location that confers cross resistance to dibenzoylhydrazine insecticides. Mosallanejad and Smagghe (2009) produced methoxyfenozide resistance in cotton leafworm when larvae were continuously selected over 13 generations. The selected colony demonstrated a 5.5-fold increase in resistance to methoxyfenozide over the selection period (Mosallanejad and Smagghe 2009). The authors concluded that oxidative metabolism is the most common mechanism of resistance

to DBH-type insecticides. Methoxyfenozide is an ideal insecticide that can be easily incorporated into integrated pest management programs due to its selective toxicity to insects and relative safety to non-targets. Ultimately, with the development of resistance by the beet armyworm to methoxyfenozide in less than three years in Thailand, careful management of insect growth regulators in Mid-South agro-ecosystems is essential to preserve this class of insecticide.

Due to the widespread adoption of methoxyfenozide and the history of insecticide resistance by the soybean looper, this research is important to determine instances of methoxyfenozide resistance in the Mid-South and to give producers and researchers insight into the efficacy of currently registered insecticides for soybean looper.

### **Objectives**

The objectives of this research were to evaluate the efficacy of labeled insecticides for use on soybean loopers by: establishing methoxyfenozide baseline resistance levels in regional soybean looper populations through the use diet incorporated bioassays, and evaluating residual efficacy of foliar insecticides through the use of defoliation and mortality over time.

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## CHAPTER 2

### RESPONSES OF FIELD COLLECTED COLONIES OF SOYBEAN LOOPER TO METHOXYFENOZIDE USING A DIET INCORPORATED BIOASSAY

#### Introduction

Soybean, *Glycine max* (L.) Merr, is one of Louisiana's major agronomic crops. In 2010 Louisiana producers planted 1,030,000 acres of soybeans yielding 41,820,000 bushels (USDA NASS 2011). Soybeans also provide a significant contribution to Louisiana agricultural economy through their use as a rotational crop (Heatherly and Hodges 1999). One quality contributing to soybeans success in Louisiana is the crop's adaptability to diverse soil and climactic conditions (Morrison and McCormick 1996).

Soybeans grown in states along the Gulf Coast have the greatest amount of insect pressure (Way 1994). Several economically important insects infest Louisiana soybeans including the threecornered alfalfa hopper, *Spissistilus festinus* (Say), corn earworm, *Helicoverpa zea* (Boddie), bean leaf beetle, *Cerotoma trifurcata* (Förster) and a complex of stink bugs that infest soybeans during important physiological development (Pedigo and Buntin 1993). The most damaging defoliating insects are the soybean looper *Chrysodeixis includens* (Walker), velvetbean caterpillar *Anticarsia gemmatilis* (Hübner) and green cloverworm *Plathypena scabra* (Fabricius). In 2010, in Mississippi, soybean loopers infested 1.7 million acres of soybeans and caused a 19% total loss plus cost of control to producers (Musser et al. 2011).

Soybean loopers are the prominent Plusiinae noctuid species in Louisiana and the Mid-South. This pest is highly polyphagous with hosts including many agricultural crops produced in the Southeastern United States. Soybeans are the optimum host for feeding and oviposition (Herzog et al. 1980). Adult soybean loopers are believed to migrate from overwintering

reservoirs in areas of southern Texas, Florida and the Caribbean to soybean production areas around the United States (Herzog et al 1980, Boethel et al. 1992).

Integrated pest management of the soybean looper utilizes multiple control tactics. Biological control employs natural enemies and pathogens to keep damaging soybean looper populations below economic thresholds. Harding (1976) collected 29 species of parasitoids from *C. includens* and *T. ni* in the Lower Rio Grande Valley of Texas with *Trichogramma* spp. being the most dominant. Richman et al. (1980) demonstrated control of soybean looper egg and larvae by 16 species of predators commonly found in soybean fields, the greatest levels of control were obtained by nabids and predatory pentatomids. Similarly, Similarly, Burleigh (1972) found two species of fungal pathogens *N. rileyi* and *Massospora* spp. controlling soybean looper populations at high levels in cotton-soybean agro-ecosystems. However, despite the high level of suppression attained by natural control agents, populations of soybean looper often exceed economic thresholds in mid-southern states forcing producers to apply insecticides (Boethel et al. 1992).

Historically, soybean loopers have been controlled with the use of broad spectrum insecticides (Boethel et al. 1992). Overuse and dependence on these compounds has led to several documented cases of resistance with instances of control failures observed throughout the southeastern United States since the 1960s (Boethel et al.1992). Resistance to DDT, cyclodienes, carbamates and organophosphates was thought to have occurred prior to 1970 (Boethel et al. 1992). Producers regained the ability to adequately control soybean loopers with the registration of permethrin in 1982 yet five years after its adoption, instances of resistance were documented in Louisiana and Mississippi (Felland et al. 1990, Leonard et al. 1990). Since the loss of permethrin, producers have been limited in their ability to adequately control soybean

looper populations. In 2001, methoxyfenozide (Intrepid® 2F, 22.6% [ai wt/v]; Dow Agrosiences, Indianapolis, IN) received full registration for use on cotton and in 2006 for use on soybeans (Moulton et al. 2002, EPA 2007). Methoxyfenozide provided control of soybean looper larvae while minimizing adverse effects on non-target organisms (Dhadialla and Jansson 1999).

In 2008, failure of methoxyfenozide to control soybean loopers below economic threshold in Louisiana was reported in arthropod management trials by Hardke et al. (2009). The authors reported control percentages of 60, 62 and 75% when sampled at 2, 5, and 8 days after treatment with the highest label rate of 8 fl oz/acre (Hardke et al. 2009). This evidence, in conjunction with producer reports of field control failures, warranted investigation.

The objective of this study was to evaluate methoxyfenozide resistance levels on strains of soybean loopers collected from Louisiana, Mississippi and Texas using diet incorporated bioassays. These data will provide a more thorough understanding of soybean looper methoxyfenozide resistance and provide a historical baseline to which future resistance management data can be compared.

## **Materials and Methods**

**Soybean Looper Colonies.** Soybean looper larvae were collected from soybean fields during August and September of 2009 and 2010 at locations throughout Louisiana, Texas and the Mid-South (Table 2.1). Larvae were collected using a standard 38.1cm sweep net from interior portions of soybean fields. Larvae were transported to the lab in 29.6ml plastic diet cups with Southland Product's artificial meridic soybean looper diet (Southland Products, Lake Village, AR) and segregated from all other colonies until pupation. Diseased and parasitized loopers were identified using external morphological characteristics of parasitoids and pathogens based

reference samples obtained from the Louisiana State University soybean entomology lab (Table 2.2) (Daigle et al. 1990). Pupae were placed in plastic rearing containers (30cm x 62cm); upon eclosion, adults were given a 10% honey solution and provided oviposition sheets (Mascarenhas and Boethel 2000). All colonies were maintained at 26°C with 75% RH and 14:10 (L:D) photoperiod. Oviposition sheets were changed every other day and placed in plastic bags (15cm x 7cm-1cm x 38cm plain clear non-vent) until eclosion. Upon eclosion, two neonate larvae were brushed into one ounce diet cups using a fine camel hair paint brush with 15 ml of artificial diet. The cups were capped and 100 cups were placed on cafeteria trays (43cm x 55cm). Soybean looper larvae were allowed to complete development to third instar.

In addition to the field colonies, a lab reared reference colony was maintained for use in bioassays. This colony (LSU1) was obtained in 1975 and has never been exposed to methoxyfenozide. (Newsom et al. 1980). Two other laboratory colonies were used for methoxyfenozide resistance comparison; LSU2 (Baur and Boethel 2002) and MR08. The MR08 colony was collected from the Macon Ridge Research Station in Winnsboro, LA during the summer of 2008 when methoxyfenozide, applied at 0.09 lb ai/acre, resulted in only 66% control of soybean loopers. A list of laboratory reared and field collected colonies can be found in Table 2.1

Table 2.1. Description of soybean looper field-collected colonies by code, year and location information

| Code  | Year | Location  |
|-------|------|---|
| LSU 1 | 1975 | South Louisiana                                   |
| MR08  | 2008 | Macon Ridge Research Station, Franklin Parish, LA |

Table 2.1 Cont.

|       |      |   |
|-------|------|---|
| WIN09 | 2009 | Macon Ridge Research Station, Franklin Parish, LA             |
| BH09  | 2009 | Ben Hur Research Station, East Baton Rouge Parish, LA         |
| NI09  | 2009 | Iberia Research Station, Iberia Parish, LA                    |
| DL09  | 2009 | Dean Lee Research Station, Rapides Parish, LA                 |
| MO09  | 2009 | Delta Research Center, Pemiscot County, MO                    |
| WIN10 | 2009 | Macon Ridge Research Station, Franklin Parish, La             |
| BH10  | 2010 | Ben Hur Research Station, East Baton Rouge Parish, LA         |
| NI10  | 2010 | Iberia Research Station, Iberia Parish, LA                    |
| DL10  | 2010 | Dean Lee Research Station, Rapides Parish, LA                 |
| MO10  | 2010 | Delta Research Center, Pemiscot County, MO                    |
| MH10  | 2010 | Production Field, Morehouse Parish, LA                        |
| TX10  | 2010 | Texas Agrilife Research and Ext. Center, Jefferson County, TX |
| MS10  | 2010 | Delta States Research Center, Washington County, MS           |

**Diet-Incorporated Bioassays.** Diet incorporation bioassays as described by Rodriquez et al. (2001) were conducted to evaluate the effects of methoxyfenozide (Intrepid® 2F, 22.6% [ai wt/v]; Dow Agrosiences, Indianapolis, IN) on field-collected and laboratory reared soybean looper larvae. Concentrations of insecticides including a distilled water control were used on each population of soybean looper larvae. Stock solutions (1000 ppm in distilled water) were prepared based on the active ingredient (Mascarenhas and Boethel 2000). Serial dilutions in distilled water were also used to obtain treatment concentrations (Mascarenhas and Boethel

2000). Dosage mortality concentrations were obtained either from 100 or 400 ppm solutions. Concentrations selected were expected to kill 95% of the individuals at the highest concentration and 10% at the lowest concentration. Each concentration consisted of a control, 0.02, 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, and 5.0 ppm. Discriminating dosages were determined from concentration mortality curves obtained from the LSU1 susceptible strain LC<sub>99</sub> (0.140 ppm).

One liter of diet was made following the manufacturer's directions. The diet was separated into 3 replicates consisting of 300 ml of liquid diet per repetition. Each repetition was composed of 30 29.57 ml plastic diet cups. After mixture using a blender (Conair, East Windsor, NJ model CB15), the diet was allowed to cool to a temperature below 60°C (Rodriguez et al. 2001). Once the diet had cooled, 5 ml methoxyfenozide concentrations were pipetted into each repetition using a pipette filler and sterile pipette. After insecticide incorporation, the diet was apportioned to each repetition at 10 ml per cup with a ketchup dispenser and allowed to cool for a minimum of 45 minutes (Rodriguez et al. 2001). Once the diet had dried for one hour, one third instar soybean looper was placed in each cup and capped. Newly molted soybean loopers were chosen for use in bioassays based on days of development and behavior. Behavior consisted of soybean looper larvae resting on the bottom of the cap sealing the diet cup upon a successful molt. Mortality was observed daily for 14 days or until pupation. Larvae were considered dead if no movement was observed after probing with a blunt object. Larvae treated with methoxyfenozide exhibited physiological symptoms of insect growth regulator poisoning including cuticular blackening, loss of hemolymph, double head capsule formation and extrusion of hindgut (Dhadialla and Jansson 1999). Previous work by Rodriguez et al. (2001), with tebufenozide, defined larval death as malformed cuticle, slipped and double head capsules, and deformed mandibles. However, we chose to use a response that could be quantified as a standard

endpoint and where previous work with diet incorporated methoxyfenozide on Noctuidae had defined death (Gore and Adamczyk 2004).

Data were analyzed as a probit analysis using POLO-PLUS (LeOra 2002). Lethal concentration values were considered to be significantly different if their 95% confidence limits (CL) did not overlap. Resistance ratios were calculated using the formula  $LC_{50}$  field strain/ $LC_{50}$  LSU 1 and MR08. Natural control mortalities were analyzed across years using analysis of variance (ANOVA) (PROC MIX, SAS Institute 2002-2005).

### **Results**

This is the first documented account of soybean looper resistance to methoxyfenozide in the United States.  $LC_{50}$ s for the 2009 colonies ranged from 0.005 (LSU 1) to 0.079 ppm (NI09), a 15.8 fold difference (Table 2.4). Ben Hur (F3), Dean Lee (F3) and New Iberia (F4) were not significantly different based on overlapping 95% confidence intervals. The F4 Winnsboro generation had the lowest  $LC_{50}$  of 0.017 ppm throughout all colonies and generations tested in 2009. Of the populations tested in 2009, Winnsboro (WIN09) and New Iberia (NI09) were significantly different between generations whereas, Dean Lee (DL09) was not significantly different between generations (Table 2.4). The Ben Hur (BH09) population did not produce sufficient individuals for a bioassay to be conducted on the F4 generation so no comparison could be made.

$LC_{50}$ s for the 2010 colonies ranged from 0.045 (BH10) to 0.122 ppm (NI10) a 24.4 fold difference from the LSU 1 reference strain (Table 2.5). All locations tested for 2010 were not significantly different from the F3 to F4 generation based on overlapping 95% confidence intervals. A population collected from Morehouse parish was added as an additional location for comparison. Morehouse parish has greater than 200,000 acres of land dedicated to production



agriculture with cotton acreages planted in close proximity to soybeans (Personal Communication with Terri Erwin). New Iberia exhibited the greatest LC<sub>50</sub> (0.122 ppm) of all populations and locations tested throughout the duration of the experiment. The F4 generation from the Ben Hur collection exhibited the lowest LC<sub>50</sub> (0.045 ppm) of all generations and locations tested in 2010. Morehouse (MH10) was found to have consistently the highest LC<sub>50</sub> values for the F3 (0.133 ppm) and F4 (0.118 ppm) generations.

Comparisons were also made between MR08 and resistant strains for both years tested. The MR08 population has been in colony for over 36 generations and methoxyfenozide resistance has been maintained within the population of soybean loopers without introductions of wild individuals. The highest resistance levels from the 2009 field colonies were 1.8 fold more resistant than the MR08 resistant standard. Whereas the highest resistant levels from the 2010 colonies were 2.8 fold more resistant than MR08. All field collected colonies tested across locations and years had significantly higher LC<sub>50</sub>s than reference colonies based on overlapping 95% confidence limits. In five bioassays, four Louisiana strains from the same locations were tested in both 2009 and 2010 and one Missouri strain from the same location was tested in 2009 and 2010 (Table 2.4 – 2.6); these included Ben Hur (BH), Dean Lee (DL), Winnsboro (WIN), New Iberia (NI) and Missouri (MO). Of the strains tested in 2009 and 2010, the WIN (F4), BH (F3), NI (F4) and DL (F4) were significantly different between generations (Table 2.4 and 2.5). No comparison could be made between the 2009 and 2010 Ben Hur F4 strains due to the 2009 colony not producing sufficient individuals for a bioassay. Also, differing generations (F2 and F3) of the Missouri strain were not tested in 2009 due to insufficient larval numbers to perform a bioassay. The 2010 Mississippi colony also failed to provide sufficient individuals for use in a bioassay on the F4 generation.

Collections for BH09-10, NI09-10, DL09-10, WIN09-10 colonies were obtained from research stations where no insecticide applications had been previously applied to soybean fields. MH10 was collected from a soybean production field that was not bordered by other commodities. MS10 and MO09-10 were also collected from soybean fields located on research stations that received no insecticide applications prior to collection.

Entomopathogens and parasitoids were identified and recorded for each year and colony collected across all locations (Table 2.2). Percent natural control mortalities were significantly different across years ( $F = 1645.58$ ;  $df = 2$ ,  $P = <0.0001$ ). *Copidosoma truncatellum* (Dalman) was the most numerous parasitoid found in all strains tested across all locations and years tested. Tachinidae, *Meteorus autographae* (Muesebeck), and *Rogas* spp. comprised the remaining parasitoids found in locations tested. Entomopathogens *N. rileyi*, *E. grammae*, and nuclear polyhedrosis virus (NPV) were the primary pathogens found infecting soybean loopers from each location and strain. *N. rileyi* provided the highest amount of pathogenic control while nuclear polyhedrosis virus provided the lowest. Natural control resulted in the least amount of mortality for the 2009 collections of soybean loopers, with the 2010 collections experiencing moderate levels of control mortality. Percent natural control was greatest in colonies collected from Louisiana during the 2011 soybean production season. All colonies collected from Louisiana in 2011 were significantly different from 2009 and 2010 collections from Louisiana, Mississippi and Texas. Unidentified secondary pathogens were also documented and preserved for possible later identification. Parasitoids and pathogens collected from all colonies were identified and discarded to prevent contamination of other laboratory and field collected colonies. As a result, no laboratory colonies could be established from the 2011 collections due to high natural control mortality.

Table 2.2. Number of parasitized and diseased soybean looper larvae from field collected colonies 2009-2011.

| Code  | Date collected | Num. collected | No. parasitized              |                                |                  |            |                    | No. diseased     |                   |                  | % Nat ctrl. |
|-------|----------------|----------------|------------------------------|--------------------------------|------------------|------------|--------------------|------------------|-------------------|------------------|-------------|
|       |                |                | <i>Meteorous autographae</i> | <i>Copidosoma truncatellum</i> | <i>Rogas</i> sp. | Tachinidae | Other <sup>a</sup> | NPV <sup>b</sup> | <i>E. grammae</i> | <i>N. rileyi</i> |             |
| WIN09 | 8/27/2009      | 117            | 0                            | 28                             | 0                | 0          | 5                  | 1                | 0                 | 0                | 27          |
| BH09  | 8/19/2009      | 105            | 3                            | 10                             | 0                | 0          | 0                  | 0                | 0                 | 0                | 12          |
| NI09  | 8/11/2009      | 102            | 1                            | 27                             | 0                | 0          | 9                  | 0                | 0                 | 0                | 36          |
| DL09  | 8/20/2009      | 90             | 0                            | 3                              | 0                | 0          | 12                 | 0                | 0                 | 0                | 16          |
| MO09  | 9/22/2009      | 180            | 0                            | 2                              | 0                | 0          | 18                 | 0                | 0                 | 25               | 25          |
| WIN10 | 7/26/2010      | 300            | 12                           | 53                             | 5                | 17         | 12                 | 0                | 0                 | 12               | 37          |
| BH10  | 8/19/2010      | 205            | 10                           | 36                             | 2                | 5          | 11                 | 0                | 0                 | 4                | 33          |
| NI10  | 8/12/2010      | 172            | 7                            | 25                             | 5                | 15         | 14                 | 0                | 3                 | 6                | 44          |
| DL10  | 7/27/2010      | 232            | 2                            | 51                             | 5                | 27         | 28                 | 0                | 1                 | 10               | 53          |
| MO10  | 10/23/2010     | 300            | 3                            | 4                              | 1                | 0          | 27                 | 0                | 0                 | 0                | 12          |
| MH10  | 7/29/2010      | 300            | 9                            | 30                             | 8                | 31         | 14                 | 0                | 3                 | 9                | 35          |
| TX10  | 8/29/2010      | 146            | 0                            | 1                              | 6                | 15         | 8                  | 0                | 4                 | 2                | 25          |
| MS10  | 9/23/2010      | 300            | 4                            | 39                             | 5                | 46         | 32                 | 0                | 12                | 9                | 49          |
| WIN11 | 9/12/2011      | 125            | 3                            | 36                             | 8                | 27         | 11                 | 0                | 4                 | 4                | 74          |
| BH11  | 8/30/2011      | 110            | 2                            | 23                             | 1                | 26         | 22                 | 0                | 3                 | 5                | 75          |
| DL11  | 8/28/2011      | 132            | 0                            | 21                             | 0                | 46         | 18                 | 0                | 2                 | 7                | 71          |
| MS11  | 9/14/2011      | 300            | 5                            | 56                             | 15               | 90         | 54                 | 0                | 10                | 34               | 88          |

<sup>a</sup>Unidentified secondary pathogens

<sup>b</sup>Nuclear polyhedrosis virus

Table 2.3. Toxicity of diet with methoxyfenozide (Intrepid 2F) to susceptible and resistant standard strains of third instar Louisiana soybean loopers.

| Strain | n <sup>a</sup> | Gen <sup>b</sup> | LC <sub>50</sub> (95% CL) <sup>c</sup> | LC <sub>90</sub> (95% CL) <sup>d</sup> | Slope (SEM) | RR  |
|--------|----------------|------------------|--|--|-------------|-----|
| LSU 1  | 2430           | ---              | 0.005 (0.002 - 0.010)                  | 0.034 (0.028 - 0.056)                  | 3.01 (0.37) | 1   |
| MR08   | 2430           | ---              | 0.043 (0.033 - 0.052)                  | 0.197 (0.158 - 0.261)                  | 1.92 (0.17) | 8.6 |

<sup>a</sup>Number of larvae tested including controls

<sup>b</sup>Generation tested

<sup>c</sup>Values expressed in ppm

<sup>d</sup>Values expressed in ppm

Table 2.4. Toxicity of diet with methoxyfenozide (Intrepid 2F) to third instar Louisiana soybean loopers 14 days after treatment 2009.

| Strain | n <sup>a</sup> | Gen <sup>b</sup> | LC <sub>50</sub> (95% CL) <sup>c</sup> | LC <sub>90</sub> (95% CL) <sup>d</sup> | Slope (SEM) | RR   |
|--------|----------------|------------------|--|--|-------------|------|
| WIN09  | 810            | F3               | 0.066 (0.056 - 0.076)                  | 0.242 (0.199 - 0.310)                  | 2.26 (0.17) | 13.2 |
| WIN09  | 810            | F4               | 0.017 (0.010 - 0.024)                  | 0.075 (0.056 - 0.114)                  | 2.01 (0.24) | 3.4  |
| BH09   | 810            | F3               | 0.034 (0.029 - 0.039)                  | 0.083 (0.074 - 0.096)                  | 3.58 (0.38) | 6.8  |
| NI09   | 810            | F3               | 0.079 (0.069 - 0.090)                  | 0.248 (0.206 - 0.313)                  | 2.57 (0.19) | 15.8 |
| NI09   | 810            | F4               | 0.047 (0.037 - 0.058)                  | 0.148 (0.119 - 0.200)                  | 2.58 (0.26) | 9.4  |
| DL09   | 810            | F3               | 0.039 (0.034 - 0.044)                  | 0.098 (0.083 - 0.122)                  | 3.21 (0.30) | 7.8  |
| DL09   | 810            | F4               | 0.032 (0.027 - 0.036)                  | 0.065 (0.056 - 0.078)                  | 4.13 (0.46) | 6.4  |

<sup>a</sup>Number of larvae tested including controls

<sup>b</sup>Generation tested

<sup>c</sup>Values expressed in ppm

<sup>d</sup>Values expressed in ppm

Table 2.5. Toxicity of diet treated with methoxyfenozide (Intrepid 2F) to third instar Louisiana soybean loopers 14 days after treatment 2010.

| Strain | n <sup>a</sup> | Gen <sup>b</sup> | LC <sub>50</sub> (95% CL) <sup>c</sup> | LC <sub>90</sub> (95% CL) <sup>d</sup> | Slope (SEM) | RR   |
|--------|----------------|------------------|--|--|-------------|------|
| WIN10  | 810            | F3               | 0.090 (0.072 - 0.109)                  | 0.359 (0.291 - 0.463)                  | 2.13 (0.18) | 18.0 |
| WIN10  | 810            | F4               | 0.076 (0.053 - 0.100)                  | 0.339 (0.269 - 0.446)                  | 1.98 (0.21) | 15.2 |
| BH10   | 810            | F3               | 0.054 (0.044 - 0.064)                  | 0.218 (0.177 - 0.283)                  | 2.10 (0.17) | 10.8 |
| BH10   | 810            | F4               | 0.045 (0.035 - 0.056)                  | 0.233 (0.184 - 0.311)                  | 5.37 (0.66) | 9.0  |
| NI10   | 810            | F3               | 0.122 (0.080 - 0.161)                  | 0.443 (0.358 - 0.583)                  | 2.29 (0.32) | 24.4 |
| NI10   | 810            | F4               | 0.098 (0.063 - 0.130)                  | 0.341 (0.275 - 0.448)                  | 2.37 (0.34) | 19.6 |
| DL10   | 810            | F3               | 0.047 (0.038 - 0.057)                  | 0.206 (0.166 - 0.269)                  | 2.01 (0.17) | 9.4  |
| DL10   | 810            | F4               | 0.049 (0.041 - 0.057)                  | 0.204 (0.166 - 0.265)                  | 2.07 (0.17) | 9.8  |
| MH10   | 810            | F3               | 0.113 (0.089 - 0.138)                  | 0.650 (0.513 - 0.865)                  | 1.68 (0.13) | 22.6 |
| MH10   | 810            | F4               | 0.118 (0.087 - 0.153)                  | 0.721 (0.563 - 0.972)                  | 1.63 (0.14) | 23.6 |

<sup>a</sup>Number of larvae tested including controls

<sup>b</sup>Generation tested

<sup>c</sup>Values expressed in ppm

<sup>d</sup>Values expressed in ppm

LC<sub>50</sub>s for the 2009-10 out of state colonies ranged from 0.028 (MO09) to 0.054 (MS10) a 10.8 fold difference (Table 2.6). Missouri (MO10) and Mississippi (MS10) had the highest LC<sub>50</sub>s and resistance ratios of the out of state colonies tested (Table 2.6). There were no significant differences between generations in Missouri (MO09-10) and Texas (TX10).

Table 2.6. Toxicity of diet treated with methoxyfenozide (Intrepid 2F) to third instar out of state soybean loopers 14 days after treatment 2009-10.

| Strain | n <sup>a</sup> | Gen <sup>b</sup> | LC <sub>50</sub> (95% CL) <sup>c</sup> | LC <sub>90</sub> (95% CL) <sup>d</sup> | Slope (SEM) | RR   |
|--------|----------------|------------------|--|--|-------------|------|
| MO09   | 810            | F2               | 0.028 (0.018 - 0.038)                  | 0.148 (0.107 - 0.238)                  | 1.78 (0.17) | 5.6  |
| MO09   | 810            | F3               | 0.026 (0.016 - 0.038)                  | 0.166 (0.118 - 0.267)                  | 1.60 (0.15) | 5.2  |
| MO10   | 810            | F3               | 0.039 (0.030 - 0.047)                  | 0.130 (0.106 - 0.169)                  | 2.44 (0.26) | 7.8  |
| MO10   | 810            | F4               | 0.047 (0.039 - 0.057)                  | 0.182 (0.148 - 0.236)                  | 2.19 (0.19) | 9.4  |
| MS10   | 810            | F3               | 0.054 (0.027 - 0.073)                  | 0.152 (0.123 - 0.202)                  | 2.83 (0.60) | 10.8 |
| TX10   | 810            | F3               | 0.045 (0.035 - 0.055)                  | 0.150 (0.122 - 0.195)                  | 2.46 (0.25) | 9.0  |
| TX10   | 810            | F4               | 0.040 (0.032 - 0.049)                  | 0.142 (0.116 - 0.185)                  | 2.34 (0.23) | 8.0  |

<sup>a</sup>Number of larvae tested including controls

<sup>b</sup>Generation tested

<sup>c</sup>Values expressed in ppm

<sup>d</sup>Values expressed in ppm

Figures 2.1-2.6 delineate percent survivorship 14 days after exposure for the standard reference colony (LSU 1), resistant standard (MR08), and the lowest and highest resistant strains of soybean looper for each year tested. Individual graphs represent selected methoxyfenozide concentrations tested for 2009-2010 and illustrate time until death for and percent survivorship for intoxicated larvae. 0.020 ppm resulted in the longest time until death and highest percent survivorship, while 5.000 ppm resulted in the shortest time until death and lowest percent survivorship. New Iberia colonies for both years exhibited greater survivorship and increased days until death when compared to the reference strain and resistant standard.

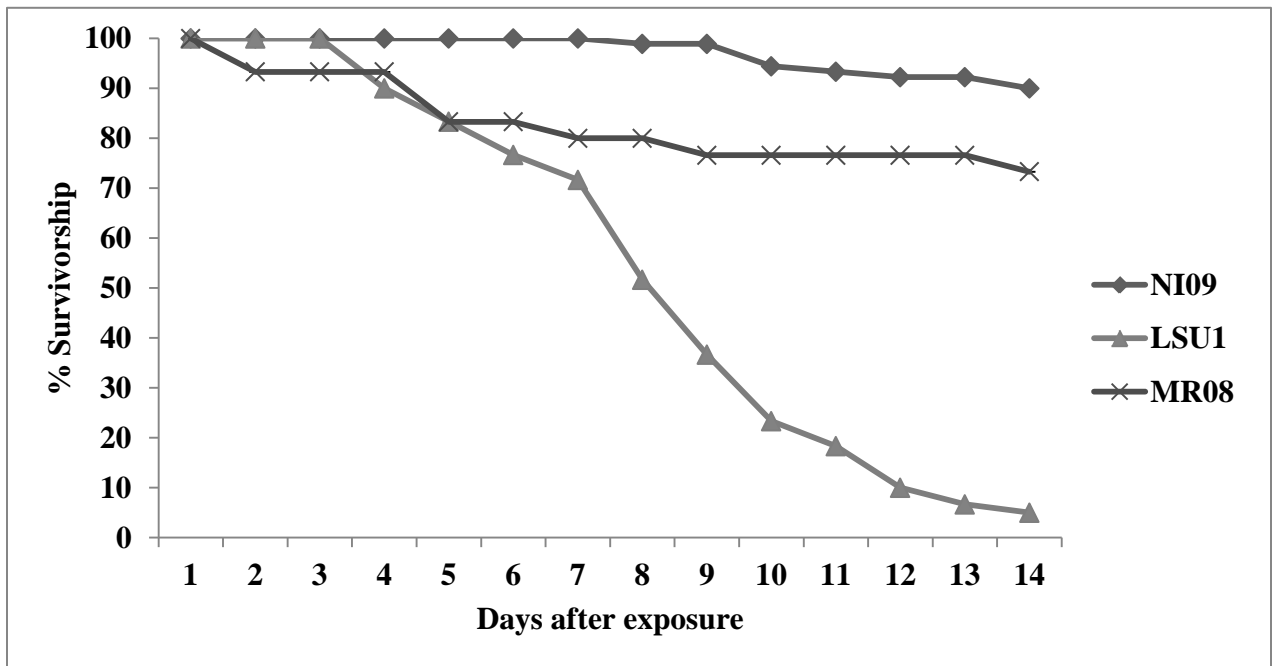


Figure 2.1. Percent survivorship of standard reference (LSU1), resistant standard (MR08), and highest (NI09) resistant strains of soybean looper tested at 0.020 ppm for 2009.

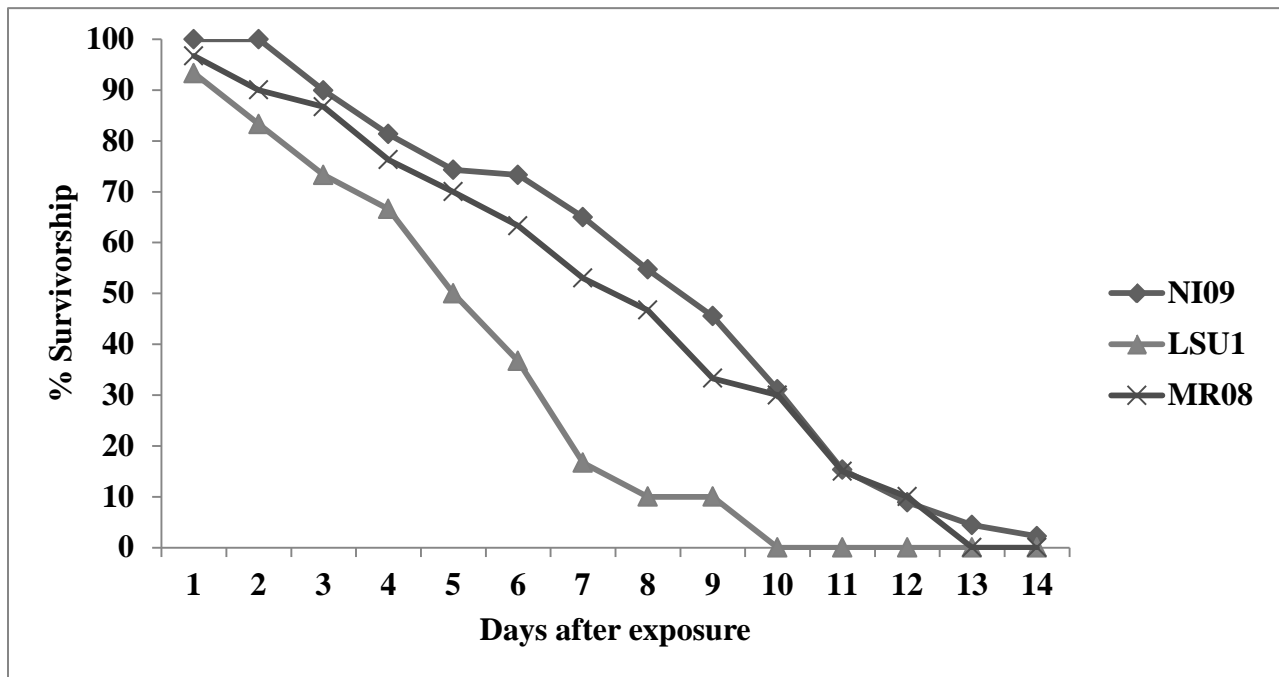


Figure 2.2. Percent survivorship of standard reference (LSU1), resistant standard (MR08), and highest (NI09) resistant strains of soybean looper tested at 0.500 ppm for 2009.

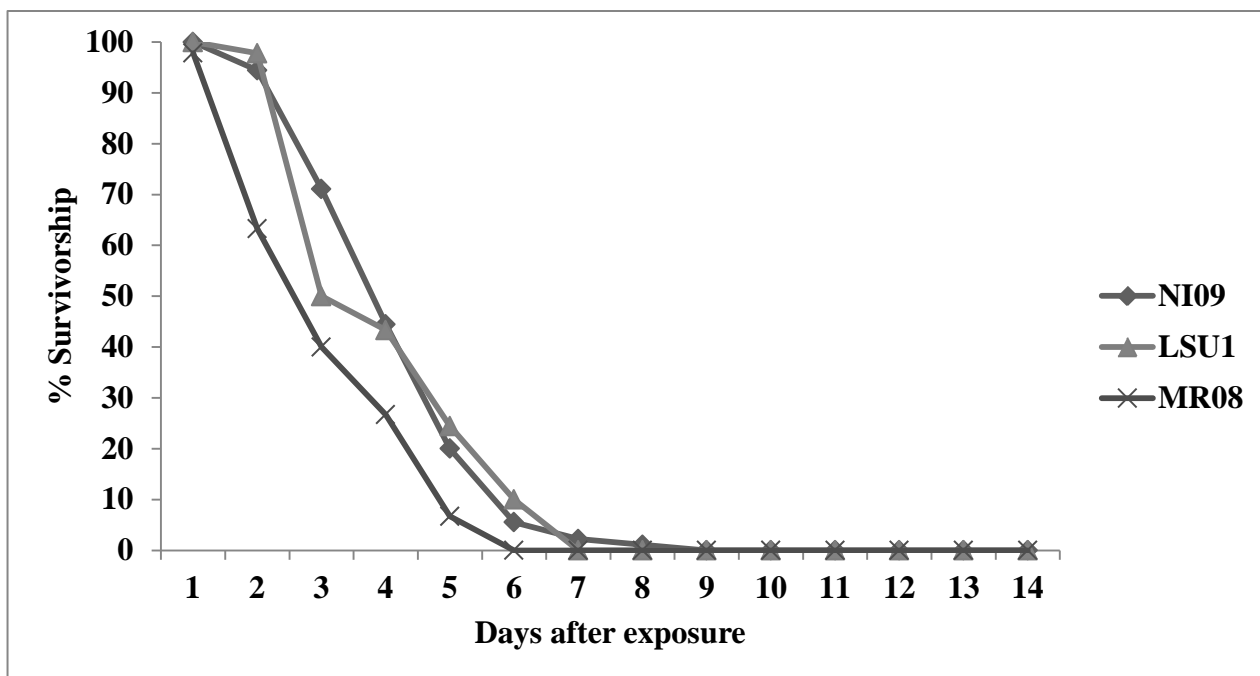


Figure 2.3. Percent survivorship of standard reference (LSU1), resistant standard (MR08), and highest (NI09) resistant strains of soybean looper tested at 5.000 ppm for 2009.

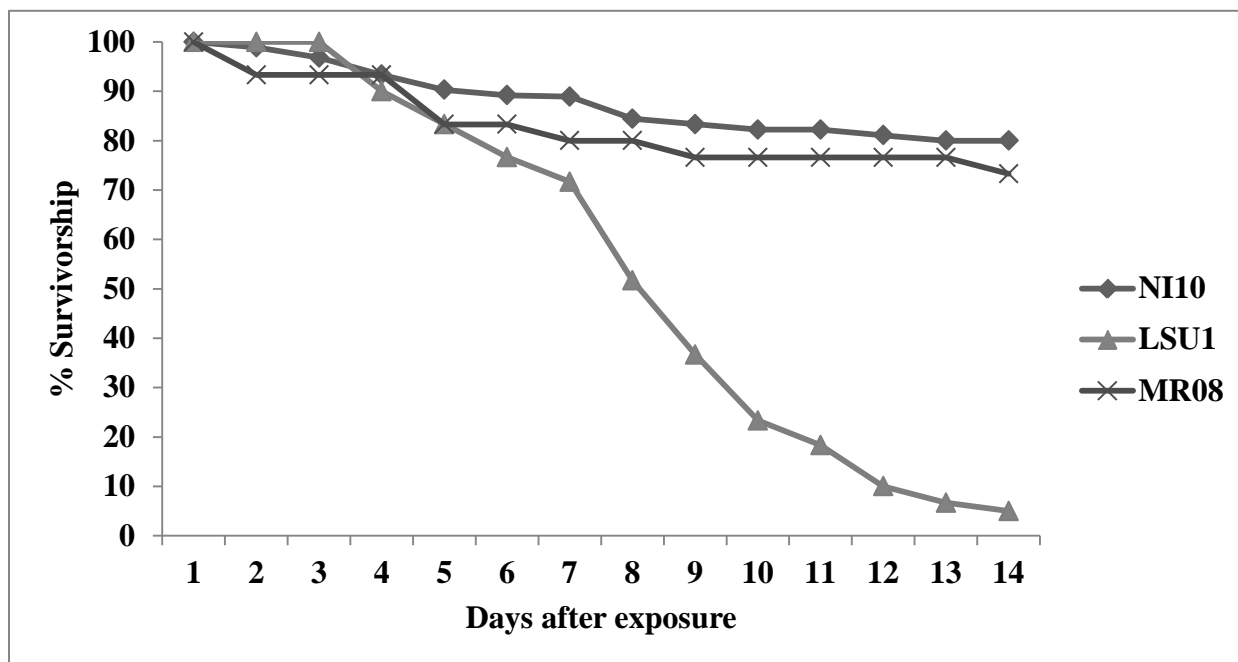


Figure 2.4. Percent survivorship of standard reference (LSU1), resistant standard (MR08), and highest (NI10) resistant strains of soybean looper tested at 0.020ppm for 2010.



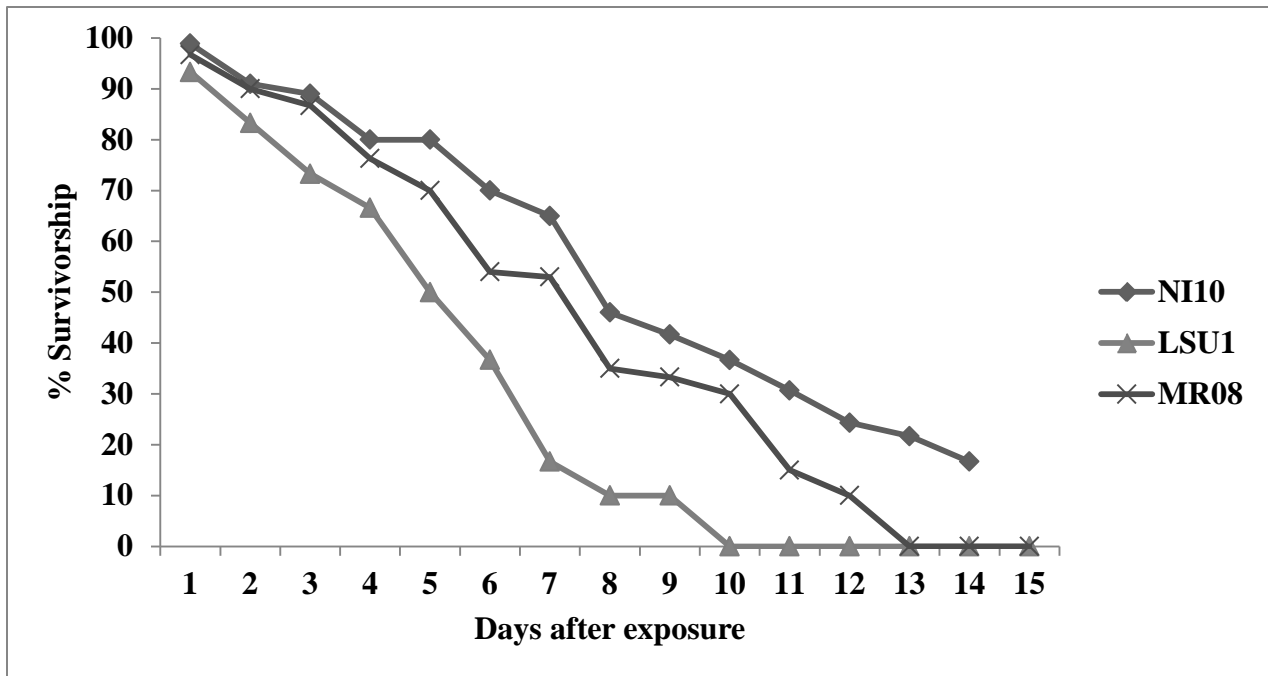


Figure 2.5. Percent survivorship of standard reference (LSU1), resistant standard (MR08), and highest (NI10) resistant strains of soybean looper tested at 0.500 ppm for 2010.

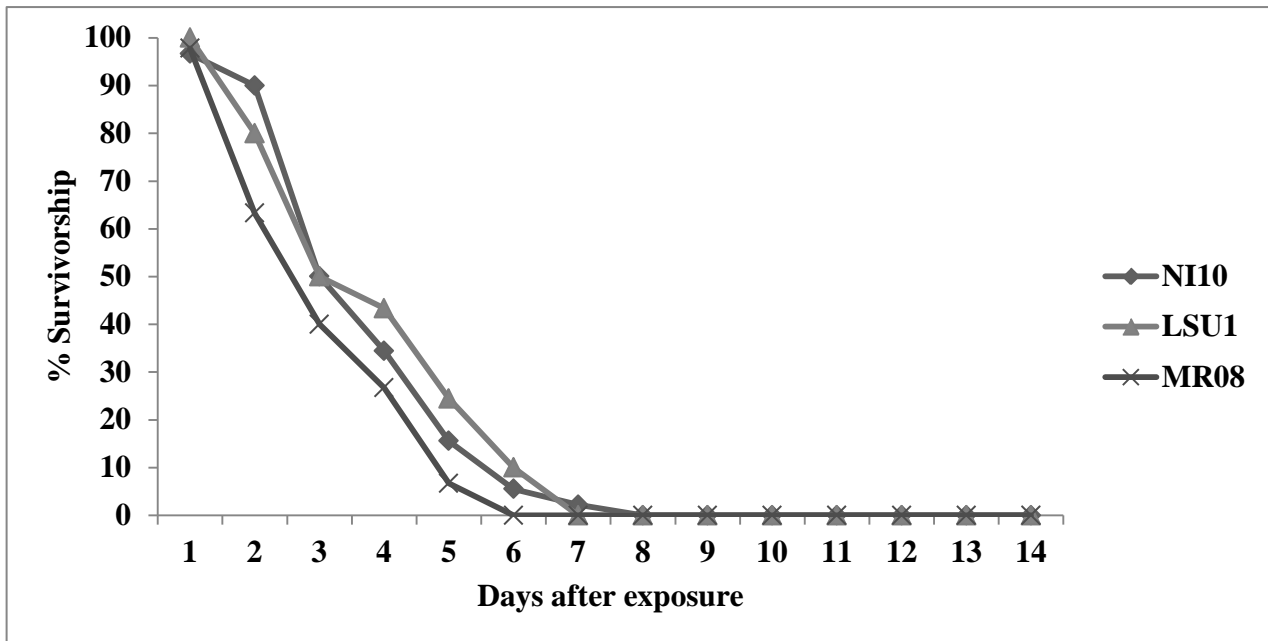


Figure 2.6. Percent survivorship of standard reference (LSU1), resistant standard (MR08), and highest (NI10) resistant strains of soybean looper tested at 5.000 ppm for 2010.

## Discussion

Soybean looper methoxyfenozide susceptibility varied significantly in populations in Louisiana, the Mid-South and Texas. These observations confirm reports of field failures seen with methoxyfenozide in Louisiana. Over all, susceptibility increased as subsequent generations were tested. In general, field collections for 2009 and 2010 from the soybean producing regions in north Louisiana (WIN09, WIN10, MH10) and the southernmost area collected from (NI09, NI10) had the greatest  $LC_{50}$ s and resistance ratios.

Natural control agents were found to be very effective in increasing soybean looper mortality. Biotic controls of soybean loopers have been found to control populations of soybean loopers in Louisiana soybeans (Richman et al. 1980, Beach and Todd 1986, Daigle et al. 1990). Parasitoids and entomopathogens resulted in mortalities ranging from 12 to 88% in field collected strains of soybean loopers. The 2009 colonies experienced the lowest control mortalities of all years tested. This may be due in part to extensive broad spectrum insecticide use in soybean fields across the Mid-South that year for stink bugs (Musser et al. 2009). However, the 2011 colonies experienced the highest levels of natural mortality with the lowest percentage of natural control being 71%. The 2011 soybean production season in Louisiana experienced very low populations of redbanded stink bugs *Piezodorus guildinii* (Westwood) with very few insecticide applications (Musser et al. 2012). This pest has resulted in a net increase of broad spectrum insecticide applications across Louisiana with adequate control requiring 3 to 5 applications of tank mixed organophosphate and pyrethroid insecticides (Bauer et al. 2010). Broad spectrum insecticides have been shown to greatly reduce the number of natural enemies found in agro-ecosystems (Croft and Brown 1975). Frequent applications of insecticides in soybean fields for pests other than soybean loopers may have deleterious effects on a wide

number of natural control agents. Natural control agents work to help keep pest populations under economic thresholds, and producers should make efforts to scout fields frequently and apply insecticides only when needed. Also, insecticides should be applied when beneficial insects are not present or are at physiological development periods that protect them from insecticide exposure.

The varying levels of resistance in Louisiana may be influenced by the soybean loopers migratory behavior. Although no evidence of reverse migration of the soybean looper back to their overwintering reservoirs has been documented, that possibility cannot be discounted. Sparks et al. (1986) placed black light traps on oil platforms 34, 72, 106 and 160 km from the shoreline south of Jeanerette, Louisiana from 11 September 1973 to 21 October 1973. Their results included a total trap capture of 1,418 individuals consisting of two species of Plusiinae Noctuidae *C. includens* and *T. ni* (Sparks et al. 1986). *C. includens* and *T. ni* comprised the overwhelming majority of insects captured during the study. Five hundred and thirty two moths were capture at the 106 km distance and 631 moths were captured at the 160 km distance (Sparks et al. 1986). Number of mated females was also counted with 22, 47, 32 and 25% of females captured at distances of 34, 72, 106 and 160 km (Sparks et al. 1986). The authors concluded that insects were transported on prevailing atmospheric conditions and meteorological factors greatly influence long and short range migration (Sparks et al. 1986). Similarly, Mason et al. (1989) conducted a study investigating the daily and seasonal values of ovarian development and whole body lipid content of the soybean looper. From these parameters, they examined the possibility of fall populations exhibiting the physiological aspects of the oogenesis-flight syndrome. Oogenetic-flight syndrome is changes based on the developing ovaries of female moths preparing to migrate (Mason et al.1989). The authors determined seasonal changes of *C.*

*includens* by sampling a laboratory colony and field populations from July - November (Mason et al. 1989). Lipid percentages remained low throughout the summer months (July - September) and increased in the fall months (October and November) (Mason et al. 1989). The authors determined that soybean loopers appear to conform to the oogenesis-flight syndrome by slowing egg maturation and storing lipids as an energy source (Mason et al. 1989). An advantage of slowing egg maturation gives females the ability to reabsorb nutrients from pre-chorionated eggs and use them as energy during sustained flight (Mason et al 1989). Male soybean loopers also stored lipids to possibly prepare for immigration back to southern source areas and ensure successful mating once the insects had reached a suitable overwintering climate (Mason et al. 1989).

Another possibility of resistance development especially in the northern regions of Louisiana may be due to proximity to cotton. Soybean looper migration is common when soybeans are planted in close proximity to cotton. Jensen et al. (1974) demonstrated a significant increase in fecundity and mating frequency when soybean looper adults were supplied a diet of cotton nectaries. Previous studies conducted by Felland et al. (1990) and Leonard et al. (1990) indicated increased soybean looper pyrethroid resistance where cotton and soybeans were grown in close proximity. Similarly, methoxyfenozide was labeled for use on cotton five years before soybeans in Louisiana and applications to cotton may have also influenced resistance formation (EPA 2007).

Methoxyfenozide resistance may also be affected by integrated pest management practices in regions outside of the United States. Newsom (1980) suggested that pest control practices in one part of the United States or other nations may have significant effects on integrated pest management programs in another. Similar commodities are grown in regions of

the United States and Mexico due to temperate climates and favorable soil conditions. Soybean loopers are a highly polyphagous pest that consumes a number of these crops requiring producers to utilize chemical control to prevent yield loss. Many of these same commodities are also damaged by several other pest insects such as the armyworm complex. Methoxyfenozide is labeled in the United States and Mexico for control of the armyworm complex on crops such as cotton, soybean, tomato, corn, peppers, and peanuts. The soybean looper has been found on soybean, cotton, corn, tomato, bell pepper and peanuts (Herzog 1980). Osorio et al. 2008 conducted studies on methoxyfenozide resistance of 5 populations of beet armyworm found in vegetable production areas with a history of continuous insecticide use which included tebufenozide and methoxyfenozide. The authors determined only one  $LC_{50}$  value to be significantly higher than values exhibited by the reference colony, and resistance ratios ranged from 2 to 13 (Osorio et al. 2008). Although their findings only yielded one colony of a beet armyworm having a significantly higher  $LC_{50}$  value, the possibility of continuous insecticide use on vegetable crops in Mexico selects for resistant individuals of beet armyworm and soybean looper. Mexico's insecticide application and worker protection regulations are weak compared to more developed nations, and the few regulations that are in place to protect the environment and slow resistance are often ignored (Abler and Pick 1993). Exacerbating the problem of insecticide resistance is the inadequate education producers have regarding insecticide labels and their use. Many of the producers in developing nations are functionally illiterate and cannot understand insecticide regulations printed in their own language leading to over-use and arbitrary applications of insecticides (Wilson and Tisdell 2001). With the bulk of insecticides being applied in developing countries, instances of resistance in these countries become likely (Wilson and Tisdell 2001).

Missouri, Mississippi and Texas colonies generally exhibited lower LC<sub>50</sub> values than colonies tested in Louisiana. Regional differences in resistance may be due to this insect's migratory behavior and soybean acreage situated along the Gulf of Mexico. In 2010, Texas produced 205,000 acres of soybeans, while Mississippi produced 2,000,000, Missouri produced 5,150,000 and Louisiana produced 1,030,000 acres of soybeans (NASS 2011). Soybean loopers migrating from overwintering reservoirs in Mexico and Central America encounter acreages of soybeans along the Gulf of Mexico in Texas and Louisiana. In 2010, Texas produced 17,400 acres of soybeans in counties situated on the Gulf of Mexico while Louisiana produced 12,000 acres of soybeans in parishes adjacent to the Gulf of Mexico (NASS 2011). Mississippi produced no soybeans in counties bordering the Gulf of Mexico (NASS 2011). Coastal acreages in Texas and Louisiana may act as targets for soybean loopers to infest as they enter soybean production areas from emigration flights. However, unlike Texas, soybean production in Louisiana begins at the Gulf Coast and continues through the central portion of the state into the Mississippi River Delta. This continuous acreage exposes populations of soybean loopers to mating opportunities and insecticide applications that influence heritable and insecticide selected resistance levels.

In conclusion, our data indicated that methoxyfenozide may no longer be an effective tool for control of soybean looper populations in Louisiana, Texas and the Mid-South. The bioassay data demonstrated greater LC<sub>50</sub>s and resistance ratios from field-collected strains when compared to a reference colony. Although some of the strains exhibited very low levels of resistance, the soybean looper's history of developing rapid insecticide resistance and lack of affordable alternatives demonstrate the importance of determining resistance at any level so proactive measures can be implemented. Laboratory bioassays as well as field evaluated foliar

applications are essential in documenting resistance. Use of diet incorporated bioassays appears to be a suitable method in evaluating resistance with insecticides utilizing stomach poisons for control of insects such as the soybean looper. Furthermore, use of insecticides with low toxicity to non-target organisms and insect selectivity are ideal components that adhere to integrated pest management practices.

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## CHAPTER 3

### RESIDUAL EFFICACY OF FOLIAR INSECTICIDES AGAINST LOUISIANA SOYBEAN LOOPER DEFOLIATION

#### Introduction

The soybean looper is an important defoliator of soybeans in the Louisiana and the Mid-South. These insects annually migrate from reservoirs in extreme southern portions of the United States and areas of the Caribbean. Damaging populations of this pest occur from early August through September in Louisiana. Soybean loopers are found throughout the entire soybean-producing regions of Louisiana and the Mid-South.

Soybean loopers have the ability to completely defoliate an entire field if left uncontrolled. Boldt et al. (1975) demonstrated that an individual soybean looper can defoliate up to 114 cm<sup>2</sup> of soybean leaf tissue through 6 instars at 25°C. Similarly, Reid and Greene (1973) found that individual soybean loopers consumed 82 cm<sup>2</sup> of soybean leaflet area and 97% of consumption occurred in the last three stadia. The preferred feeding area of the soybean looper is the lower portion of the crop canopy with defoliation spreading to the top as the crop matures.

Soybeans have the ability to withstand significant amounts of defoliation before yield loss is experienced. Board et al. 1994 concluded that late season defoliation at R 6.3 and R 6.6 resulted in yield losses of 40 and 20% when soybeans were mechanically defoliated 100%. Thomas et al. (1978) found that 40% defoliation of soybean plants at R3 and 19% at R4 resulted in yield losses of 13 and 17%. Yield loss in soybeans was found to be greatest during pod set and seed filling (R3 – R5) with losses reaching 78% when defoliation reached 100%. (Board et al. 2010). Although soybeans have the ability to compensate for damage during various stages of development, soybean looper defoliation damage must be controlled to prevent significant yield loss (Boethel 2004).

Management of defoliators in the Mid-South consists of routine field scouting and use of insecticides to prevent defoliation (Funderburk et al. 1999). Producers in Louisiana and the Mid-South have traditionally relied upon insecticides to keep damaging populations of this pest under economic threshold levels. However, producers have encountered extensive soybean looper insecticide resistance since the 1960's (Boethel et al. 1992).

The rapid development of resistance by the soybean looper to almost every class of insecticide led to laboratory bioassays to determine locations and levels of insecticide resistance. Many of these techniques including discriminating dose bioassays on field-collected insects have proved to be a cheap, effective method in determining small fluctuations in resistance levels (Forrester 1990). However, results from these experiments cannot be extrapolated to field control; resistance level is one of many components that can be attributed to the success or failure of a field spray (Forrester 1990). Resistance monitoring can only be used to detect fluctuations in resistance frequency (Mascarenhas and Boethel 1997). Therefore, insecticide efficacy must be evaluated against natural populations of insects in a field setting to quantify resistance levels observed in laboratory bioassays. Therefore, the objectives of this study were to determine the residual efficacy of foliar insecticides on soybean loopers using defoliation and mortality.

### **Materials and Methods**

Research was conducted at the LSU Agriculture Center's Ben Hur Central Research Station in Baton Rouge and at the Dean Lee Research Station in Alexandria, LA. The 2009-2010 research was only conducted in Baton Rouge and 2011 research was conducted at both Alexandria and Baton Rouge with all locations planted in early May. Pioneer 94Y90 soybean seed was used for all years and locations. For the Baton Rouge 2009 study, seeds were planted using a four row cone planter. All other locations and years tested were drill-seeded with an eight

row planter in Mhoon silty clay loam at the Ben Hur Central Research Station (East Baton Rouge Parish) and in Norwood silt loam at the Dean Lee Research Station (Rapides Parish). Test area was a series of plots, 4 rows (centered on 91.4 cm) wide, by 7.62 m long with 4 row buffers for both locations. Treatments were arranged in randomized complete block design. The 2009 study consisted of a control and five foliar insecticides used for lepidopteran control in Louisiana soybeans. The insecticides used were: flubendiamide (Belt SC<sup>®</sup>, 39.0% [ai wt/wt]; Bayer CropScience, Research Triangle Park, NC), methoxyfenozide (Intrepid 2F<sup>®</sup>, 22.6% [ai wt/wt]; Dow AgroScience, Indianapolis, IN), indoxacarb (Steward EC<sup>®</sup>, 15.8% [ai wt/wt]; DuPont Crop Protection, Wilmington, DE), thiodicarb (Larvin 3.2<sup>®</sup>, 34.0% [ai wt/wt]; Bayer CropScience, Research Triangle Park, NC), chlorantraniliprole (Coragen SC<sup>®</sup>, 18.4% [ai wt/wt]; DuPont Crop Protection, Wilmington, DE).

The 2010 study consisted of a control and seven foliar insecticides. Insecticides used were: flubendiamide, chlorantraniliprole, methoxyfenozide, indoxacarb, thiodicarb, lambda-cyhalothrin (Karate Z<sup>®</sup>, 22.8% [ai wt/wt]; Syngenta Crop Protection, Greensboro, NC), acephate (Orthene 97<sup>®</sup>, 97.4% [ai wt/wt]; AMVAC, Los Angeles, CA), + beta-cyfluthrin (Baythroid<sup>®</sup> XL, 12.7% [ai wt/wt]; Bayer CropScience, Research Triangle Park, NC). Applications for 2009-10 were made when plant development had reached R5 and when soybean loopers typically begin to infest Louisiana soybean fields.

Similarly, the 2011 study consisted of a control and four lepidopteran specific foliar insecticides. Insecticides applied were flubendiamide, chlorantraniliprole, methoxyfenozide and indoxacarb. Each of the insecticides was applied at select label rates listed in table 3.3-8. Insecticides were applied at R3 and R5 soybean development stages to coincide with tank mixed applications of fungicides made by producers in soybean production areas of Louisiana.

Insecticides for all years tested were applied using a 2 nozzle, 3 gallon, carbon dioxide hand held sprayer calibrated to deliver 75.7 liters per acre with two Teejet 8006 flat spray nozzles.

For 2009-2010, leaf samples were randomly collected from each plot at 3, 7, 14, 21 and 28 days after treatment (DAT). For 2011, leaf samples were randomly collected from soybean plants 2 DAT at the R3 and R5 development stages. Collection locations were marked in plots to prevent subsequent sampling from the same area. Standardized core samples of 11.40 cm<sup>2</sup> were taken from each leaf and placed in 100 x 15 mm petri dishes with a number one 100 x 15 mm sheet of cellulose filter paper (Whatman Inc. Sanford, Maine). One MR08 third instar soybean looper larvae collected from the Macon Ridge Research Station (Franklin Parish) near Winnsboro, LA was placed on the leaf core and allowed to feed. Insects were brushed into petri dishes with a fine camel hair paint brush to reduce injury to individual larva. Mortality and percent defoliation were assessed daily. Leaf cores were replaced when defoliation had exceeded 80%. Distilled water was apportioned to each petri dish to keep the leaf cores moist throughout the duration of the experiment and sealed with wax paper. Leaf cores that began to mold or grow fungus were replaced. Deceased insects were recorded and removed from the experiment upon determination of death. Insects surviving insecticidal treatments were discarded at the conclusion of the experiment. Insects used for the non-treated control were allowed to develop until pupation and returned to laboratory colonies.

**Data Analysis:** 2009 and 2010 data were combined and analyzed using analysis of variance (ANOVA) with means separated using Tukey's ranged test (PROC MIX, SAS Institute 2002-2005). Data from 2011 were analyzed separately from 2009 and 2010 using PROC MIXED with means separated by Tukey's ranged test (PROC MIX, SAS Institute 2002-2005).

Table 3.1. Foliar insecticides applied for soybean looper control 2009-11.

| Formulation               | Common name         | Class                   | Mode of action                    | Years Tested |
|---------------------------|---------------------|-------------------------|-----------------------------------|--------------|
| Belt SC <sup>®</sup>      | flubendiamide       | Diamide                 | ryanodine receptor modulator      | 2009-11      |
| Intrepid 2F <sup>®</sup>  | methoxyfenozide     | insect growth regulator | ecdysone receptor agonist         | 2009-11      |
| Steward EC <sup>®</sup>   | indoxacarb          | Oxadiazine              | sodium channel blocker            | 2009-11      |
| Larvin 3.2 <sup>®</sup>   | thiodicarb          | Carbamate               | inhibitor of acetylcholinesterase | 2009-11      |
| Coragen SC <sup>®</sup>   | chlorantraniliprole | Diamide                 | ryanodine receptor modulator      | 2009-11      |
| Karate Z <sup>®</sup>     | lambda-cyhalothrin  | Pyrethroid              | disruption of sodium channels     | 2010         |
| Orthene 97 <sup>®</sup>   | acephate            | Organophosphate         | inhibitor of acetylcholinesterase | 2010         |
| Baythroid XL <sup>®</sup> | beta-cyfluthrin     | Pyrethroid              | disruption of sodium channels     | 2010         |

### Results

**2009-10.** There were significant differences among treatments applied for control of soybean loopers 3, 7, 14, 21 and 28 (DAT) (3 DAT;  $F = 1921.27$ ;  $df = 7$ ,  $P = <0.0001$ ), (7 DAT;  $F = 794.45$ ,  $df = 7$ ,  $P = <.0001$ ), (14 DAT;  $F = 2721.60$ ,  $df = 7$   $P = <.0001$ ), ( 21 DAT;  $F = 1203.56$ ;  $df = 7$ ,  $P = <.0001$ ), (28 DAT;  $F = 1820.43$ ,  $df = 7$ ,  $P = <.0001$ ). In general, the lowest amount of defoliation was observed in chlorantraniliprole and flubendiamide treated leaf tissue with the highest amount of defoliation observed in non-treated leaf tissue across all dates tested per soybean looper. Defoliation ranged from: 0.20 to 15.34 cm<sup>2</sup> 3 DAT, 0.20 to 15.38 cm<sup>2</sup> 7

DAT, 0.50 to 15.10 cm<sup>2</sup> 14 DAT, 0.57 to 15.25 cm<sup>2</sup> 21 DAT, and 0.97 to 15.24 cm<sup>2</sup> 28 DAT.

Defoliated area for all treatments can be found in table 3.2.

Table 3.2. Evaluation of mean area defoliated by soybean loopers 2009-10.

| Treatment                                  | Rate <sup>b</sup> | Mean area (cm <sup>2</sup> ) consumed by soybean loopers <sup>a</sup> |         |         |         |         |
|--|-------------------|---|---------|---------|---------|---------|
|  |                   | 3 DAT   | 7 DAT   | 14 DAT  | 21 DAT  | 28 DAT  |
| Non-treated                                | ---               | 15.34 a   | 15.38 a | 15.05 a | 15.24 a | 15.25 a |
| Flubendiamide                              | 0.113             | 0.23 d  | 0.26 d  | 0.65 d  | 1.45 d  | 1.32 c  |
| Chlorantraniliprole                        | 0.119             | 0.20 d  | 0.20 d  | 0.50 d  | 0.57 e  | 0.97 c  |
| Methoxyfenozide                            | 0.134             | 0.87 c  | 1.18 c  | 1.65 bc | 1.98 cd | 2.17 b  |
| Oxadiazine                                 | 0.123             | 0.64 cd   | 1.75 bc | 1.39 c  | 2.52 bc | 2.10 b  |
| Thiodicarb                                 | 0.908             | 1.13 bc   | 1.49 bc | 1.68 bc | 2.38 bc | 2.20 b  |
| Lambda-cyhalothrin <sup>c</sup>            | 0.030             | 1.12 bc   | 2.38 b  | 2.06 b  | 2.79 b  | 2.25 b  |
| Acephate +<br>Beta-cyfluthrin <sup>c</sup> | 0.908 + 0.027     | 1.57 b  | 2.34 b  | 1.60 bc | 2.67 bc | 2.27 b  |

<sup>a</sup>Column means followed by the same letter are not significantly different (P =0.05 Tukey)

<sup>b</sup>kg/ha

<sup>c</sup>2010 addition

**2011 Ben Hur** There were significant differences among treatments for R3 ( $F = 3541.47$ ,  $df = 8$ ,  $P = <.0001$ ) and R5 ( $F = 3624.09$ ,  $df = 8$ ,  $P = <.0001$ ). Results were similar to the 2009-10 data with chlorantraniliprole having the lowest amount of defoliation at 0.19 cm<sup>2</sup> for R3 and 0.20 cm<sup>2</sup> for R5 per soybean looper tested. Non-treated control resulted in the largest

amounts of defoliation with 15.74 cm<sup>2</sup> consumed per soybean looper at R3 and 15.86 cm<sup>2</sup> consumed at R5.

Table 3.3. Evaluation of mean area defoliated by soybean loopers at select label rates from Ben Hur Central Research Station for soybeans at R3.

| Treatment           | Rate <sup>b</sup> | Mean area (cm <sup>2</sup> ) consumed by soybean loopers <sup>a</sup> |                   |         |
|---------------------|-------------------|---|-------------------|---------|
|                     |                   | R3  | Rate <sup>c</sup> | R3      |
| Non-treated         | ---               | 15.74 a   | ---               | 15.74 a |
| Flubendiamide       | 0.076             | 1.58 d  | 0.114             | 0.43 ef |
| Chlorantraniliprole | 0.079             | 0.57 e  | 0.119             | 0.19 f  |
| Methoxyfenozide     | 0.067             | 2.62 b  | 0.134             | 1.91 cd |
| Oxadiazine          | 0.050             | 2.05 c  | 0.123             | 0.51 ef |

<sup>a</sup>Column means followed by the same letter are not significantly different (P =0.05 Tukey)

<sup>b</sup>low label rate in kg/ha

<sup>c</sup>high label rate in kg/ha

Methoxyfenozide resulted in the largest amounts of defoliation of the insecticide treated leafs with 2.62 cm<sup>2</sup> at 0.067 kg/ha and 1.91 cm<sup>2</sup> at 0.134 kg/ha. Insects exhibited symptoms of insect growth regulator poisoning consisting of fused mandibles, hind gut discharge, cuticular blackening and loss of hemolymph. Oxadiazine treated leafs resulted in mean defoliation levels of 2.05 at 0.050 kg/ha and 0.51 cm<sup>2</sup> at 0.123 kg/ha. Soybean loopers exposed to oxadiazine displayed symptoms of nervous system intoxication including cessation of feeding, tremors, lethargic movements and convulsions. Core samples taken from soybeans at the R5 development stages exhibited similar results to R3 samples. Methoxyfenozide resulted in the greatest levels of defoliation with 2.09 cm<sup>2</sup> at 0.67 kg/ha and 1.09 cm<sup>2</sup> at 0.134 kg/ha. Flubendiamide resulted in



0.89 cm<sup>2</sup> at 0.076 kg/ha and 0.40 cm<sup>2</sup> at 0.114 kg/ha. Insects intoxicated by flubendiamide in all treatments ceased feeding after ingestion of treated leaf tissue within the first day with coordination and movement severely impaired. Mean defoliated area for all treatments can be found in Tables 3.3 and 3.4.

Table 3.4. Evaluation of mean area defoliated by soybean loopers at select label rates from Ben Hur Central Research Station at R5.

| Treatment           | Rate <sup>b</sup> | Mean area (cm <sup>2</sup> ) consumed by soybean loopers <sup>a</sup> |                   |         |
|---------------------|-------------------|---|-------------------|---------|
|                     |                   | R5  | Rate <sup>c</sup> | R5      |
| Non-treated         | ---               | 15.86 a   | ---               | 15.86 a |
| Flubendiamide       | 0.076             | 0.89 d  | 0.114             | 0.40 e  |
| Chlorantraniliprole | 0.079             | 0.26 e  | 0.119             | 0.19 e  |
| Methoxyfenozide     | 0.067             | 2.09 b  | 0.134             | 1.09 d  |
| Oxadiazine          | 0.050             | 2.07 b  | 0.123             | 1.56 c  |

<sup>a</sup>Column means followed by the same letter are not significantly different ( $P = 0.05$  Tukey)

<sup>b</sup>low label rate in kg/ha

<sup>c</sup>high label rate in kg/ha

**2011 Dean Lee.** There were significant differences in among treatments for both R3 ( $F = 4960.69$ ,  $df = 8$ ,  $P = <.0001$ ) and R5 ( $F = 5206.2$ ,  $df = 8$ ,  $P = <.0001$ ) at the Dean Lee location. Defoliation ranged from 0.20 to 15.17 cm<sup>2</sup> during the R3 development stage and 0.19 cm<sup>2</sup> to 15.17 cm<sup>2</sup> during the R5 development stage with chlorantraniliprole having the lowest amount of defoliation and non-treated control having the highest. Flubendiamide treated leaf tissue resulted in 0.78 cm<sup>2</sup> and 0.34 cm<sup>2</sup> at 0.076 kg/ha and 0.114 kg/ha respectively. Treatments of chlorantraniliprole resulted in the lowest defoliation across all treatments for both R3 and R5.

Chlorantraniliprole treated core samples resulted in 0.33 cm<sup>2</sup> and 0.20 cm<sup>2</sup> at 0.079 kg/ha and 0.119 kg/ha respectively. Insects intoxicated by flubendiamide and chlorantraniliprole exhibited symptoms outlined in the previous paragraph with cessation of feeding observed across all treatments within the first day of the study.

Table 3.5. Evaluation of mean area defoliated by soybean loopers at select label rates from Dean Lee Research Station at R3.

| Treatment           | Rate <sup>b</sup> | Mean area (cm <sup>2</sup> ) consumed by soybean loopers <sup>a</sup> |                   |         |
|---------------------|-------------------|---|-------------------|---------|
|                     |                   | R3  | Rate <sup>c</sup> | R3      |
| Non-treated         | ---               | 15.17 a   | ---               | 15.17 a |
| Flubendiamide       | 0.076             | 0.78 c  | 0.114             | 0.34 d  |
| Chlorantraniliprole | 0.079             | 0.33 d  | 0.119             | 0.20 d  |
| Methoxyfenozide     | 0.067             | 1.60 b  | 0.134             | 0.87 c  |
| Oxadiazine          | 0.050             | 1.77 b  | 0.123             | 1.04 c  |

<sup>a</sup>Column means followed by the same letter are not significantly different (P =0.05 Tukey)

<sup>b</sup>low label rate in kg/ha

<sup>c</sup>high label rate in kg/ha

Methoxyfenozide and oxadiazine treated leaf substrate resulted in the greatest levels of mean defoliation with 1.60 cm<sup>2</sup> and 0.87 cm<sup>2</sup> consumed at 0.067 kg/ha and 0.134 kg/ha observed with methoxyfenozide. Oxadiazine treated tissue resulted in 1.77 cm<sup>2</sup> and 1.04 cm<sup>2</sup> at 0.050 kg/ha and 0.123 kg/ha respectively. Leaf samples selected from plots at the R5 growth stage resulted in a similar outcome. Flubendiamide and chlorantraniliprole intoxicated larvae consumed significantly less foliage than oxadiazine. Methoxyfenozide controlled insect feeding

at the 0.134 kg/ha rate with no significant difference between flubendimide, chlorantraniliprole and methoxyfenozide with a high label rate at R5.

Table 3.6. Evaluation of mean area defoliated by soybean loopers at select label rates from Dean Lee Research Station at R5.

| Treatment           | Rate <sup>b</sup> | Mean area (cm <sup>2</sup> ) consumed by soybean loopers <sup>a</sup> |                   |         |
|---------------------|-------------------|---|-------------------|---------|
|                     |                   | R5  | Rate <sup>c</sup> | R5      |
| Non-treated         | ---               | 15.56 a   | ---               | 15.56 a |
| Flubendiamide       | 0.076             | 0.44 ef   | 0.114             | 0.22 f  |
| Chlorantraniliprole | 0.079             | 0.23 ef   | 0.119             | 0.19 f  |
| Methoxyfenozide     | 0.067             | 1.31 d  | 0.134             | 0.58 ef |
| Oxadiazine          | 0.050             | 2.07 b  | 0.123             | 1.69 c  |

<sup>a</sup>Column means followed by the same letter are not significantly different ( $P = 0.05$  Tukey)

<sup>b</sup>low label rate in kg/ha

<sup>c</sup>high label rate in kg/ha

**2011 Days to 100% Mortality.** Significant differences were also observed in days to 100% mortality across all treatments for both R3 ( $F = 7.0$ ,  $df = 7.0$ ,  $P = <.0011$ ) and R5 ( $F = 35.06$ ,  $df = 35.06$ ,  $P = <.0001$ ) development stages at both locations. Chlorantraniliprole at 0.11 kg/ha provided the fewest days to 100% mortality for R3 (2 days) and R5 (1 day) development stages for both locations tested. Chlorantraniliprole applied at 0.08 kg/ha was not significantly different at the R3 growth stage from the higher rate of 0.11 kg/ha at both locations. Flubendiamide was not significantly different at the R3 development stage from all other insecticides tested with the exception of chlorantraniliprole. Oxadiazine and methoxyfenozide treatments resulted in the most days to 100% mortality with a mean of 6 days for 0.55 kg/ha of

oxadiazine and 0.15 kg/ha of methoxyfenozide at R3. Results were similar for R5 with 0.50 kg/ha of oxadiazine and 0.15 kg/ha of methoxyfenozide resulting in 5.7 and 4.3 days to 100% mortality. Methoxyfenozide, , consistently resulted in greater mean days to mortality; this may be due to its specific mode of action as an insect growth regulator.

Table 3.7. Evaluation of mean days to 100% mortality for Ben Hur Central Research Station and Dean Lee Research Station at R3.

| Treatment           | Rate <sup>b</sup> | Mean days to 100% mortality |                   |       |
|---------------------|-------------------|-----------------------------|-------------------|-------|
|                     |                   | R3                          | Rate <sup>c</sup> | R3    |
| Non-treated         | ---               | ---                         | ---               | ---   |
| Flubendiamide       | 0.076             | 5.0 a                       | 0.114             | 4.7 a |
| Chlorantraniliprole | 0.079             | 4.3 ab                      | 0.119             | 2.0 b |
| Methoxyfenozide     | 0.067             | 6.0 a                       | 0.134             | 5.3 a |
| Oxadiazine          | 0.050             | 6.0 a                       | 0.123             | 4.7 a |

<sup>a</sup>Column means followed by the same letter are not significantly different (P =0.05 Tukey)

<sup>b</sup>low label rate in kg/ha

<sup>c</sup>high label rate in kg/ha

Results from field studies indicate that chlorantraniliprole and flubendiamide provided satisfactory control of soybean loopers across all locations and all dates tested; while methoxyfenozide and oxadiazine resulted in significantly longer mean days to death. Flubendiamide and chlorantraniliprole only significantly differed in defoliated area 21 DAT and cessation of feeding occurred rapidly after soybean loopers ingested treated leaf substrate. Insects were considered dead upon probing with a blunt object. Total days to 100% mortality can be found in tables 3.7 and 3.8.

Table 3.8. Evaluation of mean days to 100% mortality for Ben Hur Central Research Station and Dean Lee Research Station at R5.

| Treatment           | Rate <sup>b</sup> | Mean days to 100% mortality <sup>a</sup> |                   |         |
|---------------------|-------------------|--|-------------------|---------|
|                     |                   | R5                                       | Rate <sup>c</sup> | R5      |
| Non-treated         | ---               | ---                                      | ---               | ---     |
| Flubendiamide       | 0.076             | 3.0 cde                                  | 0.114             | 2.0 ef  |
| Chlorantraniliprole | 0.079             | 2.3 de                                   | 0.119             | 1.0 f   |
| Methoxyfenozide     | 0.067             | 4.3 b                                    | 0.134             | 3.3 bcd |
| Oxadiazine          | 0.050             | 5.7 a                                    | 0.123             | 4.0 bc  |

<sup>a</sup>Column means followed by the same letter are not significantly different (P =0.05 Tukey)

<sup>b</sup>low label rate in kg/ha

<sup>c</sup>high label rate in kg/ha

### Discussion

Foliar applications used primarily for control of pod and stem feeding guild of insects such as the stink bug complex and three cornered alfalfa hopper do not work well for controlling soybean looper damage. Acephate + beta-cyfluthrin and lambda-cyhalothrin kept defoliation reduced to 1.57 cm<sup>2</sup> and 1.21 cm<sup>2</sup> 3 days after treatment respectively. However, defoliation control was not sustained past 7 days after treatment with compounds tested. Resistance to pyrethroids and organophosphates by the soybean looper has been thoroughly documented across the Mid-South and applications of broad spectrum insecticides should not be relied upon for control (Boethel et al.1992).

Thiodicarb provided marginal defoliation protection against soybean loopers during 2009-10 experiment. Thiodicarb provided the greatest amount of defoliation control at the 3 days

after treatment resulting in 1.21 cm<sup>2</sup> of defoliated area. However, thiodicarb requires 72 hours after application to become rain fast leaving soybeans vulnerable to damage by soybean loopers if precipitation occurs during the 72 hour period. Similarly, methoxyfenozide did not significantly differ from thiodicarb, lambda-cyhalothrin, and oxadiazine for defoliation control 3, 7, 21 and 28 days after treatment. The highest amounts of control attained from methoxyfenozide limited defoliation to 0.87 cm<sup>2</sup> 3 days after treatment and 1.17 cm<sup>2</sup> 7 days after treatment. Oxadiazine did not significantly differ from flubendiamide and methoxyfenozide 3 days after treatment limiting defoliation to 0.64 cm<sup>2</sup>. However, defoliation significantly increased at 7 days after treatment and insecticidal efficacy declined 21 and 28 days after treatment.

Soybean loopers ceased feeding after intoxication with all compounds tested. Larvae poisoned with insecticides utilizing chemistries targeting receptors in the nervous system exhibited symptoms characteristic with broad-spectrum insecticides. These included convulsing, tremors, lethargic movement, erratic behavior, and expulsion of gut contents. Similarly, insects affected by methoxyfenozide, an insect growth regulator, exhibited slipped head capsules, malformed cuticle, cuticular blackening, hind gut discharge and cessation of feeding.

Soybean looper population dynamics often warrant the use of foliar insecticides to keep damaging levels of this pest under economic thresholds. Our results suggest that reduced efficacy of methoxyfenozide documented in Chapter 2 exists in populations of Louisiana soybean loopers outside the confines of a laboratory. Overuse and reliance on insecticides has been shown throughout history to drive insecticide resistance formation. The widespread use of methoxyfenozide in Louisiana soybeans, as well as its possible misuse, has limited the efficacy producers relied upon for control of soybean loopers. While methoxyfenozide and thiodicarb are

currently labeled for use on soybean loopers in Louisiana, more efficacious products are available (Baldwin et al. 2011). Although differences by product ranged in small increments of  $\text{cm}^2$ , the cumulative damage sustained by individual soybean plants can result in substantial yield loss if residual activity is inadequate.

Flubendiamide and chlorantraniliprole provided consistent protection from defoliation resulting in an overall decrease in mean days to mortality. Hannig et al. (2009) concluded that chlorantraniliprole intoxication caused rapid cessation of feeding in a variety of lepidopteran pests. Chlorantraniliprole induced feeding cessation in a time span comparable to broad spectrum insecticides methomyl, lambda-cyhalothrin and esfenvalerate (Hannig et al. 2009). The ectotoxicological profile of chlorantraniliprole is favorable for the preservation of beneficial organisms while reducing risks to the applicator (Hannig et al.2009). Both flubendiamide and chlorantraniliprole provide excellent activity against a large spectrum of lepidopteran pests while possessing an acute oral  $\text{LD}_{50}$  of  $>2,000$  mg/kg in mammals. This insecticidal chemistry has promise to help replace older, more toxic, methods of control relied upon by producers for control of soybean looper.

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## CHAPTER 4

### SUMMARY AND CONCLUSIONS

Soybeans serve as a host for a broad range of insect pests, and a few species cause economic losses that require control with insecticides. Hemipteran and lepidopteran pests comprise the majority of economically important insects in Louisiana soybeans. The soybean looper *Chrysodeixis includens* (Walker) limits soybean quality and yield across the Mid-South.

Soybean loopers are only known to overwinter in southern Florida and Texas in the continental United States. Soybean loopers are strong fliers and migration from states where overwintering occurs to soybean production areas of the Mid-South is common. Invasions of soybean-producing areas are thought to originate from these reservoirs or from areas in Central or South America and the Caribbean. Soybean loopers occur across a broad range of commercially produced commodities and are exposed to insecticide applications in areas where yearlong growing seasons are common. Subsequent applications of insecticides in regions outside of the United States may influence resistance levels inherited by the soybean looper and annual migratory flights expose regional soybean acres to highly resistant populations of insects. Louisiana and mid-southern soybean producers have relied on an insect growth regulator, methoxyfenozide, for consistent control of the soybean looper and other lepidopteran insects. Recently, Louisiana soybean producers have reported instances of control failures and reduced efficacy of methoxyfenozide against soybean loopers. If the soybean looper has developed resistance to this insecticide, it is important for agricultural researchers and producers in the Mid-South to know where resistance occurs and at what levels.

In order to determine if soybean looper resistance has emerged against methoxyfenozide multiple studies were proposed. The objectives of these studies were to determine soybean

looper resistance to methoxyfenozide using diet incorporated bioassays, and assess residual efficacy of foliar insecticides used for control of pest insects in Louisiana soybeans

Soybean looper populations collected from Louisiana, Texas, Mississippi and Missouri in 2009 and 2010 were exposed to nine dosage mortality concentrations of methoxyfenozide, ranging from 0.020 to 5.000 ppm. Insecticide concentrations were incorporated into artificial soybean looper diet and one third instar soybean looper was placed in each cup and allowed to feed for 14 days. Each concentration consisted of 3 repetitions with 30 individual diet cups (n=90) totaling 810 insects for each generation tested. A total of 26,730 insects were tested throughout the duration of this study. Resistance levels for Louisiana insects were highest in New Iberia for 2009 (0.079 ppm) and 2010 (0.122 ppm). Soybean looper resistance levels for out of state colonies were highest in Missouri for 2009 (0.028 ppm) and Mississippi for 2010 (0.054 ppm).

With control options for soybean looper becoming more limited and positive identification of resistance to methoxyfenozide by soybean loopers in all regions tested, insecticide stewardship is essential to the preservation of this class of chemistry. Although very effective alternative options are available for soybean looper control, these new insecticide chemistries cost substantially more than methoxyfenozide. However, integrated pest management practices outside of the confines of the United States will continue to create resistance management challenges for researchers and producers southern row crop agriculture.

The efficacy of foliar insecticides used for the control of the pod and stem feeding guild and lepidopteran defoliators were evaluated. Individual plots were treated in 2009-2010 at Ben Hur Central Research Station. Leaf samples were randomly collected from plots and standardized 11.57 cm<sup>2</sup> tissue cores were taken from each treatment. Significant differences were

detected across treatments for both years ( $P < 0.0001$ ). In general, the diamide compounds retained the longest residual activity limiting defoliation to less than 1.0 cm<sup>2</sup> for chlorantraniliprole and 1.5 cm<sup>2</sup> for flubendiamide. Lambda-cyhalothrin and acephate+baythroid allowed significantly greater defoliation than other compounds tested. These findings are analogous with published reports of resistance exhibited by the soybean looper to broad spectrum insecticides. For 2011, applications were made at two locations Ben Hur Central Research Station and Dean Lee Research Station. Insecticides consisted of lepidopteran specific compounds at select rates. Results were similar to previous years tested with diamide chemistry giving the greatest protection from defoliation. Methoxyfenozide and oxadiazine provided the least amount of defoliation protection. Days to 100% mortality were significant ( $P < 0.0001$ ). Chlorantraniliprole had the greatest insecticide efficacy limiting days to mortality to 2.0 at 0.119 kg/ha for R3 and 1.0 at 0.119 kg/ha for R5 soybean development stages. Methoxyfenozide resulted in longest days to 100% mortality taking an average of 5.3 days at 0.073 kg/ha for R3 and oxadiazine resulted in an average of 4.0 days to 100% mortality at 0.133 kg/ha for R5 development stages.

Foliar insecticidal residual activity is important for quantifying the resistance findings previously mentioned in Chapter 2. Methoxyfenozide resulted in significantly longer mean days to 100% mortality. This insecticide option may still be an effective tool for soybean looper control, however use of the low label rate of 0.067 kg/ha may not offer sufficient protection against soybean looper infestations. In general, the newer insecticide chemistries for lepidopteran control offer producers another avenue in control of soybean loopers with superior residual activity and reduced non-target effects.

## **VITA**

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