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Effects of Elicitor Induced Host Plant Resistance on Lepidopteran Insecticide Efficacy

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ABSTRACT

Soybean looper (SBL), *Chrysodeixis includens* (Walker), is an important defoliating Lepidopteran pest of southern U.S. soybean and utilizes other agronomic crops and weeds as hosts. With increasing resistance to insecticides, alternative control strategies such as induced host plant resistance were evaluated against SBL. Jasmonic acid (JA) is an elicitor of host plant resistance, and was selected to determine its fit in an IPM plan for SBL.

JA was applied to the top of meridic SBL diet and fed to SBL; no effects were found. JA applied as an exogenous elicitor to cotton, sweet potato, okra, cowpea, and soybean did result in differences. Less leaf area was consumed on all JA treated hosts aside from sweet potato, where SBL larvae consumed 10% less leaf area from control plants. Larval weight was reduced on all JA hosts except cowpea.

To assess impacts of JA induction on insecticide efficacy, larvae were fed induced or uninduced host plant tissue for seven days and then transferred to diet incorporated with or without methoxyfenozide. The number of days to adult emergence was longer on JA treated cotton (1.8) and soybean (0.9), while shorter on sweet potato (1.1). However, JA treatment to host plants did not affect methoxyfenozide efficacy.

Another pesticide that may induce plants is the herbicide, glyphosate. Glyphosate was applied to glyphosate resistant soybeans in the field, and in the greenhouse to glyphosate resistant cotton, Palmer amaranth, and soybeans to test induction effects on SBL survival, weight gain, and defoliation. Life table studies revealed non-induced Palmer amaranth could be an alternative host for SBL. However, consumption was half the leaf area and pupal weights were lower than larvae placed on soybean.

A glyphosate diet overlay bioassay revealed SBL neonates had lower weights after seven days than those that fed on control diet and SBL 3rd instars were not affected. On foliage from glyphosate treated host plants after 7 days, SBL third instar weights were similar across treatments. On cotton, SBL consumed more leaf area on glyphosate treated leaves than on non-treated leaves but on soybean, consumed less leaf area of glyphosate treated vs non-treated leaves.

Chapter 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Soybean Production and Soybean Pests

Soybeans have a very important role in human digestion and nutrition and for this reason they are one of the top five most widely grown commodity crops in the U.S. (USDA ERS 2016). In Louisiana, soybeans are the largest and most valuable field crop and, in 2016, soybean acreage reached 485,622 hectares with an average yield of 48.5bu/A with an estimated €489,213,204 in revenue (USDA-NASS 2016). Soybean oil is used in frying, baking, and as a main ingredient in many food products (United Soybean Board 2014). The U.S. food industry uses 5.4 million tons of soybean oil a year (United Soybean Board 2014). Consumers view soy-based food products as healthier because studies have shown that isoflavones contained in soy products are nutritionally beneficial (Higdon et al. 2017) and have been shown to help prevent breast cancer and treat osteoporosis (Messina 1999).

Soybeans grown in Gulf Coast states are attacked by a complex of insect pests (Way 1994). These pests are generally broken up into two types of feeding guilds; pod feeders and defoliators. Pod feeders include Southern green stink bug (*Nezara viridula* (L.)), redbanded stink bug (*Piezodorous guildinii* (Westwood)), brown stink bug (*Euschistus servus* (Say)), dusky stink bug (*Euschistus tristigmus* (Say)), and the green stink bug (*Chinavia hilaris* (Say)). Defoliators consist of the soybean looper (*Chrysodeixis includens* (Walker)), velvetbean caterpillar (*Anticarsia gemmatalis* (Hübner)), and green cloverworm (*Plathypena scabra* (Fabricus)). Of these defoliators, the most yield limiting is soybean looper (SBL). In 2013, 95% of the soybean acreage in Louisiana was treated for SBL and the yield loss plus cost of insecticide applications reached $26 million dollars (Musser et al. 2013). A single SBL can consume 114 cm^2 of foliage during its growth and development (Boldt et al. 1975) and 97% of that

consumption will occur in the last 3 stages of larval development (Reid and Greene 1973). Soybean plants can tolerate some injury from SBL (Fehr et al. 1971, Turnipseed and Kogan 1987). Before R3 growth stage, soybeans can withstand 35% defoliation without significant yield loss (Turnipseed and Kogan 1987). However, at the R5 to R6 stage, defoliation should not exceed 20%.

1.2 Soybean Looper Biology

SBL adults migrate annually from Florida, Central and South America, and the Caribbean Islands to the southeastern U.S. where they arrive and feed on a variety of species (Mitchell et al. 1975, Newsom et al. 1980). This pest can occur throughout the U.S. with reports of findings ranging from New York all the way to California. However, economic infestations in soybeans have rarely been reported north of the southeastern U.S. (Herzog 1980). Though SBL is an economic pest of soybeans, it is polyphagous and has been recorded feeding on a variety of agronomic hosts including beans, cabbage (*Brassica oleracea*), okra (*Abelmoschus esculentus*), sweet potatoes (*Ipomoea batatas*), tobacco (*Nicotiana tabacum*), and tomatoes (*Solanum lycopersicum*) (Bottimer 1926; Folsom 1936; Wolcott 1936). SBL undergoes four generations per year (Turnipseed and Kogan 1976) with highest populations occurring during July and August (Harding 1976). In Louisiana, SBL has been shown to be very successful on cotton and cowpea and can utilize these alternative host plants to build up populations (Moonga and Davis 2016). Moths need a carbohydrate source to maintain fecundity, fertility, and longevity. This has been thought to be one reason why large outbreaks of this pest may occur in soybean systems where cotton is present in the same agro-ecosystem, as cotton may be providing a more nutritious nectar source (Turnipseed and Kogan 1976). SBL have been found to have higher total progeny production, intrinsic rate of increase, and net reproductive rate in later instars on

cotton compared to soybean (Moonga and Davis 2016). This may be another reason there may be larger SBL outbreaks in soybean-cotton agro-ecosystems.

1.3 Alternative Soybean Looper Host Plants

Agronomic crops are not the only host for SBL. Weeds can also serve as alternative hosts including: morning-glory (*Ipomoea purpurea*), lambsquarter (*Chenopodium album*), dock (*Rumex spp.*), and Palmer amaranth (*Amaranthus palmeri*) (Harding 1976). Weeds are controlled through herbicide use and normally would not be present in large numbers in soybean fields. However, many weed species have become resistant to herbicides. A consultant's survey concluded that Palmer amaranth and morning-glory are the two most problematic weeds in soybeans in Louisiana and the Midsouth due to their fast growth rates, high seed production, and ability to successfully compete for resources (Riar et al. 2013).

Glyphosate is a non-selective broad-spectrum herbicide that inhibits the plant's ability to produce the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (NPIC OSU 2010). EPSPS is involved in the shikimic pathway and the biosynthesis of three aromatic amino acids; tyrosine, tryptophan, and phenylalanine (Maeda and Dudareva 2012). These amino acids are the forerunners for secondary metabolites that are involved in plant phenolic production and plant defense (Buchanan et al. 2000). Glyphosate resistant Palmer amaranth can overcome the inhibition of EPSPS by increasing its production of the enzyme EPSPS (Gaines et al. 2011). When glyphosate resistant weeds increase their production of EPSPS, they may increase production of secondary plant compounds which could cause insect pests that are more resistant to plant defenses to be more tolerant to insecticides. Insect metabolic resistance is often due to overproduction of detoxification enzymes that metabolize xenobiotics (Després et al. 2007). For example, *Spodoptera frugiperda* can feed on plant allelochemicals and be more tolerant to

insecticides (Yu and Ing 1984). The over production of EPSPS increases production of enzymes correlated to plant defense against herbivory. If insects can overcome these defenses through metabolic means it is possible insecticide efficacy will be compromised.

Palmer amaranth is an important weed in the Southeastern U.S. It has become increasingly important because it has become resistant to glyphosate (Stephenson 2010). Palmer amaranth can produce up to seven million seeds per plant, and as resistance is spread through pollen, a non-resistant glyphosate parent can produce a glyphosate-resistant offspring (Stephenson 2010). Glyphosate resistant Palmer amaranth was first confirmed in Georgia and Arkansas in 2005 (Culpepper et al. 2006; Norsworthy et al. 2007), North Carolina in 2006 (Culpepper et al. 2008), and Louisiana in 2010 (Stephenson 2010). Glyphosate resistance in Palmer amaranth is problematic for multiple reasons. First, it is a pest of soybean crops, competing for sunlight and resources, causing lower soybean yield (Culpepper et al. 2006). It also can harbor plant diseases during non-growing months, allowing for quick re-infestation of the disease when growing season begins (Culpepper et al. 2006). Finally, glyphosate resistant Palmer amaranth will persist in a soybean field throughout the entire growing season which can cause major damage to farm equipment during pesticide applications and harvest (Culpepper et al. 2008). Since Palmer amaranth can persistently occur as a volunteer during the non-growing season, what can it serve as a host?

A survey done by Harding (1976) showed that Palmer amaranth is a host for soybean looper. It showed that the insect could survive and reproduce on the plant as eggs, larvae, and pupae were found on the plant species. Palmer amaranth seemed to serve as a species maintenance host so that the loopers could build up their population (Harding 1976). However, there has been little to no research on how glyphosate applications may affect soybean looper

survival and reproduction on Palmer amaranth. Fortunately, there is some research on the family Noctuidae that shows great survival success on Palmer amaranth. In a study that took five different host plants (cabbage, cotton, bell pepper, pigweed, and sunflower) and tested survivorship of *Spodoptera frugiperda*, larval survival was highest on pigweed (Greenberg et al. 2001). Larvae that fed on pigweed had lower consumption and took longer to reach pupation. Pupal weights that fed on pigweed were the highest and pupation only took 12 days compared to the 14 to 16 days pupation took on the other host plants (Greenberg et al. 2001). Another study done by Sappington et al. (2001) tested *Spodoptera exigua* (beet armyworm) egg oviposition in canopies of cotton and pigweed host plants. This was an interest to these researchers because pigweed seems to be a preferred host over cotton for the beet armyworm (Howard 1907; Wene and Sheets 1965; Tingle et al. 1978). This study revealed that beet armyworm preferred laying eggs on the pigweed host; almost a 30% difference from cotton (Sappington et al. 2001). Less is known about the weed morning glory (*Convolvulaceae*).

1.4 SBL IPM

In order to control SBL, all tools and tactics must be taken into consideration and utilized through an integrated pest management (IPM) plan. Current strategies involve scouting and monitoring populations and applying chemical controls only when economic thresholds are reached in order to protect natural enemies (Kogan and Herzog 1980). Natural enemies of SBL include predators, parasites, and fungal pathogens. Some of the main predators that prey on SBL include three species of *Nabis*, *Chrysoperla plorabunda* (Fitch), two species of *Geocoris*, *Calleida decora* (F), *Stiretrus anchorago* (F), and *Arilus cristatus* (L.) (Richman et al. 1980). The main parasites that utilize SBL are in the families Tachinidae, Braconidae, Chalcididae, and Ichneumonidae (Harding 1976). The fungal pathogens that are most documented on SBL are

Mesochorus spp., Spicaria rileyi (Farlow), and *Massospora spp.* (Burleigh 1972). There is one soybean looper virus that is found in the southeastern U.S. in the family Baculoviridae, a single-nuclear capsid type nuclear polyhedrosis virus (Ali and Young, 1991). This virus was introduced to SBL in the U.S. in the late seventies as a control tactic (Kogan and Herzog 1980). The virus was successful in bringing down populations at first but mortality never exceeded 50%. The virus kills younger instars at a much higher rate; however, when the virus was first deployed it was applied to the top of soybean leaves (Ali and Young, 1991). Small SBL instars typically feed on the underside of soybean leaves and older SBL instars feed on the top of the leaf, meaning the target instar stage was not directly consuming the virus (Ali and Young, 1991). Today the virus occurs in the field as another natural enemy to SBL (Fuxa et al. 1992).

SBL chemical treatments are applied when there are 150 larvae per 100 sweeps (LSU AgCenter 2015) or 24 loopers per row meter (Heatherley 2014) or when defoliation has reached 30% defoliation prior to R2 or 20 at R3 to R6 (LSU AgCenter 2012). Unfortunately, SBL has developed resistance to almost all classes of insecticides including carbamates, cyclodienes, organophosphates, DDT, and pyrethroids (Boethel et al. 1992). Louisiana soybean producers are now facing the problem of SBL being resistant to methoxyfenozide. Resistance ratios to methoxyfenozide have been documented at 24 times the susceptible control (Brown 2012).

1.5 Constitutive Host Plant Resistance

Host plant resistance (HPR) is one method that can be used in IPM programs. There are many definitions of HPR in the literature. In 1951, it was defined by Painter as “the relative amount of heritable qualities possessed by the plant which influence the ultimate degree of damage done by the insect in the field” (Painter 1951). The definition has evolved and now includes the impact herbivore activity has on the plant and the natural enemies of the herbivore

(Strauss and Agrawal, 1999). Constitutive HPR are plant related resistance traits that are always active in the plant with or without herbivore attack. Examples of constitutive defenses include morphological and biochemical expressions such as trichomes (Mauricio, 2005), thick epicuticular waxes (Jenks et al; 2002), allocating resources to tissues that are inaccessible to the herbivores (Tao and Hunter, 2012), and secondary compounds (Osbourn et al; 2003). Soybeans naturally possess a trypsin inhibitor that has been shown to stunt SBL growth when ingested (McManus and Burgess 1995).

Past research has focused on breeding insect resistant soybean cultivars. Beach and Todd (1988) showed resistance to SBL and velvetbean caterpillar, using insect-resistant bred soybean lines (PI 229358 and GatIR 81-296). These two soybean lines reduced larval growth rates in VBC and reduced pupal weights in SBL (Beach and Todd 1988). Smith showed that the soybean variety PI 227687 was very resistant to sixteen different lepidopteran pests including SBL. The main effect this variety had on SBL was on the final instar where effects included reduced weight gain, a decrease in growth rates, and an increase in mortality (Smith 1985). However, although breeding efforts have produced resistant varieties, none have found grower acceptance due to poor yields and later maturity.

Host plants' constitutive defenses can drastically affect insecticide efficacy. When HPR is incompatible with insecticides, some defensive plant compounds will reduce the efficacy of insecticides. Brattsten et al. (1988) found that 2-tridecanone is toxic against corn earworm, but its presence reduces the effectiveness of carbaryl because when corn earworm defends against 2-tridecanone, it increases its detoxification enzymes which assists in overcoming carbaryl. Some allelochemicals have been found to interfere with an insects' resistance to insecticides. When fall armyworm (*Spodoptera frugiperda*) feeds on maize it becomes more tolerant to insecticides,

maize secondary chemical defense has been connected to insecticide tolerance (Yu and Ing, 1984). Also, when fall armyworm fed on cowpea, they were twice as tolerant to organophosphorous insecticides than those that fed on soybean (Yu and Ing, 1984). Furthermore, when corn earworm was exposed to the allelochemical xanthotoxin, their offspring was more tolerant to the pyrethroid α-cypermethrin (Li 2000). Certain plant toxins can also have an effect on insecticide efficacy, fall armyworm that fed on corn were much less susceptible to insecticides compared to larvae that fed on soybeans (Yu and Ing 1984).

1.6 Induced Host Plant Resistance

Induced HPR is defined as defenses that are produced when an herbivore attacks the plant (Kant et al. 2015). These defenses are induced in response to herbivore injury and decrease the negative fitness impacts on the plant (Karban and Myers 1989) such as reallocation of resources, increasing growth rates, and decreasing photosynthesis production which all can cause yield loss. When herbivory occurs, plants can respond by producing tannins and phenols which are toxic to insects and are products of the shikimic acid pathway in plants (War et al; 2012). When production of these enzymes increases, the concentration of secondary metabolites also increases (Karban and Meyers 1989).

Secondary metabolites are not necessary for basic plant functions and are low in concentration in the plant (Balandrin et al; 1985). However, they are responsible for the mediation between plant interactions and the environment including interactions with herbivores. These interactions are defined as allomonal functions. An allomone is an allelochemical that is advantageous to the producing organism and not the receiving organism. For example, soybeans produce a chemical called sapogenin that deters oviposition by *Callosobruchus chinensis* (Ignacimuthu and Jayraj, 2005). The opposite is a kairomone which is advantageous to the

receiving organism (Finch 1978). For example, soybeans produce a chemical called 3-octanone which attracts the SBL parasitoid *Microplitis demolitor*. After feeding on soybean, SBL deposits frass containing 3-octanone back onto the leaf surface, alerting *M. demolitor* to the presence of SBL (Ramachandran et al; 1991).

While secondary chemicals can be induced naturally by insect feeding, there are exogenous elicitors that have been documented in over 100 plant species to temporarily activate plant resistance by inducing secondary metabolites (Stout et al, 2002). One of these is jasmonic acid (JA). JA is a phytohormone that mediates plant responses to wounds and when it is applied exogenously to plants, it elicits a response that is similar to responses that are induced by insect herbivory (Stout et al. 2002).

1.7 HPR in IPM

Over the years, scientists have studied HPR extensively in more than one scientific discipline. There are three major ways in which HPR is studied throughout the science disciplines. The first approach is the ecological/evolutionary approach. This approach focuses on insect-plant interactions (IPI). IPI strives to show the ecological and evolutionary relationship between plants and insects while answering what is responsible for the vast diversity in plants and herbivorous insects and what factors are regulating insect populations. The two main ideas in this approach are that secondary metabolites in plants have evolved primarily to provide defense against insect herbivores and that there is a co-evolutionary relationship between plants and insects; they have evolved in response to one another.

The second approach to studying HPR is the plant biology approach. Over the past 25 years, the focus of this approach has been phenotypic plasticity, the ability of one genotype to produce more than one phenotype when exposed to different environments. The main question is

what is the molecular basis of the plant insect interactions? The third approach to studying HPR is the applied agricultural approach. This approach focuses on intraspecific variation in resistance in crops and the differences in resistance across crop varieties (Stout 2013). Host plant resistance (HPR) in this approach is compatible with other IPM tactics, being cheap and easy for producers to apply (Stout and Davis 2009).

HPR can be incorporated into an IPM program by selecting a resistant variety or cultivar to an arthropod pest. For example, certain wheat (*Triticum aestivum* (L.)) varieties are resistant to the Hessian fly (*Mayetiola destructor*) by causing cell death and solidification of the cell wall around the nutritious tissue that the fly feeds on (Harris et al. 2003). Some sugarcane species (*Saccharum* spp.) are resistant to the sugarcane borer (*Diatraea saccharalis* F.)) due to epidermal silicon that prevents the borer from feeding (Posey et al. 2006). There are some soybean species (*Glycine max* (L.)) that are more resistant to defoliating Lepidopterans (Zhu et al. 2008) by producing toxic flavonoids that if ingested can cause mortality in larvae (Piubelli et al; 2005). While plants have the ability to defend on their own, this is not always enough protection from a large insect attack, so plants may deploy alternative defense methods for increased protection.

In recent years, many studies have shown that herbivore cues can trigger an increase in production of metabolites (Korpita et al. 2014). There are many examples of HPR being used in field applications. Extensive work has been done in tomatoes, looking at volatile emissions from the plant and cues from the herbivore. A study by Korpita et al. (2014) looked at the cues from a specialist herbivore that increased tolerance to defoliation in tomato. A study showed that when *Manduca sexta* regurgitant was applied to a tomato plant it caused a decrease in plant vertical growth, increased stem growth, and the plant recovered more quickly from defoliation than undamaged plants (Korpita et al. 2014). During an attack, overall host plant quality may

decrease due to the plants response. An attack signals the plant to begin reallocating resources to areas free from attack, which moves nutritional chemicals away and toxic chemicals to feeding sites. One study gave strong evidence that the quality of the host plant is very determinant of how reproductively successful an insect will be as well as insect oviposition choice (Awmack et al. 2002). This is a good example of how resistance can be a non-preferential trait.

Natural enemies and quality of the host plant regulate herbivorous insect populations. These two regulations are termed "top-down" and "bottom-up" factors (Stout et al. 2002). Bottom-up factors can be triggered through herbivory; the plant releases a toxic secondary metabolite that will deter herbivores from feeding on the plant or signal predators that prey is present (Stout et al. 2002). Top-down factors are when the predators and prey are signaled or alerted to the herbivores presence (Beck 1965). The understanding of the signaling pathways in plants has led to synthetic chemicals called elicitors, which can induce chemical responses in plants that occur naturally in response to herbivory (Karban and Kuc´, 1999). Jasmonic acid (JA) is one of these elicitors that can cause a plant to express toxic chemicals at a higher rate (Després et al; 2007). JA has been found to be a signaling agent in the transduction pathways when wounding occurs either mechanically or by an herbivore (Staswick and Lehmann 1999). When JA is applied exogenously, it can elicit a response in the plant that is similar to an herbivory response (Stout et al. 2002). For example, exogenous JA has been shown to increase meristem thickness in potatoes, which imitates resource allocation due to an herbivore attack (Cenzano et al. 2003). In Arabidopsis, applications of exogenous JA caused the plants to produce more trichomes on the leaves (Traw and Bergelson 2003). HPR is important to understand from an IPM perspective in order to design a compatible management program among all forms of control. One control tactic that has been working for control of a pest for many years may be

rendered ineffective if a new control tactic is introduced suddenly without any prior research. This can become a problem when a new insecticide is introduced into the market. HPR can be compatible or incompatible with insecticides. When HPR is compatible with insecticides it can slow insect development and reproduction and repel the insect, making them move off the plant, which leads to less insecticide applications (Panda and Khush, 1995). When HPR is incompatible with insecticides, the defensive plant compounds can reduce insecticide efficacy. For example, coumestrol in soybeans has been found to increase the efficacy of acephate and fenvalerate to SBL but also reduce the toxicity of methomyl to SBL (Rose et al. 1988). A study involving fall armyworm showed that many plant phenolic compounds have been shown to inhibit glutathione S-transferases (GST) in larvae. GST is a detoxification enzyme that is present in many insects, and is used for detoxifying harmful plant chemicals. This study found that when this enzyme was inhibited in fall armyworm, insecticide efficacy increased (Yu and Abo-Elghar 2000).

1.8 Objectives

The objectives of this thesis were first to determine if JA can induce resistance to SBL in a variety of agronomic hosts, answering the question, can exogenous induction of HPR be used as an IPM control tactic? Second, to determine whether exogenous HPR is compatible with insecticide applications, answering the question, can more than one IPM control tactic for SBL be deployed simultaneously and still be effective at control? Thirdly, to determine if glyphosate can induce HPR to SBL and if glyphosate-resistant Palmer amaranth plays a part in SBL successfully completing a life cycle. By understanding these interactions better, we hope to improve soybean looper IPM.

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CHAPTER 2
THE EFFECTS OF JASMONIC ACID INDUCED HOST PLANT RESISTANCE ON *CHRYSODEIXIS INCLUDENS* BIOLOGY AND INSECTICIDE EFFICACY

2.1 Introduction

Soybeans are the largest commodity crop in Louisiana, reaching 1.2 million acres in 2016, and soybean looper (SBL) is a major defoliator of soybeans in this state. Currently, chemical control and natural enemies are the only control methods for SBL. Chemical control of SBL can be challenging due to their resistance to almost all classes of insecticides including carbamates, cyclodienes, organophosphates, DDT, and pyrethroids (Boethel et al. 1992). Louisiana soybean producers are now facing the problem of SBL being resistant to methoxyfenozide. Resistance ratios to methoxyfenozide have been documented at 24 times the susceptible control (Murray and Davis 2014), so all tools and tactics for control must be taken into consideration to create an integrated pest management (IPM) plan. Current strategies involve scouting and monitoring populations and applying chemical controls only when economic thresholds are reached (Kogan and Herzog 1980). The lack of control options for SBL is an increasing problem as insecticide resistance in SBL continues to build. Different control methods are needed to improve SBL control and reduce insecticide use. Induced host plant resistance is one alternative control method that could be utilized in soybean IPM.

The term host plant resistance (HPR) is generally used when describing an IPM tactic in which the variation in plant resistance is taken advantage of to achieve pest management (Stout and Davis 2009). HPR is defined in the literature as the sum of all genetically inherited traits that reduce the impact of potential herbivory by changing an aspect of the plant-insect interaction. Plants have many different ways to defend themselves from herbivory and are either constitutive

(basal) defenses or induced defenses. Constitutive defenses are always expressed in the plant, where induced defenses are facultatively expressed (Karban and Baldwin 1997). Some constitutive defenses include leaf trichomes that deter insects from feeding, allocating resources to sites that the herbivore cannot access, and altering plant architecture to make feeding or oviposition more difficult (Kant et al. 2015). These resistance mechanisms are not always expressed at the highest level and are sometimes induced to optimal levels by various stimuli, including herbivory (Stout et al. 2002). Plant defenses can often be induced from insect herbivory and are often favored by natural selection when herbivory is not uniform as constitutive defenses can be costly relative to the benefits (Åström and Lundberg 1994).

Induced responses are plant responses that cause a decrease in choice or reproductive success of an herbivore that feeds on an injured plant (Agrawal and Karban 1999). Plant resistance may reduce insect attack with morphological (Levin 1973) or chemical (Fraenkel 1959) traits. Chemical traits are often expressed through secondary metabolites; chemicals that the plant produces but do not have a vital role in any primary functions in the plant (Seigler 1998). Secondary chemicals can be classified as kairomones and allomones. Kairomones are defined as chemicals that give an adaptive advantage to the receiving organism such as an herbivore searching for hosts. The codling moth (*Cydia pomonella*) uses multiple host kairomones to find hosts including kairomones produced by apple and pear fruit (Landolt and Guédot 2008). An allomone is defined as a chemical that gives an adaptive advantage not to the receiving organism but to the producing organism like a chemical that a plant produces to repel an herbivore. Secondary chemical production increases when a plant is attacked by an herbivore or pathogen (Schultz 2017). Research has begun to look at how to induce these plant defenses

before herbivory occurs, mostly with exogenous elicitors. In the past, there has been little research on induced resistance to insect herbivory in large acreage crops.

The discovery of chemical elicitors of induced responses in recent years has led to the questions of can and how to use induced resistance in crop protection. Applying elicitors of induced responses could activate plants direct and indirect defenses which in turn may heighten biological control of the herbivore and host plant resistance (Stout et al. 2002). Jasmonic acid (JA) as an elicitor that has been widely studied for its potential to be utilized in induced HPR. JA is an organic compound that is found naturally in higher plants (Creelman and Mullet 1995). It is in the jasmonate class of plant hormones and is biosynthesized from linolenic acid via the octadecanoid pathway (Lyons et al. 2013). Jasmonates play a role in regulating universal changes to gene expression after mechanical or insect wounding. Jasmonates also help regulate tritrophic interactions, aid in host plant resistance to phloem feeders, pathogen resistance, and regulating the release of defense signals (Howe and Jander 2008). When an insect attacks a plant, endogenous JA increases and regulates the expression of many different genes, some which are found to increase resistance to arthropods (Stout et al. 2002) by increasing proteinase inhibitors, polyphenol oxidases, lipoxygenases, volatile organic compounds, and nicotine (Staswick and Lehmann 1999). JA increases nicotine content when plants are attacked (Baldwin 1998), and nicotine is very toxic to herbivores because it interacts with the acetylcholine receptors in the nervous system of animals (Steppuhn et al., 2004). Proteinase inhibitors make up one of the most diverse classes of proteins in plants and interfere with insects' digestive processes (Ussuf et al., 2001). Plant volatiles can attract predators and parasites to the herbivores. Volatiles may also induce defense reactions in adjacent plants (Paré and Tumlinson, 1999).

When JA is applied to the plant exogenously, it has been shown to induce direct resistance to herbivores in cabbage, tomato, tobacco, and cotton (Stout et al. 2002

Although plants are good at self-defense, this is not enough to protect against large pest outbreaks. For this reason, insecticides are widely used to control pests. One class of insecticides, insect growth regulators (IGR), are widely used in crop pest management because they are much more selective and are less likely to harm other organisms including natural enemies (Krysan and Dunley 1993). IGR discovery was based on the observation of how insects grow and develop. Since IGRs interact with insects' unique physiology, they are more selective than broad spectrum insecticides (Krysan and Dunley 1993). IGR's mimic hormones in immature insects, disrupting the insects' growth patterns and subsequently their reproduction success rates (NPIC 2015). They prevent egg-hatch and molting but are rarely fatal to mature insects (NPIC 2015). There are three types of IGR's: chitin synthesis inhibitors, juvenile hormone analogs and mimics, and anti-juvenile hormone agents (Krysan and Dunley 1993). Methoxyfenozide is a juvenile hormone mimic and has been widely used in the past to control lepidopteran pests (Cantoni et al; 2004). When larvae ingest the chemical, it binds to the ecdysone receptors and accelerates the molting process (Wing et al. 1988), disrupting the hormonal balance (Palli et al. 1996). The larvae develop dark colored bands between their body segments and become inactive (CDPR 2003). Methoxyfenozide is a diacylhydrazine, which is highly selective to lepidopterans making it a good fit for IPM, as it is non-harmful to non-target species (Smagghe and Degheele 1998). What is less understood is whether or not exogenously induced HPR and insecticides are compatible or incompatible in an IPM program.

HPR has been used in crop management in some form since 1870. Resistant grape-rootstocks were used against the pest *Phylloxera* sp., which resulted in widespread use across

France. This resistance lasted for more than 100 years (Wiseman 1994). HPR and insecticide interactions have had varying results in the past, being compatible or incompatible. An example of HPR that is compatible with insecticides is the sweet corn hybrid variety 471-U6 X 81-1. This hybrid is tolerant to corn earworm damage up to the larvae completing its lifecycle without causing significant damage to the corn ear (Wiseman et al. 1973). This leads to less pesticide applications (Wiseman et al. 1972). Another example of HPR that is compatible with insecticides is sorghum and the sorghum midge (*Contarinia sorghicola*). Sorghum has a contact oviposition deterrent in its spikelet that causes females to probe longer when ovipositing, reducing fecundity (Teetes 1985). This resistance-related trait leads to less insecticide applications (Teetes 1984). HPR can in some cases be incompatible with insecticides as it was with sorghum hybrids that were resistant to the greenbug (*Schizaphis graminum*). This hybrid gave way to low insecticide dosages (Cate et al. 1973), however this caused the resurgence of the greenbug on the resistant variety (Reissig et al. 1982).

There are some examples of how HPR and insecticides can fit into an IPM program; however as stated before there are few examples of how exogenously induced HPR and insecticides fit together into an IPM program.). Direct resistance is usually shown through a decrease in herbivore fecundity, growth, preference, and survivorship as well as some pathogen resistance (Stout et al. 2002; Cohen et al. 1993). The numerous benefits that exogenous JA brings to the host plant are reasons to continue looking into this tool as part of an IPM strategy. IPM is only successful if control methods will work together to knock down pest populations. A search of the literature reveals little to no research on how exogenously inducing a plant defense will affect insecticide efficacy on an insect. This research was done to better understand the induced response of a host plant through an elicitor and the effect it has on SBL and insecticide

efficacy. The intent of this study was to determine whether applying an exogenous elicitor to a host plant could increase or decrease HPR to SBL and increase or decrease efficacy of insecticides against SBL.

2.2 Materials and Methods

2.2.1 SBL colony and management

C. includens used in this study (MR08) were obtained from the Soybean Entomology Laboratory (Louisiana State University Agricultural Center, Baton Rouge). This colony was established in 2008 from larvae that were collected at the Macon Ridge Research Station in Winnsboro, LA (Brown 2012). This colony is maintained using the following protocol. Larvae were placed in 30 ml plastic diet cups (2 larvae/cup) with 10 ml Southland Product's artificial meridic soybean looper diet (Southland Products, Lake Village, AR) until pupation. The cups were placed in a rearing room which is maintained at 22°C with 55% RH and a 14:10 (L:D) photoperiod (Mascarenhas and Boethel 2000). Once pupation occurred, SBL were transferred to a large plastic cylinder container (32 cm X 62 cm) with 30 g of vermiculite (Sun Gro, Bellevue, WA) and upon eclosion were free to mate and oviposit on paper sheets. Paper sheets (8 x 23 cm) were placed on the inside of the container and the container was sealed with a plastic and muslin cover (Jensen et al. 1974). The adults were fed a 10% honey water solution in cotton wadding. Egg sheets and honey water were changed every two days and egg sheets were placed in a plastic bag (15cm x 7cm-1cm x 38cm plain clear non-vent) until eclosion. When the neonates emerged two to three were placed into each diet cup

2.2.2 JA Diet Overlay

In order to test the effects of JA alone on SBL, diet overlay experiments were conducted which included an untreated check, EtOH, and JA + EtOH. To prepare the JA solution, 0.042mg

of JA was dissolved in 1 ml EtOH and then added to 100 ml of distilled water to get a 2 mM JA solution. For EtOH solution, 1 ml of EtOH was added to 100 ml of distilled water. For untreated check, unmanipulated SBL diet was used. In order to get full surface coverage, 500 μl of stock solution (JA solution; EtOH solution) was placed onto the top of the diet in each cup, swirled to ensure even distribution, and allowed to dry overnight. SBL third instar larvae that previously fed on a meridic soybean looper diet from Southland Products, Inc. (Lake Village, AR, USA) were weighed (to ensure third instar status) and then randomly placed onto one of the three treatments (untreated check, EtOH, and JA + EtOH). There were four simultaneous replications, 50 insects per treatment per replicate. Insects were monitored daily for mortality and stadia (time spent in each instar) over seven days. After seven days, surviving insects were weighed.

2.2.3 JA Field Application and Bioassay

Soybean (*Glycine max* L.) var. Asgrow 5332 (Monsanto Company, St. Louis, MO), cowpea (*Vigna unguiculata* L.) var. California Buckeye (Seed Savers Exchange, Decorah, IA), cotton (*Gossypium hirsutum* L.) var. DP174RF (Monsanto Company, St. Louis, MO), common bean (*Phaseolus* vulgaris) var. Tiger Eye (Seed Savers Exchange, Decorah, IA), sweet potato (*Ipomoea batatas* L.) var. Beauregard (LSU AgCenter, Baton Rouge, LA), and okra (*Abelomoschus escuelentus* L.) var. Silver Queen (Seed Savers Exchange, Decorah, IA) were grown according to production standards at the LSU AgCenter Burden Research Station in Baton Rouge, LA. Seeds were hand planted in 6 m rows with 1.5 m alleys. Each host plant was arranged in a split plot design, with 4 replications.

Previous research has shown that a 2-mM solution of JA will induce changes in cotton and decrease growth in *S. frugiperda* larvae without causing phytotoxicity to the plant (Mézáros et al. 2011). This concentration was used for all host plants. To prepare the JA application, 42

mg JA (Sigma-Aldrich, St. Louis, MO, USA) was weighed (OHAUS precision standard electronic balance) in a 20 ml Pyrex beaker. A pipet was used to add 1 ml EtOH to dissolve the JA and then mixed with 100 ml of water to get a 2-mM JA solution. Plants were sprayed four weeks after emergence. Each host plant had a JA treatment group (1.5 m long row) and a control treatment group (1.5 m long row) replicated four times. The JA treatment group was sprayed with a portable Preval sprayer (Power unit 97.6 ml Jar/cap 168.6 ml; Preval, CA Acquisition, LLC., Coal City, IL, USA) until runoff. The control treatment group was sprayed with the same volume of control solution (1 ml EtOH in 100 ml water) until runoff. The control plants had a 1 to 1.5 m buffer zone from the JA plants to avoid contamination. Leaves were collected 72 hr after application. JA content is highest in new growth so leaf tissue was taken from the top part of the plant where new growth was occurring (Creelman and Mullet, 1995). Fifty leaves were collected from both the control and JA treatments (50/JA group; 50/control group) per host plant. The collected leaves were cored with a No. 149 Arch Punch (Osborne and Co., Harrison, NJ), which created an 11.34 cm^2 leaf core. Cores were placed onto sterile petri dishes (VWR polystyrene disposable, 100X15 mm) on moistened Whatman 90mm (#1) filter paper. A single soybean looper neonate that had been feeding on artificial diet (Southland Products, Lake Village, AR) for 24 hr to decrease handling mortality was placed onto the leaf core with a fine camel hair paintbrush. The filter paper was moistened with distilled water to prevent the leaf from drying. Leaf tissue was changed as leaf integrity deteriorated and the filter paper was wetted every day. There were 50 neonates per treatment per replication for a total of 150 neonates for each host plant replicated four times. The plates containing the insects were placed in a growth chamber (Percival Intellus Environmental Controller, Model No. I-36VL, Percival Scientific, Perry, IA) at 25°C with 75% RH and a 14:10 (L:D) photoperiod. Insects were fed leaf

tissue for seven days and then were weighed. Insects were monitored daily for mortality and area eaten (cm^2) was recorded when leaf tissue was changed. To estimate leaf area eaten by each larva, defoliation percentage of each leaf core was visually approximated based on the defoliation estimates for foliage feeding pests (Baldwin et al. 2011; Fig. 2.1). and total area of the leaf core (11.32 cm^2).

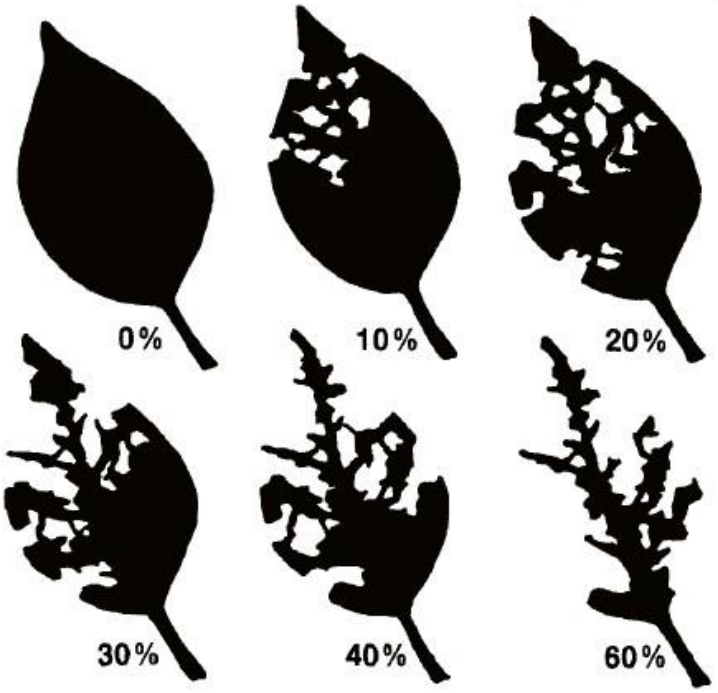

Figure 2.1 Defoliation estimates for foliage feeding pests

2.2.4 Diet incorporated Insecticide Assay

In order to understand the effects of JA induction on insecticide efficacy, a diet incorporated insecticide assay was conducted using soybean looper previously fed JA induced leaf tissue. After insects fed on leaf cores for seven days, they were transferred to artificial diet and artificial diet incorporated with insecticide. A 20% mortality was anticipated so, insects were divided among treatments into numbers of 20 for a total of 40 from the control group and 40 for

the JA treated group. Twenty insects were placed on control diet and 20 insects were placed on diet incorporated with insecticide. There were four different test groups. There was a control group (diet without insecticide) for insects that fed on JA induced leaf tissue and a control group for insects that fed on control treated leaf tissue. There was an insecticide treated group for insects that fed on JA induced leaf tissue and insects that fed on control treated leaf tissue. Diet was prepared as described previously in section 2.2.1. To incorporate methoxyfenozide (Intrepid 2F, Dow AgroSciences, Indianapolis, IN, USA) into SBL diet, diet was blended (Conair Corp., Stamford, CT, Waring commercial Model No. CB15) and separated into Pyrex glass beakers, 300 ml per beaker, to allow the diet to cool prior the addition of insecticide.

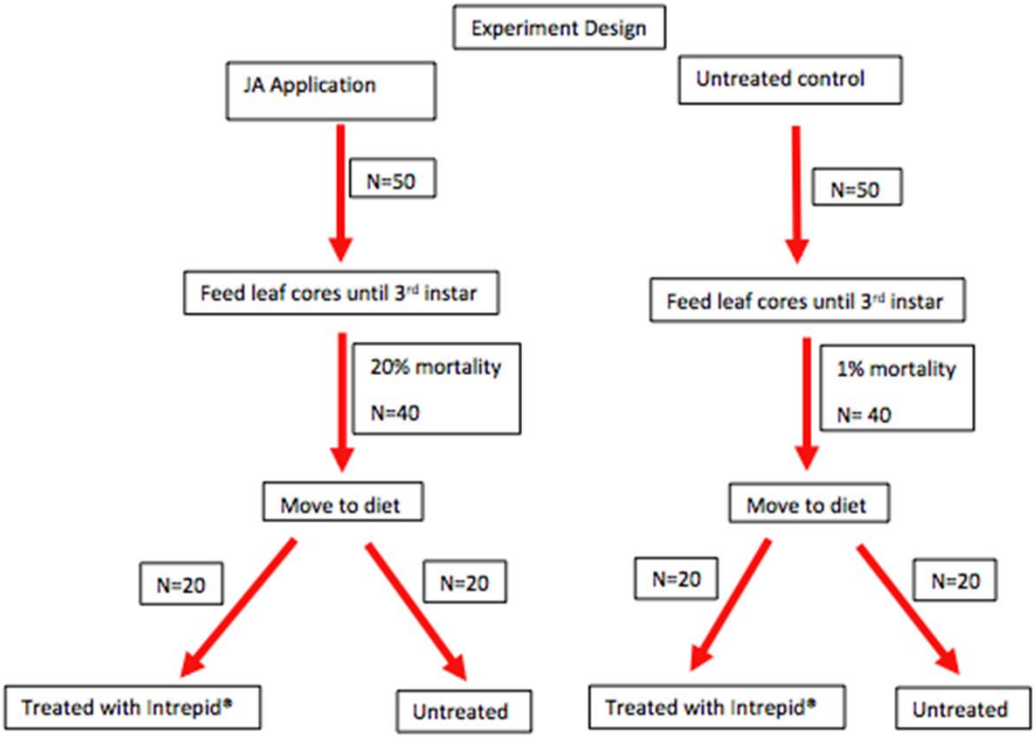

Figure 2.2 Illustrates experimental design for JA induction

To obtain a 0.05 ppm final diet mixture, a stock solution of 100 ppm was made with 110.6 μl of formulated methoxyfenozide in 250 ml of water. Once the diet cooled, 150 ml (0.05

ppm) of methoxyfenozide stock solution was added to 300 ml of diet and mixed. Diet was dispensed into diet cups at 10 ml per 30 ml cup (Dart Solo) for each repetition (20 per treatment) using a ketchup dispenser. The diet cooled for two hours and then third instar SBL were placed into each diet cup, one per cup. Figure 2.2 illustrates the experimental design. Insects were monitored daily for mortality, pupal formation, and adult emergence. Pupae were weighed. If emergence occurred, it was observed and recorded and total accrued days to reach adult from third instar were determined.

2.2.5 Data analysis

Statistical analyses were performed using JMP software (SAS Institute Inc. Cary, NC, USA 2016, v. 13). In the first experiment conducted, a two-factor analysis of variance (ANOVA) using Least Squares Fit (LSF) was used to analyze the effects of JA treated diet on SBL growth and survivorship, SBL instar stage, days to instar stage, mortality, third instar initial weight, and larvae weight after seven days were recorded. Relative growth rate was calculated using the formula**:** $RGR= (\ln W_2 - \ln W_1)/ (t_2 - t_1)$, where: ln is natural logarithm, t_1 is time one (in days), t_2 is time two (in days), W_1 is average weight at time one, and W_2 is average weight at time two (Philipson et al., 2012). Means of third instar initial weights and weights after seven days were separated by a Student's t-test at $\alpha=0.05$ level.

In the second study, a two-factor analysis of variance (ANOVA) using LSF was used to determine the effects of JA applied to various host plants on SBL growth and survivorship. SBL instar stage, days to each instar stage, leaf tissue area eaten (cm^2), third instar weights (g), and mortality were recorded. Area eaten (cm^2), third instar weights (g), and mortality data were all analyzed by two factor analysis of variance (ANOVA) using LSF to determine differences

among treatments by host as the main factors. Means were separated using Student's t-test at α=0.05 level.

In the third study conducted a two-factor analysis of variance (ANOVA) using LSF was used to determine if JA treatment to various hosts affects the efficacy of methoxyfenozide incorporated diet to SBL instar stage, days to each instar stage, mortality, pupal weights (g), and days to emergence or death were recorded. Mortality observed on methoxyfenozide diet was corrected by replication for natural mortality using mortality observed in the control treatments based on Schneider-Orelli's formula (Püntener 1981):

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treated plot - Mortality \% in control plot}}{\text{100 - Mortality \% in control plot}} \right) * 100$$

Pupal weights, days to emergence, and transformed mortality data were analyzed by one-way analysis of variance (ANOVA) using LSF to determine differences among treatments by host. Means were separated using Student's t-test at α=0.05 level.

2.3 RESULTS

2.3.1 JA Diet Overlay

The application of JA and EtOH to SBL larval diet had no effect on larval growth rate and weight gain while feeding on diet. Initial larval weights and their weights after feeding 7 days on surface treated diets were similar across treatments (P = 0.509 and P = 0.073, respectively). Additionally, the percent relative growth rates for each treatment were not significantly different (Control- 29%, EtOH- 28%, and JA- 30%, P = 0.102).

2.3.2 Induced HPR Bioassay

Larvae placed on plant tissue and treated with JA did have a significant effect on third instar weights. The two factor ANOVA revealed significant effects of each (Host and

Treatment; $P < 0.001$ and $P < 0.001$) and the interaction of Host by Treatment ($P < 0.001$). Larval weights (F = 2.91, df = 4, n = 200 P = 0.343; Fig 2.2.) were significantly lower on JA treated hosts compared to non-treated hosts except for cowpea. The JA induced foliage reduced third instar weights after feeding seven days on soybean (F = 2.15; df = 1, $P = 0.009$), okra (F = 6.95; df = 1, $P = < 0.001$), cotton (F = 19.38; df = 1, $P = < 0.001$), and sweet potato (F = 3.07; df = 1, $P = 0.005$; Fig 2.3). Larval weights on control treated plants were greatest on okra (0.022 g ± 0.003), followed by soybean (0.017 g ± 0.001), and the lowest mean weight occurred on sweet potato (0.010 g ± 0.001).

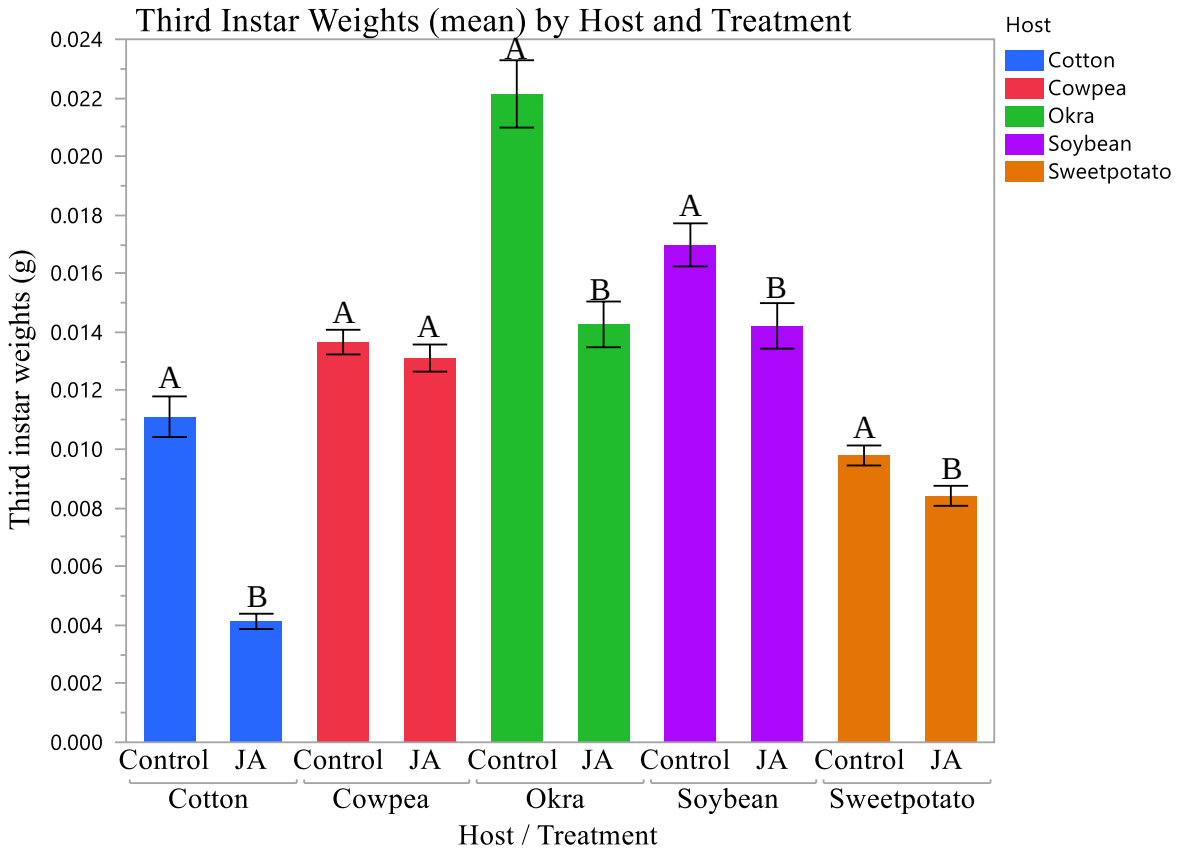

Figure 2.3 The weight (mean (g)) of 3rd instar SBL after feeding seven days on JA induced or control host plant tissue. The standard error is represented by the error bar, and the different letters above each bar within host denote significant differences (Student's t test, α=0.05).

The greatest JA treatment effect on reducing larval weights was on okra and cotton (36% and 64% reduction, respectively), while on cowpea, a small but non-significant reduction occurred (1%).

Larval mortality from neonate to 3rd instar was also significantly affected by host and treatment. The two factor ANOVA revealed significant effects of each factor (Host and Treatment; $P < 0.001$ and $P = 0.031$) and the interaction of Host by Treatment ($P < 0.001$) on larval mortality.

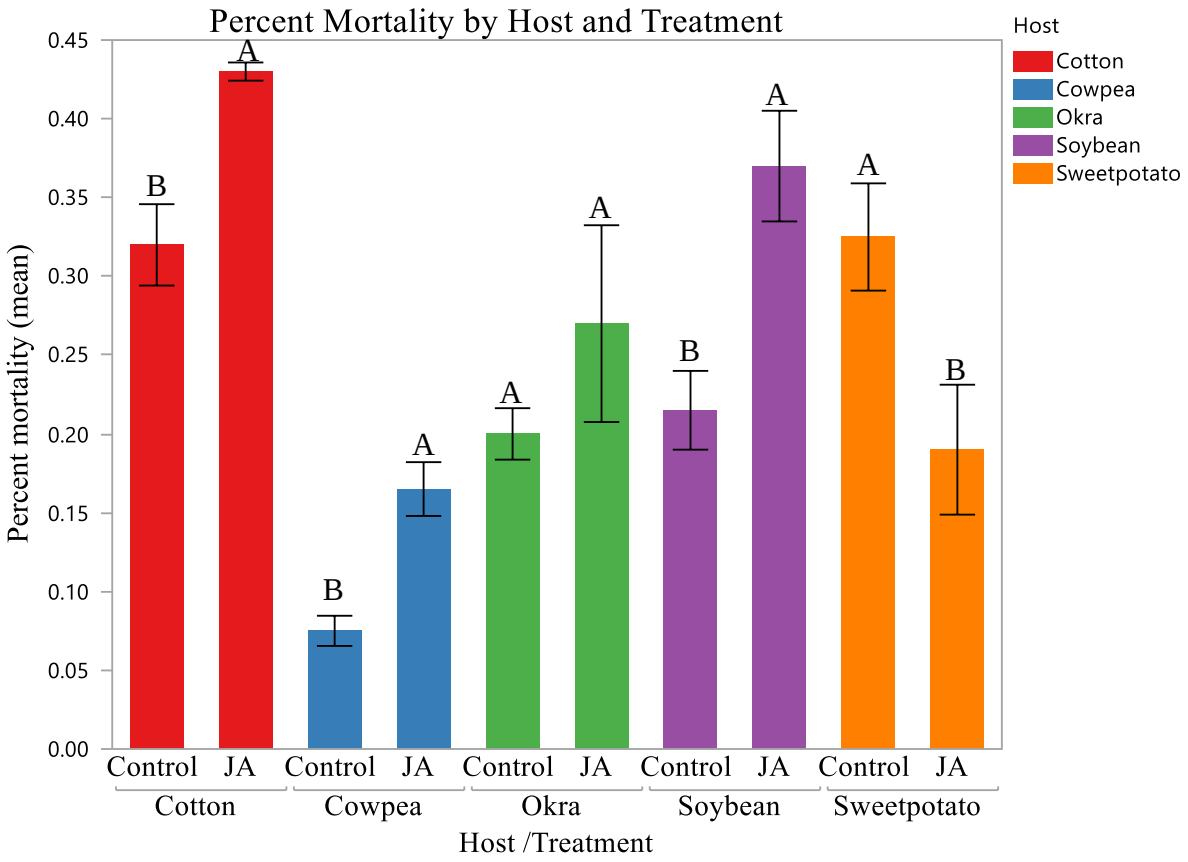

Figure 2.4 The percent mortality (mean) of SBL from neonate to 3rd instar after feeding for seven days by treatment and host plant. The standard error is represented by the error bar, and the different letters above each bar within host denote significant differences (Student's t test, α=0.05).

Higher mortality occurred on JA treated plants on all but sweet potato and on this host, larvae that fed on JA treated plants had significantly lower mortality than those on control plants ($F = 7.61$, $df = 2$, $P = 0.023$; Fig 2.4). Larval mortality on control plants was lowest on cowpea (8%) and highest on cotton (32%). The greatest increases in mortality on the 5 hosts treated with JA over the control treated plants was on soybean, cotton and cowpea (42%, 26% and 54% increase, respectively) to slight increases on okra (25%; Fig. 2.4).

Larvae placed on various plants and treated with JA did have a significant effect on area eaten over seven days by SBL. The two factor ANOVA revealed significant effects of each factor (Host and Treatment; $P < 0.001$ and $P < 0.001$) and the interaction of Host by Treatment ($P < 0.001$). On sweet potato, SBL larvae consumed approximately 10% less foliage from control plants ($F = 3.63$, $df = 1$, $P = 0.030$; Fig 2.5) than from JA treated sweet potato leaves. On the other hosts treated with JA, consumption after 7 days was significantly lower than on control plants; soybean ($F = 2.28$; $df = 1$, $P = 0.034$, okra ($F = 5.69$; $df = 1$, $P = 0.001$), cowpea ($F = 4.97$; $df=1$, $P=0.003$), and cotton ($F = 36.2$; $df = 1$, $P = < 0.001$; Fig 2.5). Area eaten (cm^2) on control treated plants was greatest on okra ($4.31 cm^2$), followed by cowpea ($3.07 cm^2$), and lowest occurred on cotton ($2.46 cm^2$), a little over half of area eaten on okra (Fig. 2.5). The greatest JA treatment effect on reducing area eaten was on cotton (46% reduction, respectively) followed by okra (16% reduction).

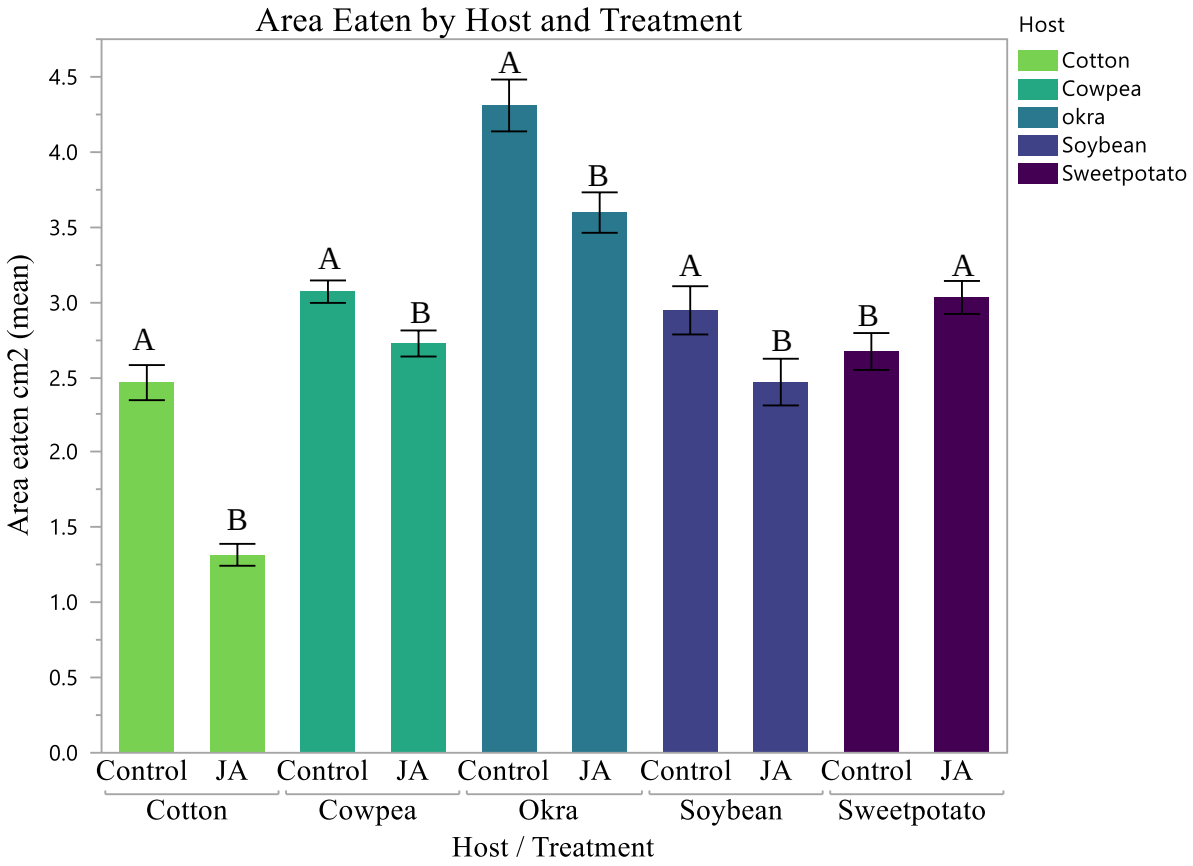

Figure 2.5 The leaf consumption (area eaten cm^2; mean) of SBL larvae after feeding seven days on JA induced or control host plant tissue. The standard error is represented by the error bar, and the different letters above each bar within host denote significant differences (Student's t test, α=0.05).

2.3.3 Diet Incorporation Bioassay

Third instar larvae that that reached pupal stage were weighed to determine the effects of feeding for 7 days on JA and control plant foliage. Pupal weights were not significantly different when 3rd instar SBL had fed for 7 days on JA treated or untreated hosts (Table 2.1).

Of the survivors through pupal stage, the number of days to adult emergence was significantly longer on JA treated cotton and soybean by 1.8 and 0.9 days, respectively while significantly shorter on sweet potato (1.1 days; Fig 2.6). On the other two hosts, okra and cowpea, SBL adult emergence was numerically ($P > 0.060$) longer by about 0.5 days when larvae had fed on JA treated plant foliage.

Table 2.1 Mean (± se) (pupal weights (g) of SBL after feeding for 7 days on untreated and JA treated hosts.

Host Plant	UTC	JA	*P*-value[1]
Cotton	0.233 ± 0.006	0.235 ± 0.006	0.879
Cowpea	0.223 ± 0.005	0.226 ± 0.005	0.680
Okra	0.221 ± 0.008	0.226 ± 0.008	0.720
Soybean	0.221 ± 0.006	0.205 ± 0.008	0.117
Sweet potato	0.198 ± 0.006	0.214 ± 0.006	0.069

[1]Within host, pupal weight comparison by treatment was determined using a Student's t-test, alpha=0.05.

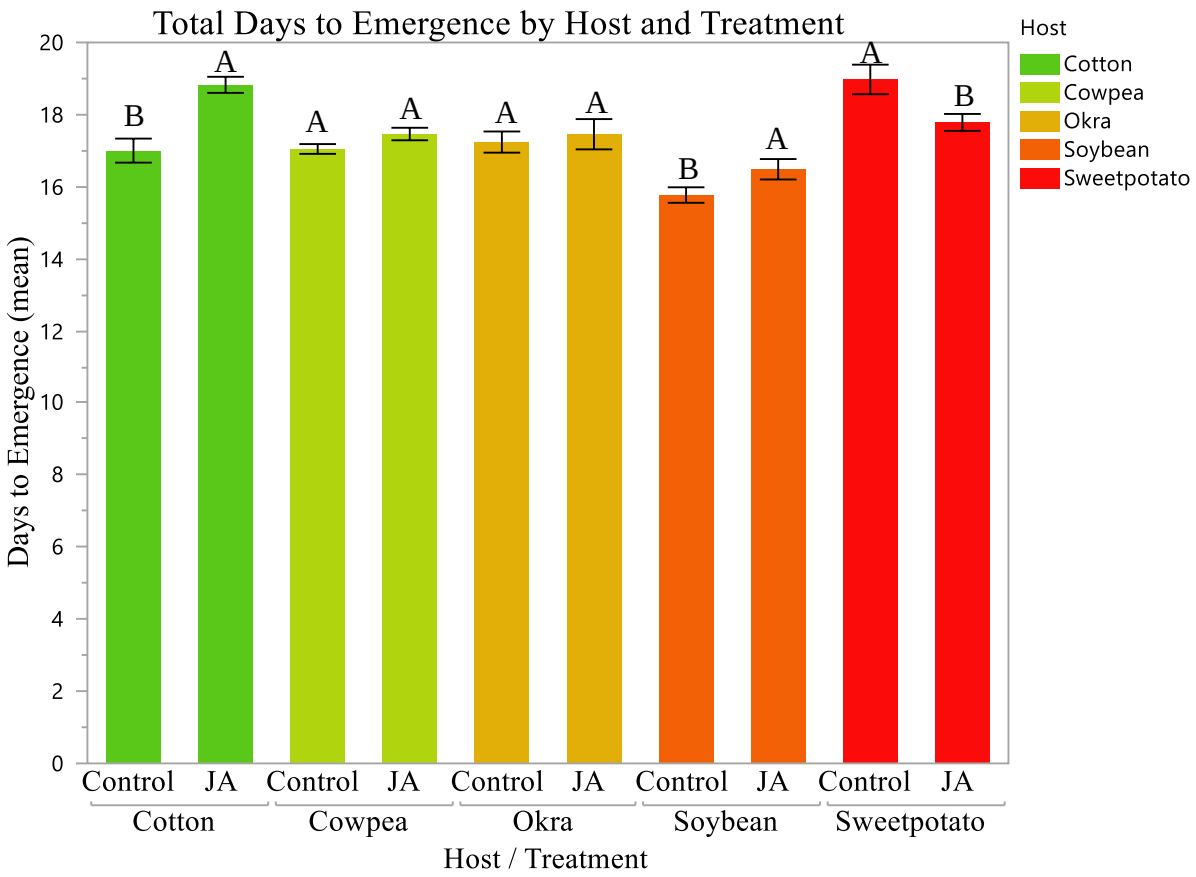

Figure 2.6 The effect of JA induction on various host to plants to SBL number of days (mean) until SBL adult emergence occurred after feeding for 7 days as 3rd instars on untreated and JA treated hosts. Within host, average days to adult emergence comparison by treatment was determined using a Student's t-test, alpha=0.05.

In the insecticide efficacy experiment, JA treatment to host plants did not affect insecticide efficacy (Fig 2.7). Percent mortality was significantly higher on all SBL fed host plant tissue then fed methoxyfenozide incorporated diet: okra ($P = 0.050$), cotton ($P < 0.001$), soybean ($P < 0.001$), sweet potato ($P = 0.009$) and cowpea ($P < 0.001$) (Fig 2.7). SBL placed on methoxyfenozide incorporated diet had much higher percent mortality than the control groups (Fig 2.7).

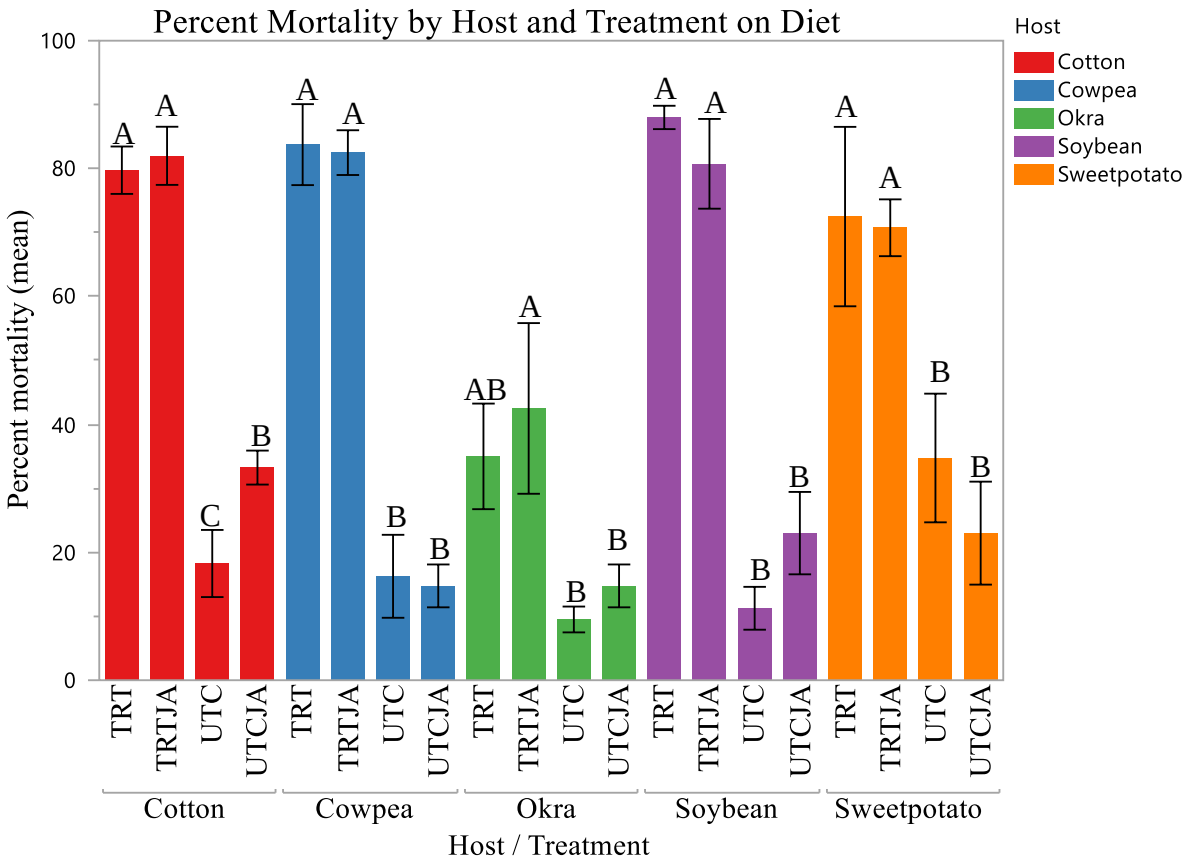

Figure 2.7 Percent mortality of SBL 3rd instar larvae after five days of exposure to methoxyfenozide incorporated diet (TRT) or untreated diet (UTC). Larvae that were pre-exposed to JA then exposed to Treated (TRTJA)and Untreated diet (UTCJA) prior to placement on diet. Within host, percent mortality five days after application comparison by treatment was determined using a Student's t-test, alpha=0.05. Data were transformed using the Schneider-Orelli's formula to obtain corrected percent mortality for TRT and TRTJA groups.

2.4 Discussion

Plants have many unique ways in which they defend or protect themselves against herbivore attack. JA has been found to be naturally occurring in most plants and is mainly involved in wound response by herbivores (Thaler, 1999). A study done by Rodriguez et al. (2001) showed that exogenous JA applications can induce volatile emissions in cotton plants. Although there are various examples of the role JA plays in plant defense there are little examples of how JA fits into an IPM strategy.

A study done in California showed that exogenous JA helped significantly reduce leafminer length and number of mines on celery (Black et al. 2014); however, this study did not address how JA may be interacting with conventional pest management strategies that are already in place for celery crops. This current study aimed to show the possible outcomes JA may have with SBL on various hosts plants and how that may affect insecticide efficacy. Like the study conducted in California, JA was applied in field setting and taken one step farther by exposing SBL to insecticide after consumption of JA to see the affect.

In the JA diet overlay trial, there were no significant differences among treatments. Relative growth rates did not differ between JA, EtOH, and control treatments suggesting no effect. These results suggest that JA consumption alone has no effect on SBL.

The induced HPR bioassay suggests that when JA is applied to different host plants, it can have an effect on SBL growth and survival. The results of this study reveal that larval weights were significantly reduced on JA treated okra, soybean, cotton, and sweet potato, indicating activation of plant resistance. However, cowpea larval weights were not significantly different. Results also show that area eaten was lower on all JA treated host leaf tissue except for sweet potato. Results also reveal that JA has an effect on SBL mortality on all host plants tested

except for sweet potato. Sweet potato had higher mortality and less area eaten on control treatments than JA treatments. Sweet potato growing conditions were stressful as there was a large amount of weed competition. Since JA is beneficial to a plant when not under attack, it promotes growth (Zhang et al. 2017), this may be a reason why SBL did better on JA treated plants as the plant quality may have been higher (Zhang et al. 2017).

When okra is attacked or wounded by pests, the total phenolic content in the leaves increase while it decreases in the roots and stems (Sharma and Rao 2013). Many phenolics have no effect on insects but some have been found to decrease digestion and consumption as well as cause some mortality in Lepidopterans (Lindroth and Peterson 1987). When JA is applied to okra the same effect may happen since the shikimic pathway is activated and may cause the total phenolic content in leaves to be higher. Okra has morphological characteristics of host plant resistance such as leaf toughness, trichomes, silica content, and leaf size, all which deter feeding and/or insect preference (Abang et al. 2016). Okra is a known host plant of soybean looper however this variety of okra, Silver Queen, has many hairs on the surface of the leaves. The density of the trichomes may have deterred or made it difficult for SBL to feed on the okra leaves. While okra is a host to SBL, there is no information available on the performance, survival, and fecundity of SBL on okra. Okra contains carotenoid, sugar, starch, chlorophyll a and b, and proteins in their leaf tissues (Ames and Macleod 1990). When okra is attacked by pests, total phenolic concentration in leaves increases while decreasing in the stems and roots (Sharma and Rao, 2013). Although okra followed the pattern that JA effects SBL growth and survivorship, overall, SBL consumed more, weighed more, and had a lower mortality rate than all other hosts which could suggest that okra is a very optimal host for SBL.

Soybeans produce allelochemicals that affect SBL weight gain and molting (Rose et al. 1989; Smith 1985). One of the allelochemicals soybeans contain is coumestrol, a known secondary plant chemical which is associated with insect resistance (Rose et al. 1989). The soybean variety Asgrow 5332 contains R*hg,* a gene for soybean cyst nematode resistance, which allocates plant resources to wounded roots (Liu et al. 2016). In the event of insect attack, soybeans produce chalcone synthase (Creelman et al. 1992), which has insecticidal properties and acts as an insect deterrent (Diaz et al. 2016). These may be factors in soybean resistance to SBL.

Cotton has chemical defenses to herbivory including gossypol and related sesquiterpene aldehydes. Gossypol is a phytotoxin that disrupts gut activity and digestion in lepidopterans (Tao et al., 2012). Lepidopterans have the ability to overcome gossypol by increasing their production of detoxification enzymes. This may be why SBL had lower weights on JA induced cotton. JA increases the production of gossypol, which may have slowed down the larval weight and leaf consumption leading to mortality.

Sweet potato showed significantly higher weights, more area consumed, and lower mortality on JA treated leaves. Sweet potato produces a protease inhibitor used for defense against herbivory attack called sporamin, which is used as a storage protein when the plant is not under attack (Yeh et al. 1997). Sporamin is a trypsin inhibitor, which, when ingested by herbivores, breaks down proteins, which aid in digestion. In a recent study, sporamin expression was dependent upon JA and the systemin-mediated-JA-signaling pathway (Rajendran et al. 2014). However; they also found that when lepidopteran feeding was the cause of attack, salicylic acid levels rose in the leaf tissue, which suppressed sporamin expression (Rajendran et al. 2014). In addition, sweet potato can recognize a lepidopteran attack from insect oral

secretions and thus salicylic acid production (Rajendran et al. 2014). The same may have happened with the sweet potato in this trial; the JA treated sweet potato may have had higher levels of JA and sporamin, but the untreated sweet potato may have had higher levels of salicylic acid. Salicylic acid is part of the plants first line of defense against herbivores while JA and sporamin's primary functions are plant growth and storage.

JA induction did not affect insecticide efficacy. Methoxyfenozide killed all SBL larvae by the time control treatments had reached adults. The diet incorporation bioassay revealed SBL total days to adult emergence on JA induced soybean and cotton host plant material took significantly longer, while the opposite occurred on SBL fed JA induced sweet potato plant tissue took less time than the control to reach adult emergence. SBL had lower percent mortality on control treated cotton, cowpea, okra, and soybean but on sweet potato, mortality was lower on JA induced host material. These results suggest that JA may have some prolonged effects on SBL larvae.

Our data shows that a single field application of JA can induce host plant resistance to SBL but is host dependent. Tomato is one example of JA induced HPR (Thaler et al. 1996); one application of JA to tomato induced tomato glandular trichomes, allelochemicals, and volatiles (Thaler et al. 1996). Plant species plays an important role in the effect JA will have on direct or indirect resistance, if any effect at all (Dixon et al. 1994). The results also suggest that when SBL ingests JA without feeding on a plant there is no effect on larval weight or mortality. These results correlate with a study that found JA alone has no effect on larval survival or success (Accamando et al.;2012) This may be strong evidence that JA is only the inducer and the plant response is what is affecting SBL growth and survival.

Plants produce allelochemicals that defend against herbivores, and herbivores have evolved mechanisms of resistance to avoid, detoxify, or sequester the plant defenses (Dermauw et al. 2012). Not only have herbivores developed resistance to plant defenses but they have also developed resistance to insecticides. Spider mite *Tetranychus urticae* was able to overcome insecticides after feeding on tomato plants and it is believed that chemicals from the plant may have aided in the mites' resistance to insecticide (Dermauw et al. 2012). Since every plant species has its own unique set of defenses, exogenous HPR induction will have varying results due to the different ways in which SBL may detoxify the chemicals produced by the host plants. In order to better understand the role JA can play in insect pest management, further field trials are needed. It is important to know how the insect and JA is interacting with the plant and its' natural defenses.

In conclusion, our studies looking at JA treated diet and JA treated agriculturally important crops have shown that JA alone did not significantly alter SBL larval fitness, but JA and host plant interaction, will have varying results on SBL fitness that may be positive, negative or indifferent. These studies are important in in integrating alternative pest management tools against phytophagous pests.

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CHAPTER 3
STATUS OF GLYPHOSATE RESISTANT *AMARANTHUS PALMERI* AS AN ALTERNATIVE HOST FOR *CHRYSODEIXIS INCLUDENS* AND THE EFFECTS OF GLYPHOSATE ON *CHRYSODEIXIS INCLUDENS* POPULATION GROWTH RATES

3.1 Introduction

Palmer amaranth (*Amaranthus palmeri* S.Wats.) is native to the southwest U.S. and Mexico (Legleiter and Johnson 2013). It has spread to the southeastern U.S. and has become a major weed problem for Louisiana soybean growers. Palmer amaranth is drought tolerant and grows and survives under dry conditions (Ehleringer 1983). In addition, it grows in shaded areas allowing it to compete with plants for light in dense crop canopies (Whitaker et al. 2010). One Palmer amaranth plant per 0.125 m row of soybean has been documented to cause up to 91% yield loss (Bensch et al. 2003). While Palmer amaranth affects agronomic yield, it may also be affecting insect pest populations by providing an alternative host. Harding (1976) documented that Palmer amaranth was a host for soybean looper (SBL).

In 1997, Roundup® Ready (glyphosate resistant) soybeans were introduced on a commercial scale. Shortly thereafter, Roundup® Ready corn was released in 1998 (J Dekker 1999). Glyphosate became the main herbicide used for weed control because it was used as a pre-and post-emergence product (Nandula 2010). Not only is glyphosate convenient, it also has many other beneficial factors for farmers. Glyphosate is useful for a no-till agriculture design system (Nandula 2010). When a farmer uses glyphosate to kill weeds at the roots there is no longer a need to plow the soil, which helps with sustainability. Applying glyphosate before planting also cuts back on plant disease, parasite, and insect intermediate hosts, cutting down overall production costs (Nandula 2010). With the new glyphosate resistance technology for

crops, the use of glyphosate skyrocketed, causing weeds to develop herbicide resistance, including Palmer amaranth.

Glyphosate resistant Palmer amaranth has been found in Louisiana, Mississippi, Arkansas, and Tennessee (Riar et al 2013). Glyphosate kills or suppresses all plant types including, perennials, grasses, vines, shrubs, and trees by inhibiting the production of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Nichols et al. 2007). Palmer amaranth can overcome this inhibition by increasing the production of the enzyme EPSPS (Gaines et al. 2011). The resistance gene can then be spread to a susceptible plant in a remote field through pollen. Resistant Palmer amaranth is able to withstand glyphosate toxicity from the production of resistance alleles during random DNA mutations (Powles and Yu 2010). These resistance alleles regulate very efficient constitutive defense mechanisms that keep herbicides from inhibiting important metabolic pathways (Vila-Aiub et al. 2015). These mechanisms can impair herbicide uptake by leaves or translocation within the plant through sequestration or reduced cellular uptake, or change the herbicides chemical properties through detoxification (herbicide-enhanced metabolism) (Vila-Aiub et al. 2015). There are also target-site resistance defense mechanisms that involve changes in the amino acid sequence of the herbicide target protein, minimizing herbicide binding (Powles and Yu 2010), and gene overexpression, which results in overproduction of herbicide sensitive target proteins (Dinelli et al. 2006). A search of the literature reveals little about how these defense mechanisms may be affecting herbivory defense in the plant. Are these herbicide constitutive defense mechanisms also activating or acting as constitutive defense mechanisms to insect attack?

When glyphosate was released for commercial use, many carcinogenicity studies were done on various species of animals and the EPA classified it as a Group E (Franz et al. 1997),

meaning it is unlikely to have carcinogenic effects on humans or animals. In 2015, a study done by the International Agency for Research on Cancer (IARC) showed that glyphosate may be a carcinogen to humans. The IARC put out a yearlong study on glyphosate that classified it as a Group 2A which means probably carcinogenic to humans (Tarone 2016). After this happened, the World Health Organization called for the Environmental Protection Agency to re-evaluate the registration of glyphosate as a product fit for commercial use (Tarone 2016). The registration is currently still underway. Initial studies done to get glyphosate on the market suggest that there is no bioaccumulation in mammals, due to relatively high water solubility (Franz et al. 1997). Not only is glyphosate being investigated for its effects on humans, it has and is being researched for the impacts it has on the environment when used in large quantities (Schuette 1998). When glyphosate first was approved as commercial product all initial studies showed that it was relatively harmless to the environment. It wasn't persistent in water for a long period of time and it was found to be non-toxic to birds, insects, and mammals (Franz et al. 1997). It is unclear if it was shown conclusively to be harmful to fish life (Franz et al. 1997). There are many who argued that the weeds being controlled in certain areas were not weeds but vital agents in the success of particular ecosystems (Gover et al. 2007). Many weedy species are known to be food sources for beneficial insects (Chandler et al. 1998). When these weedy species are removed from roadways and fields, it raises concern as to how impactful weed removal is to the natural ecosystem.

One of the concerns people have with transgenic crops is whether or not they are having a negative impact on colony collapse disorder (CCD) in bees (Han et al. 2010). Science literature currently says there is no evidence that transgenic crops have any responsibility for CCD (Kluser and Peduzzi 2007). A second concern over the use of transgenics is whether or not transgenes

can be transferred to wild plant populations (Cerdeira et al. 2006). If gene transfer is occurring among weed species and crops, it could give rise to glyphosate resistant weed species (Landry 2015). Transgenic plants are much more likely to be problematic as volunteer plants in cropping systems (Smythe et al. 2002) with the potential to be alternative hosts to pests (Gitlin 2012).

A concern for farmers when using glyphosate on their transgenic crops is that studies have shown glyphosate applications can give rise to plant pathogens (Johal and Huber 2009). When a transgenic crop is treated with glyphosate, it may indirectly induce weakening of the plant defenses (Johal and Huber 2009). When glyphosate is applied, the plant is predisposed to disease because specific micronutrients needed for disease resistance are immobilized (Johal and Huber 2009). Along with immobilizing vital micronutrients, glyphosate also reduces plant growth and vigor, alters physiological efficiency, and modifies the soil microflora, which affects nutrient availability (Johal and Huber 2009).

However, the concern that resistant weeds could be a byproduct of transgenic crops remains. Not only is gene transfer a concern (Smythe et al. 2002) but after transgenic crops were introduced to the market, glyphosate was used as a post-emergent tool on a much larger scale, giving rise to glyphosate resistant weed species (Boerboom et al. 2006). When transgenic crops were introduced and the price of glyphosate dropped, it was not only used for burndown purposes but also for post-emergence weed control (Nandula 2010). This increased the amount of acreage that was being treated with glyphosate which may have increased the selection for glyphosate resistant weeds (Purdue Extension 2006). The economically damaging glyphosate resistant weeds in the U.S. today are *Ambrosia artemisiifolia* L., *Ambrosia trifida* L., *Amaranthus palmeri* S Watson., *Amaranthus rudis* JD Sauer, *Amaranthus tuberculatus* (Moq) JD Sauer, and many *Conyza* and *Lolium* spp. (Powles 2008).

Could glyphosate resistant Palmer amaranth be an alternate host for SBL? If so, what potential impacts will it have on SBL populations? This experiment assessed the effects glyphosate resistant Palmer amaranth (pigweed) has on SBL growth rate, survival, and fecundity. It also assessed if glyphosate applications to glyphosate resistant palmer amaranth and Roundup® Ready soybean alter SBL populations.

3.2 Materials and Methods

3.2.2 SBL Colony Rearing

C. includens used in this study were DL12 from the Soybean Entomology Laboratory (Louisiana State University Agricultural Center, Baton Rouge). This colony was established in 2012 from larvae that were collected at the Dean Lee research station in Alexandria, LA (J. Davis 2008). This colony is maintained using the following protocol. Larvae were placed in 30 ml plastic diet cups (2 larvae/cup) with 10 ml Southland Product's artificial meridic SBL diet (Southland Products, Lake Village, AR) until pupation. The cups were placed in a rearing room which is maintained at 22°C with 55% RH and a 14:10 (L:D) photoperiod (Mascarenhas and Boethel 2000). Once pupation occurred, SBL were transferred to a large plastic cylinder container (32 cm X 62 cm) with 30 g of vermiculite (Sun Gro, Bellevue, WA) and upon eclosion were free to mate and oviposit on paper sheets. Paper sheets (8 x 23 cm) were placed on the inside of the container and the container was sealed with a plastic and muslin cover (Jensen et al. 1974). The adults were fed a 10% honey water solution in cotton wadding. Egg sheets and honey water were changed every two days and egg sheets were placed in a plastic bag (15 cm x 7 cm-1 cm x 38 cm plain clear non-vent) until eclosion. When the neonates emerged two to three were placed into each diet cup

3.2.4 SBL Life Tables

Glyphosate resistant Palmer amaranth (growing in the soybean field) and soybean (*Glycine max*) var. AG5332 (Asgrow, Monsanto, St. Louis, MO) which was grown according to production standards at the LSU AgCenter Dean Lee Research Station in Alexandria, LA. The Palmer amaranth grew in the soybean field after glyphosate applications. Leaf tissue was collected from these plants as needed.

Palmer amaranth and soybean leaves were collected weekly from the top of the plant where new growth was abundant. Fifty leaves were collected per host plant (50 soybean; 50 Palmer amaranth). The collected leaves were cored with a No. 149 Arch Punch (Osborne and Co., Harrison, NJ), which created an 11.34 cm^2 leaf core. Cores were placed onto sterile petri dishes (VWR polystyrene disposable, 100 X 15 mm) on moistened Whatman 90 mm (#1) filter paper. A single SBL neonate that had been feeding on artificial diet (Southland Products, Lake Village, AR) for 24 hr to decrease handling mortality was placed onto the leaf core with a fine camel hair paintbrush. The filter paper was moistened with distilled water to prevent the leaf from drying. Leaf tissue was changed as leaf integrity deteriorated and the filter paper was wetted every day. There were 50 neonates per host per replication for a total of 150 neonates for each host plant replicated four times. The plates containing the insects were placed in a growth chamber (Percival Intellus Environmental Controller, Model No. I-36VL, Percival Scientific, Perry, IA) at 25°C with 75% RH and a 14:10 (L:D) photoperiod. Insects were monitored daily for mortality and area eaten (cm^2) was recorded when leaf tissue was changed. The area eaten was recorded using a LiCor-3100 area meter (Li-Cor Inc. Lincoln, NE). Insects fed on leaf tissue until either death or pupation occurred. When pupation occurred, they were sexed (Shorey et al. 1962) and weighed. The sexed pupae were randomly put into pairs (one male and one female)

for a total of ten pairs and placed into a 3.8 L-rearing container (Huhtamaki Foodservice, De Soto, KS) with 30 ml of vermiculite (Sun Gro, Bellevue, WA) for mating and oviposition for a total of 20 pairs per host plant. The containers were maintained as previously described above. Egg lay was counted every two days using a head magnifying glass and egg hatch was counted as it occurred. Egg hatch ratio was calculated by dividing the number of egg hatch by the total number of eggs laid. Host plant effects on mortality, leaf consumption, and pupal weights were compared by a student's t-test ($\alpha = 0.05$). Egg lay and egg hatch were analyzed through analysis of variance (ANOVA). A partial life table was created to compare growth rate, survival, and fecundity on each host plant.

3.2.5 Glyphosate diet overlay procedure

In order to test the effects of glyphosate alone on SBL, diet overlay experiments were conducted which included an untreated check and glyphosate treatment. To prepare the glyphosate solution, 5 ml of glyphosate (calculated from recommended field rate for soybeans; 2 qts/10 gal water) was added to 100 ml of distilled water. For untreated check, unmanipulated SBL diet was used. In order to get full surface coverage, 500 μl of glyphosate solution was placed onto the top of the diet in each cup. Treatments were applied to the top of the diet, swirled to ensure even distribution, and allowed to dry overnight. SBL third instar larvae that previously fed on a meridic SBL diet from Southland Products, Inc. (Lake Village, AR, USA) were weighed (to ensure third instar status) and then randomly placed onto one of the two treatments (untreated check and glyphosate). There were four simultaneous replications, 50 insects per treatment per replicate. Insects were monitored daily for mortality and stadia (time spent in each instar) over seven days. After seven days, surviving insects were weighed.

3.2.6 Glyphosate application procedure to plant tissue

Palmer amaranth, soybean var. AG5332 and cotton (*Gossypium hirsutum* L.) var. DP174RF (Monsanto, St. Louis, MO) were grown in the greenhouse from seed in 13 cm plastic pots with Miracle-Gro soil (Merryville, OH) and Osmocote fertilizer (Merryville, OH). Host plants were sprayed with glyphosate (Cornerstone Plus, WinField Solutions, LLC. St. Paul, MN, USA) at the recommended field rate for soybeans; 2 qts/10 gal water, which calculates to 38.36 ml of glyphosate per 500 ml of water. The control plants were sprayed with water only to serve as a control. Plants were sprayed with a portable Preval sprayer (Power unit 97.6 ml Jar/cap 168.6 ml; Preval, CA Acquisition, LLC. Coal City, IL, USA) until runoff. After 48 hours, leaves were collected randomly from the top of the plants where new growth occurs. Fifty leaves were collected from both the control and glyphosate treatments (50/glyphosate group; 50/control group) per host plant. The collected leaves were cored with a No. 149 Arch Punch (Osborne and Co., Harrison, NJ), which created an 11.34 cm^2 leaf core. Cores were placed onto sterile petri dishes (VWR polystyrene disposable, 100 X 15 mm) on moistened Whatman 90 mm (#1) filter paper. A single SBL neonate that had been feeding on artificial diet (Southland Products, Lake Village, AR) for 24 hr to decrease handling mortality was placed onto the leaf core with a fine camel hair paintbrush. The filter paper was moistened with distilled water to prevent the leaf from drying. Leaf tissue was changed as leaf integrity deteriorated and the filter paper was wetted every day. There were 50 neonates per treatment per replication for a total of 150 neonates for each host plant replicated four times. The plates containing the insects were placed in a growth chamber (Percival Intellus Environmental Controller, Model No. I-36VL, Percival Scientific, Perry, IA) at 25°C with 75% RH and a 14:10 (L:D) photoperiod. Insects were fed leaf tissue for seven days and then were weighed. Insects were monitored daily for mortality and area

eaten (cm^2) was recorded when leaf tissue was changed. To estimate leaf area eaten by each larva, defoliation percentage of each leaf core was visually approximated based on the defoliation estimates for foliage feeding pests (Baldwin et al. 2011; Fig. 2.1). and total area of the leaf core (11.32 cm^2).

3.2.7 Data analysis

Statistical analyses were performed using JMP software (SAS Institute Inc. Cary, NC, USA 2016, v. 13). In the first experiment conducted, a one way analysis of variance (ANOVA) using Least Squares Fit (LSF) was used to analyze the effect Palmer amaranth has on SBL growth, survival, and fecundity. SBL instar stage, days to instar stage, mortality, pupal weight, leaf consumption (cm^2), pupal sex, egg lay, and egg hatch were recorded.

In the second experiment conducted, a two-factor analysis of variance (ANOVA) using Least Squares Fit (LSF) was used to analyze the effects of glyphosate treated diet on SBL growth and survivorship, SBL instar stage, days to instar stage, mortality, third instar initial weight, and larvae weight after seven days were recorded. Relative growth rate was calculated using the formula**:** $RGR= (\ln W_2 - \ln W_1)/ (t_2 - t_1)$, where: ln is natural logarithm, t_1 is time one (in days), t_2 is time two (in days), W_1 is average weight at time one, and W_2 is average weight at time two (Philipson et al., 2012). Means of third instar initial weights, third instar weights after seven days, and neonate weights after seven days were separated by a Student's t-test at $\alpha=0.05$ level.

In the third experiment conducted, a two-factor analysis of variance (ANOVA) using LSF was used to determine the effects of glyphosate applied to various host plants on SBL growth and survivorship. SBL instar stage, days to each instar stage, leaf tissue area eaten (cm^2), third instar weights (g), and mortality were recorded. Area eaten (cm^2), third instar weights (g),

and mortality data were all analyzed by two factor analysis of variance (ANOVA) using LSF to determine differences among treatments by host as the main factors. Means were separated using Student's t-test at α=0.05 level.

3.3 Results

3.3.1 Life Table

In this study, results suggest that Palmer amaranth can act as an alternative host for SBL but is not as optimal a host as soybean. SBL had significantly lower egg lay ($P < 0.001$), egg hatch ($P < 0.001$), leaf consumption ($P < 0.001$), and pupal weights ($P < 0.001$) on Palmer amaranth than on soybean (Table 3.1). It took almost four more days (18 days total) for SBL larvae to reach pupal stage on Palmer amaranth than the 14-day average for SBL that fed on soybean (P= 0.041).

Table 3.1 Soybean vs. Palmer amaranth as a Host

Host	No. eggs laid/female (mean ± se)	% egg hatch (mean ± se)	Leaf consumption (mean (cm^2) ± se)	Pupal Weights (mean (g) ± se)
Soybean	302.4 ± 3.12	80.1 ± 1.57	82.3 ± 1.62	0.203 ± 0.004
Palmer amaranth	216.2 ± 2.95	68.4 ± 1.11	45.7 ± 1.06	0.171 ± 0.002

3.3.2 Diet overlay procedure

The application of glyphosate to SBL larval diet had no effect on 3rd instar larval growth rate and weight gain while feeding on diet. SBL weights after feeding 7 days on surface treated

diets were similar across treatments ($P = 0.050$; Table 3.2). Additionally, the percent relative growth rates for each treatment were not significantly different (Control- 34% and Glyphosate- 35%).

Table 3.2 Glyphosate diet overlay effects on SBL third instar weights (mean (g) ± se) after feeding for seven days

Treatment	Initial weight	Final weight
Control	0.025 ± 0.001	0.270 ± 0.004
Glyphosate	0.025 ± 0.001	0.281 ± 0.004

In contrast, the application of glyphosate to SBL larval diet did have an effect on SBL neonate growth rate and weight gain while feeding on diet. On glyphosate treated diet, SBL weights after feeding 7 days were significantly lower than SBL weights feeding on control diet for seven days ($P < 0.001$; Table 3.3) SBL neonates that fed on diet with glyphosate had an average mortality of 18% and SBL neonates on diet with no manipulation was 6% ($P = 0.084$).

Table 3.3 Glyphosate effects on SBL neonate weights (mean ± se) over seven days

Treatment	Weight (g) after 7 days	% mortality
Control	0.018 ± 0.001	0.067 ± 0.032
Glyphosate	0.014 ± 0.001	0.183 ± 0.032

3.3.3 Glyphosate application procedure

The application of glyphosate to glyphosate resistant soybeans had an effect on SBL growth and fecundity. SBL fed treated glyphosate leaves consumed on average 6.11 cm^2 ±

0.340 of leaf tissue daily, where SBL fed soybean leaves consumed on average 4.72 cm^2 ± 0.340 (*P* - value < 0.001). SBL pupal weights were significantly higher on the glyphosate treated leaves (0.199 ± 0.003 g) than on untreated leaves (0.159 ± 0.005 g) (*P* - value < 0.001; Table 3.4).

Table 3.4 Effects of glyphosate treated soybean leaves vs. soybean leaves to SBL

Host	No. eggs laid/female (mean ± se)	% egg hatch (mean ± se)	Leaf consumption (mean (cm^2) ± se)	Pupal wt (mean (g) ± se)
Soybean	77 ± 1.13	0 ± 0.001	6.11 ± 0.057	0.159 ± 0.005
Glyphosate	97 ± 1.25	58 ± 1.02	4.72 ± 0.032	0.199 ± 0.003

SBL had no significant differences in third instar weights among host plants on glyphosate treated leaves vs. untreated leaves (Table 3.5).

Table 3.5 Third instar weights by host plant (3rd instar wt (g) ± se)

Host Plant	Glyphosate	Control	*P*-value
Soybean	0.020 ± 0.002	0.025 ± 0.002	0.118
Cotton	0.012 ± 0.001	0.015 ± 0.001	0.179
Palmer amaranth	0.006 ± 0.002	0.007 ± 0.0012	0.605

Larvae placed on various plants treated with glyphosate did have a significant effect on area eaten over seven days. On cotton, SBL consumed significantly more leaf area on glyphosate treated leaves (4.32 ± 0.336) than on control treated leaves (3.31 ± 0.345; *P* = 0.038; Fig. 3.1). On soybean, SBL consumed significantly less leaf area on glyphosate treated leaves

(5.34 ± 0.412) than on control leaves (8.32 ± 0.379; $P < 0.001$; Fig. 3.1). Palmer amaranth did not have any significant effects among treatments ($P = 0.063$; Fig. 3.1).

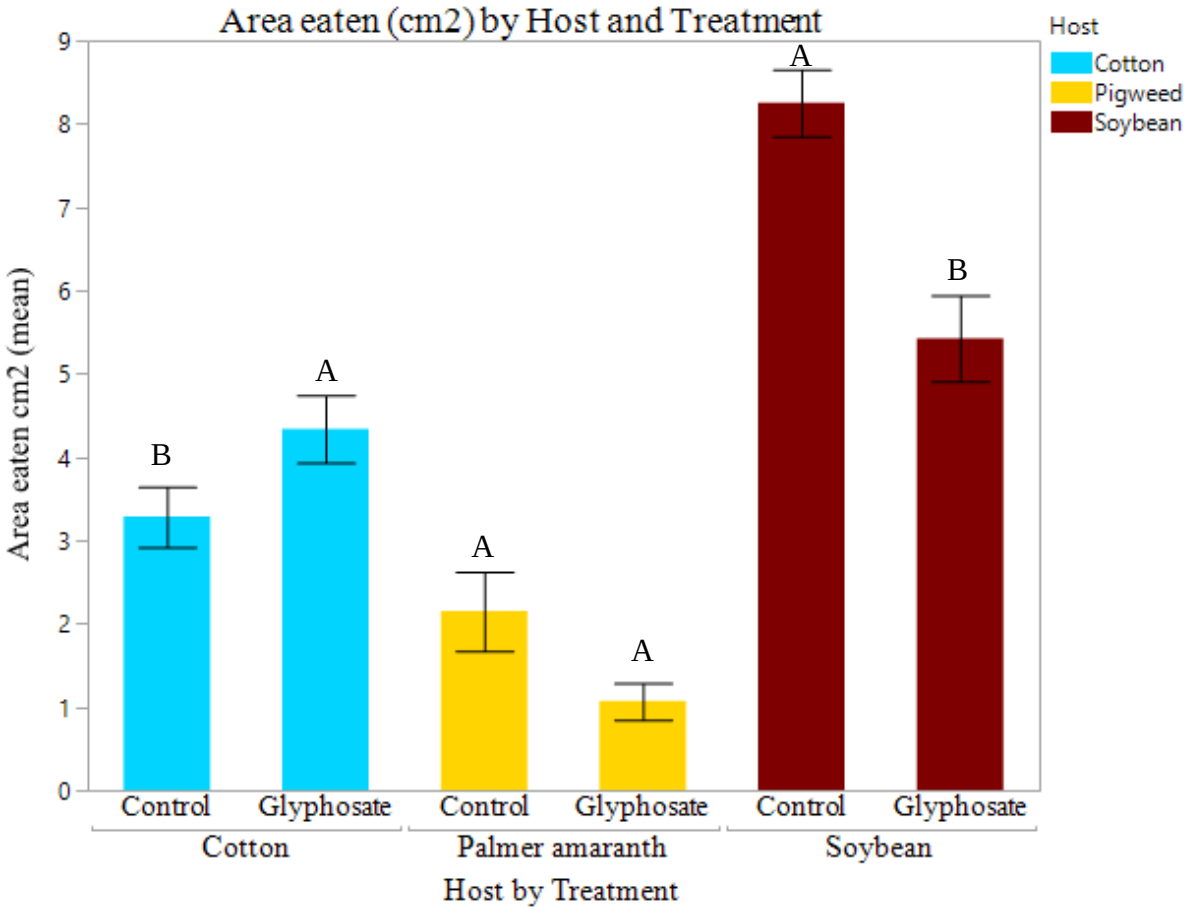

Figure 3.1 The leaf consumption (area eaten cm^2; mean) of SBL larvae after feeding seven days on glyphosate treated or control host plant tissue. The standard error is represented by the error bar, and the different letters above each bar within host denote significant differences (Student's t test, α=0.05).

3.4 Discussion

The results of this study provide evidence that Palmer amaranth is a host for SBL and has the potential to use this weed as bridging host. Harding (1976) found that Palmer amaranth

supports SBL life and reproduction. The life table procedure supports that SBL can utilize Palmer amaranth as a host, but that population growth rates will be less on Palmer amaranth than on soybean. This may be problematic for farmers in the future because not only is Palmer amaranth a weedy pest of soybeans, it is a potential alternative host for SBL which may aid in the build-up of SBL populations and serve as a reservoir for SBL regardless of crop rotation plans (Schroeder et al. 2005). Palmer amaranth that is left to grow in or near fields that are not occupied by a crop could harbor SBL populations. When the crop is planted, SBL adults could then move to its preferred host (Capinera 2004). If a Palmer amaranth plant persists in the field during growing season due to herbicide resistance it has the potential to aid in the build-up of SBL population densities (Capinera 2004) by providing nutrients where the crop plant may be lacking due to weed competition (Powles 2008). Current IPM strategies for Palmer amaranth control include rotating different tillage practices, rotating different herbicides, and controlling weeds around fields to decrease the spread of herbicide resistant weeds for the next growing season (Beckie 2011). Because Palmer amaranth is a host for SBL, the biological interactions needed to be explored further in order to develop a better IPM strategy.

The glyphosate diet overlay study revealed that SBL neonates have higher mortality and lower weights after ingesting glyphosate. However, glyphosate did not have an effect on SBL third instar weights or mortality. Neonate mortality may have been caused by their inability to digest anything with low-digestibility content (Kogan and Cope 1974). The neonates may not have been able to develop enough to overcome the possible lack of nutrients they were receiving and died. Older SBL larvae are not affected by glyphosate, and may be a reason to suggest that growers treat crops with glyphosate at earlier stages of SBL development. The next step would be to find if or how SBL detoxifies glyphosate, as this would give a more complete picture into the many

interactions going on among the insect, plant, and chemicals. With a more complete picture a better IPM strategy can be developed.

Glyphosate field application results suggest that when you apply glyphosate to a soybean crop, SBL consume more and weigh more. This may not be just because of the glyphosate, there may be other factors to consider. One thing that may have affected this study was the untreated soybean were hand weeded on a weekly basis. Hand weeding is not as effective of a control method for weeds as is glyphosate (Hasanuzzaman et al. 2009) so the plants may have had more competition from weeds around them for resources.. Alston et al. (1991) found that *Helicoverpa zea* is more abundant in weed free soybean systems, supporting the notion that less competition from weeds provides a better environment for SBL populations to thrive. If the nutritional value was lower in untreated soybean plants, this could explain why growth rates and pupal weights were lower. SBL require a high nutritional value in order to complete their life cycle and morph into an adult (Kogan and Cope 1974). It has been shown that there is a strong link between plant quality (nitrogen, sulphur, phosphorous levels) and SBL survival success (Busch and Phelan 1999). Without the proper levels of nutrients, SBL will never be able to complete a life cycle on a detrimental plant (Kogan and Cope 1974).

When glyphosate was applied to soybean, Palmer amaranth, and cotton and fed to SBL, third instar weights were not significantly different than the control treated third instar weights. SBL that fed on glyphosate treated cotton consumed more leaf tissue than SBL fed control treated cotton leaves. SBL fed glyphosate treated soybean leaves consumed less leaf tissue than SBL fed control treated soybean leaves. SBL fed glyphosate treated Palmer amaranth had no significant effects on area consumed.

In conclusion, this study supports the idea that Palmer amaranth is a suitable host for SBL to maintain its population. However, it neither proves nor disproves whether glyphosate is helpful or detrimental to SBL. This study helps support the idea that glyphosate is not toxic to later instar lepidopteran, but may have some deleterious effects on young instar lepidopterans. This study also suggests that at times using glyphosate in the field may benefit herbivores because the plant will have less competition from weeds for resource and better nutritional value. This may be important to consider when developing an IPM strategy. If glyphosate is not controlling weeds, is causing resistance in weed species, and creates a better environment for insect pests there may be a better herbicide solution for your IPM plan. The benefits of using glyphosate in your crops to control weeds may outweigh this finding.

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Summary and Conclusion

Soybeans are the second largest grown commodity in the U.S. and is continuously growing. In 2016 soybean growers planted 83.7 million acres of soybean, a 1% increase from 2015 (NASS 2016). This just shows how vital good management strategies are and will be in the future for soybeans, strategies that will aid in the management of herbivores while simultaneously optimizing resources for the best yield. Integrated Pest Management (IPM) is the most widely recommended way to achieve a sustainable agroecosystem. The soybean looper (SBL) has become resistant to many insecticides and is the most expensive pest for Louisiana soybean farmers. SBL resistance to diamides has been reported up to 1000x the recommended field rate. Alternative strategies to managing SBL have been explored in recent years, most notably induced host plant resistance (HPR). The goal of HPR is to reduce herbivore fitness, fecundity, survival, growth, and population rates. Exogenous elicitors have been the focus of recent induced host plant resistance to reduce herbivore population studies. Jasmonic acid is the most widely studied elicitor for induced resistance. Our studies looking at JA treated diet and JA treated agriculturally important crops and weeds, have shown that JA alone did not significantly alter SBL larval fitness, but based on host plant, will vary on SBL fitness that may be positive, negative or indifferent. Also, induction of secondary compounds when treated with JA, may play a role in the efficacy of certain insecticides used to control SBL larvae. The results indicate that JA induced resistance in some hosts and these impacts had lasting impacts on insecticide efficacy.

Weeds have always been a pest of soybeans, now they are potentially becoming alternative hosts for insects because they are resistant to glyphosate. Palmer amaranth has become resistant to glyphosate in the Southern U.S. and this is of concern to farmers because it

will compete for resources and sunlight and cause lower crop yield and quality. Palmer amaranth can act as an alternative host to soybean looper and may provide a reservoir to SBL during times of crop rotation and tillage to maintain populations. This study helps support the idea that glyphosate is not toxic to insects, and more specifically Lepidopteran species. It does however suggest that using glyphosate in the field may benefit the insects because the plant will have better nutritional value. This may be important to consider when developing an IPM strategy. If glyphosate is not controlling weeds, is causing resistance in weed species, and creates a better environment for insect pests there may be a better herbicide solution for your IPM plan. This study also indicated that using glyphosate in the field may be beneficial to soybean looper on soybeans as well as on cotton. These studies are important in our next step to better understand factors that may be useful in integrating pest management tools against this important polyphagous pest, SBL.

Vita

Abigail Cox is the daughter of Dr. David and Becky Cox. She was born in Phillipsburg, New Jersey, United States of America. Abigail graduated from High school in June of 2009. She received her Bachelor of Science degree in plant and soil systems with a concentration in pest management from Louisiana State University in May 2014. Abigail has been a graduate student at Louisiana State University Agriculture and Mechanical College under Dr. Jeffrey A. Davis since June 2014. Abigail is currently a candidate for a Master of Science degree in the Department of Entomology at Louisiana State University.