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# Effects of Induced Plant Resistance and Potassium Fertilization Rates on Soybean Looper (*Chrysodeixis Includens*) Development in Soybean

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EFFECTS OF INDUCED PLANT RESISTANCE AND POTASSIUM  
FERTILIZATION RATES ON SOYBEAN LOOPER (*CHRYSODEIXIS*  
*INCLUDENS*) DEVELOPMENT IN SOYBEAN

A Thesis

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in

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## ABSTRACT

Soybean looper, *Chrysodeixis includens* (Walker), is one of the most destructive pests of soybean in the southern U.S. It is highly resistant to most insecticides. Induced host plant resistance and cultural control are sought as alternative control tactics. In this study, effects of induced resistance by exogenous application of elicitors were estimated on soybean looper development in soybean. Three different known elicitors of SAR (systemic acquired resistance), Actigard 50WG (acibenzolar-S-methyl), Regalia (extract of *Reynoutria sachalinensis*) and methyl jasmonate (MeJA), were applied to soybean plants at R2 and R5 stages to determine if these chemicals could induce plant resistance and lower soybean looper fitness in the field. All of the SAR elicitors had significantly adverse effects on soybean looper development time, defoliation, and pupal weight. In particular, applications of MeJA significantly reduced pupal weight by 6.8% and delayed larval development by 14.3% compared to untreated checks. The results suggest that exogenous elicitors can trigger plant resistance against herbivores and may have a place in integrated pest management.

Inorganic fertilizers increase yield but sometimes also stimulate insect outbreaks. Potassium (K) fertilizer plays an important role in crop production. In this study, different rates of K fertilizer were used to detect their effects on soybean yield and soybean looper development. Soybean plants were supplied with six different rates of K (0, 33.6, 67.3, 100.9, 134.5, and 168.1 kg/ha as K<sub>2</sub>O) in field trials. Soybean leaves were sampled at R1, R3, R5, and R6 stages and fed to soybean looper in laboratory bioassays. Soybean looper consumed more tissues and spent less time completing larval development when fed on foliage from treatments of 134.5 and 168.1 kg/ha. Pupal weight significantly increased at 134.5 and 168.1 kg/ha. Soybean yield and 100 seed weight were also significantly enhanced at 168.1 kg/ha, but seed

protein and oil content were not affected by K fertilization. Higher K fertilizer rates can enhance soybean yield but also improve soybean looper development. Therefore, K fertilizer rates will need to be balanced between increased yields and reduced herbivore populations.



## **CHAPTER 1**

### **INTRODUCTION AND LITERATURE REVIEW**

Soybean, *Glycine max* (L.) Merrill, is an important crop worldwide and soybean seed is a significant source for human and animal to obtain nutrition of protein, oil, and minerals (Kolar and Grewal 1994). In the United States, soybean is the second most planted field crop, with a total of 77.2 million acres planted in 2013 (USDA-NASS 2013). Soybeans are the world's largest protein feed source for animals and the second largest source of vegetable oil for human beings (USDA-ERS 2012). In Louisiana, soybean production includes several hundreds of thousands of hectares and plays an important role in the agricultural economy (Boyd and Boethel 1998). It is one of the most adaptable crops for varying soil types and climatic conditions in Louisiana (Morrison and McCormick 1996).

Like other agricultural crops, soybean plants can be damaged by a variety of insect pests which feed on leaves, stems, roots, nodules, pods and seeds, from emergence to harvest (Turnipseed and Kogan 1987). In general, occurrence of soybean pest insects is greatest in the South, especially in the Southern states neighboring the Gulf of Mexico and the Atlantic Ocean, because the warm climate facilitates high survival and multiple generations each year (Gianessi 2009). In Louisiana, numerous insect pest species consume soybean. The most damaging defoliating insects of soybean in the southern U.S. are the green cloverworm, *Plathypena scabra* (Fabricius), velvetbean caterpillar, *Anticarsia gemmatilis* (Hübner), and soybean looper, *Chrysodeixis includens* (Walker) (Higley and Boethel 1994). Soybean looper cost growers more than \$10/acre in expenses and yield loss during 2012 (Musser et al. 2013). In Louisiana, 95% of the soybean acreage was treated for this pest with a yield loss plus cost of insecticide treatment reaching \$26 million (Musser et al. 2013). In addition, soybean looper is highly resistant to most insecticides and few products exist for control (Leonard et al. 1990). Alternative control

strategies are needed.

### **1.1 Soybean Looper Life Cycle**

Soybean looper, *Chrysodeixis includens* (Walker), is a leaf defoliator that belongs to the family Noctuidae in the order Lepidoptera. Its life cycle includes egg, larval, pupal, and adult stages. The average duration of the egg, larval and pupal stages is 2.7, 13.4 and 7.0 days under rearing conditions at  $26.5 \pm 1$  °C (Canerday and Arant 1967), and adult males and females can live for 13.5 and 14.1 days respectively at 26.7 °C (Mitchell 1967). Larvae typically develop through six stages (Canerday and Arant 1967).

Soybean looper eggs are small, round, and greenish-white, laid singly on the underside of leaves of the mid-to-lower canopy. Soybean looper larvae are pale green or green with longitudinal white stripes along the sides with small dark spots on the abdomen. Larvae have pale hairs and can reach 45 mm in length. The body of larvae has three pairs of prolegs, tapers gradually from the rear to the head, and crawl with a distinct looping motion. Larvae spin loose silken cocoon under leaves and then pupate in it. The pupa is greenish or greenish-white with irregular black spots in it. The adult has mottled brown forewings and dusty brown hind wings. There are various brown and gray shades and silvery white markings at the forewings. The wingspan measures about 30 to 39 mm (Canerday and Arant 1967). The forewings are darker in color than the hindwings and a silver figure-8 marking appears near the middle of the distinctive forewings (Smith 1994).

### **1.2 Soybean Looper Biology**

Soybean looper is a polyphagous species that has 73 known host-plant species in 29 families (Herzog 1980) including important agricultural crops such as sweet potatoes, tomatoes, okra, and beans (Bottimer 1926). However, soybean is the most preferred host for its feeding and

oviposition (Herzog 1980). Soybean looper larvae attack soybean plants by feeding on the foliage, with the lower two thirds of the crop canopy as a preferred feeding area for this insect (Herzog 1980). Caterpillars move upward and outward from the inside of the canopy and then consume the entire plant, leaving a ragged appearance at heavy infestations (Smith 1994). For each larva, approximately 114 cm<sup>2</sup> of leaf material is consumed during its whole larval stage (Reid and Greene 1973). Initial feeding of soybean looper larvae does not cause economic damage to soybean yield because the foliage consumption is relatively minimal and soybean plants can withstand up to 30% defoliation before blooming stage (R2) (Fehr et al. 1971, Trunipseed and Kogan 1987). Later foliage consumption of soybean looper during fourth to sixth instars accounts for 95% of the total defoliation (Sullivan and Boethel 1994). This is detrimental to yield because the period of greatest leaf consumption by soybean looper coincides with soybean growth stages after R3 (pod formation) (Carter-Wientjes et al. 2004).

Soybean looper adults migrate annually from overwintering areas in Florida, southern Texas, and the Caribbean Islands (Mitchell et al. 1975) to build economically damaging populations in Gulf Coast States (Herzog 1980). Three to four generations can occur in the Mid-South in one soybean growing season (Funderburk et al. 1999). In Louisiana, this insect can be found in May or June when cotton begins to flower (Sullivan and Boethel 1994). Adult females feed on cotton nectar that provides more carbohydrates than soybean flowers for egg production. Females then move to adjacent soybean fields to lay eggs on the bottom side of soybean leaves (Jensen et al. 1974, Carter-Wientjes et al. 2004). Soybean looper outbreaks usually occur in late August and early September in the cotton-soybean ecosystem where cotton and soybean are planted as principal crops (Burleigh 1972).

### **1.3 Soybean Looper Management**

Since soybean looper is an important pest in soybean, a variety of control measures have been applied to suppress its population. Recommendations for preventing yield loss from defoliating insects in soybeans were designed not to exceed 30% defoliation in vegetative stages and 20% in reproductive stages (Layton and Boethel 1998). In addition, scouting once a week during vegetative stages and twice weekly from early flowering to late podding is recommended (Bailey 2007). The economic threshold was established at 8 and 4 worms that were longer than one half inch per row foot during vegetative stages and pod filling stages, respectively (Kogan 1976).

Historically, broad-spectrum insecticides have been the major tools to control soybean loopers. These include cycloidenes, organophosphates, carbamates, and pyrethroids (Boethel et al. 1992). However, soybean loopers have become difficult to manage in recent years (Temple et al. 2008). Overuse of these insecticides has led to resistance (Leonard et al. 1990). Insecticides have also resulted in non-target effects on natural enemies which flare populations (Temple et al. 2008). Although soybean looper is a migratory pest insect, this species can be used as a host by native parasitoids and fungi in southern soybean fields (Gianessi 2009). Parasites and fungal pathogens provided some impact on reducing soybean looper population in the field. Burleigh (1972) reported that seven different parasite species and two fungal pathogens attacked soybean looper in three different agricultural ecosystems, suggesting that some of these biotic control factors may be potentially useful for soybean looper management when the population density reached a high level. However, economic damage usually occurs before these natural control agents can reduce soybean looper populations (Gianessi 2009). Smith (1994) also reported that naturally occurring parasites and pathogens have not provided much help in controlling soybean looper in recent years because they only affect a small portion of soybean looper populations.

Soybean plant breeding for insect resistance to herbivory has been studied since 1970s, but there is limited success probably due to the quantitative nature of resistance (Narvel et al. 2001). Three soybean varieties with defoliator resistance were commercialized in the United States: ‘Crockett’, ‘Lamar’, and ‘Lyon’ (Boethel 1999). However, none of these were widely accepted by growers due to low yield, late maturity, and inadequate resistance levels (Lambert and Tyler 1999, Gianessi 2009).

#### **1.4 Induced Host Plant Resistance**

In order to protect themselves from pathogens and herbivores, plants have developed a wide range of strategies. These strategies consist of two categories of defenses: constitutive defenses and induced plant defenses. Constitutive defense is resistance that is expressed regardless of the previous history of the plants, which includes physical and chemical barriers that are always present and active in plants. In contrast, induced defenses include defensive mechanisms that become activated after plants are attacked by herbivores (Karban and Myers 1989). Induced defense can be categorized into direct and indirect resistance. Direct induced resistance is characterized by reducing host plant quality and preference for subsequent herbivorous insects through changes in host plant biochemistry, physiology, or morphology, which could negatively impact the herbivore’s physiology and could reduce the preference or performance of feeding herbivores (Karban and Baldwin 1997). Except for the direct induced resistance, plants also defend themselves indirectly by releasing volatile organic compounds (VOCs) that may attract natural enemies of herbivorous insects to reduce the populations of insect pest. Induced plant resistance has been well studied and reported in more than 100 plant species, which included agricultural crops like soybean (Lin and Kogan 1990), tomato (Thaler et al. 1996), and cotton

(Karban and Carey 1984). These induced resistance strategies could provide significantly ecological and economic benefits to global agriculture and environment.

### **1.5 Elicitors of Induced Plant Resistance**

Plants protect themselves against herbivores by reducing plant suitability or palatability to reduce the performance and preference of herbivores directly, and by attracting natural enemies of herbivores indirectly with VOCs. In natural systems, because the direct and indirect induced defenses are not presented at maximum levels all the time; various stimuli are needed to induce higher levels of resistance (Karban and Badwin 1997, Walling 2000). Multiple biotic and abiotic challenges such as drought, nutrient deficiency, low/high temperature, and previous damage by herbivores can result in various plant responses (Karban and Myers 1989). Herbivores can produce elicitors that trigger plant gene expression to create toxic secondary metabolites that reduce herbivore feeding, growth, fecundity, and fertility, and also produce VOCs that attract parasites and predators (Walling 2000, Stout et al. 2002, Heil et al. 2001). Likewise, elicitors, natural or synthetic chemicals, have the similar or even equal roles in inducing plant responses as natural herbivores (Karban and Kuć 1999, Stout et al. 2002). Induced responses caused by elicitors can potentially protect crop plants by activating direct and indirect resistance against pests at proper time during the development of crops (Stout et al. 2002). These elicitors provide an opportunity to investigate induced resistance at the absence of natural herbivores (Stout et al. 2002).

Jasmonic acid (JA) and salicylic acid (SA) are the best documented elicitors for inducing resistance to herbivores. JA is reported to involve in signaling transduction pathways in response to mechanical wounding and damage by some species of herbivores (Staswick and Lehmann 1999). Plants respond to a broad range of pathogens and some insects by those

signaling pathways that are related to SA (Dempsey et al. 1999). In addition to JA and SA, a number of other synthetic chemicals have been used as resistance elicitors (Buzi et al. 2004); such as Actigard 50WG (acibenzolar-S-methyl) (Syngenta Crop Protection, Greenboro, NC), Regalia (extract of *Reynoutria sachalinensis*) (Marrone Bio Innovations, Davis, CA), and MeJA (methyl jasmonate) (Bedoukian Research, Danbury, CT). Actigard 50WG, Regalia, and MeJA are well known elicitors of SAR, systemic acquired resistance, which is defined as the mechanism by which plants acquire a variety of systemic immunity when responding to previous localized infections (Ross 1961).

Actigard 50 WG has been registered as a plant fungal and bacterial pathogen protectant and has been reported to induce resistance to cereal, tobacco, cucumber and soybean pathogens by activating the plants' own defense mechanisms (Srinivas and Danielson 2001). Actigard 50 WG is a novel compound that mimics host-pathogen interactions in plants in responding to pathogens (Perez et al. 2003). Gorlach et al. (1996) demonstrated that a single application of Actigard 50 WG provided long lasting protection in wheat and rice against fungal diseases. In addition, Actigard 50WG has been reported to induce resistance to insects. Exogenous application of Actigard 50 WG reduced the population of leaf miner in tomatoes and this insect preferred to consume untreated leaves in field (Inbar et al. 1998). Furthermore, the exogenous use of Actigard 50 WG in soybean can induce resistance to bean leaf beetle and reduce its defoliation (Srinivas and Danielson 2001). Thus, an application of Actigard 50WG has the potential to protect plants against both pathogens and herbivores.

Regalia, extract of *Reynoutria sachalinensis*, is a wettable powder formulation that has been commercialized for control of powdery mildew and other pathogens (Daayf and Bédanger 1995). Applying Regalia as a foliar spray in plants was very effective for controlling a wide range of

fungus and bacterial diseases by the unique mechanism of induced plant resistance (Su et al. 2012). Daayf and Bélanger's study (1995) showed that exogenous application of *Regalia* in Long English cucumber significantly reduced the incidence of powdery mildew. In addition, treatments with extracts from *R.sachalinensis* have been proposed to prevent infection of tomato, apple, and begonia against powdery mildew in greenhouse (Herger and Klingauf 1990).

MeJA, methyl ester of jasmonic acid, is a volatile organic compound used in a wide range of developmental pathways such as seed germination, flowering, fruit development, and leaf senescence. MeJA and JA are found in a variety of plant species and they are also defined as endogenous regulators of wound-induced compounds in plants (Wasternack and Hause 2002). In addition, jasmonates are considered potent elicitors or signaling agents, and they are viewed as mediators of plant responses to herbivores (Staswick & Lehman 1999). Previous research has documented that MeJA is involved as a signaling agent in plant cellular responses, plant-and-plant interactions, as well as plant-and-herbivore interactions, (Cheong and Choi 2003). Franceschi et al. (2002) demonstrated that exogenous use of MeJA to spruce tree could induce defense response to bark-beetle attack. In addition, lima beans treated with JA and MeJA produced a volatile mixture, which was the same as plants damaged by the two-spotted spider mite *Tetranychus urticae* Koch (Dicke et al. 1999).

Some of these elicitors have been commercialized and used to control plant diseases, with few focusing on the herbivores. Therefore, more research is needed to study the effectiveness of elicitors on herbivore management (Tally et al. 1999, Lyon and Newton 1999, Stout et al. 2002).

## **1.6 Potassium (K) Fertilizer**

Fertilizer is an important input for increased agricultural productivity (Yardtm and Edwards 2003). Inorganic fertilizers, especially nitrogen (N), phosphorus (P) and Potassium (K) are



critical nutrients in maintaining or improving crop yields (Belay et al. 2002). Potassium (K) is one of the primary crop nutrients and its function on plants has been studied since the 1950s (Dibb 1998). It is well known that K is involved in numerous plant physiological and biochemical processes including enzyme activation, photosynthesis, assimilate transport, protein synthesis, and plant water relations (Pettigrew 2008). Wyn Jones and Pollard (1983) reported that almost 60 different enzymes involved in plant growth depend on activity of potassium and these enzymes have critical functions in organic compounds and nitrogen metabolism. These physiological roles have direct impacts on plant growth, yield production and crop quality. Insufficient potassium can cause yield loss due to reduced production of photosynthetic assimilates. Pettigrew and Meredith (1997) have reported that short plant stature is likely to be observed when grown under K-deficiency conditions, which is often accompanied by decreased number and size of leaf tissues. Besides crop yield, crop quality is also determined by K nutrition. Grain protein content and amino acid content were increased by potassium fertilization applications in maize (Usherwood 1985) and cotton fiber traits were reduced when cotton was not supplied sufficient potassium (Pettigrew 1999).

Potassium nutrition is involved in uptake and utilization of other nutrients. N is the most critical component of amino acids and proteins in plants. A positive relationship was observed between K supply and the utilization of N in protein synthesis in rice (Mengel et al. 1976) and tobacco (Koch and Mengel 1974). For the family of Leguminosae, it is well known that they can fix their own nitrogen through nodulation. Studies have been conducted to evaluate the effects of K nutrition on nitrogen fixation in the leguminous plants. Parthipan and Kulasooriya (1989) and Lynd et al. (1983) reported that K was positively related to the activation of specific

nitrogenases. Moreover, Cowie (1951) also reported that K and P increased the number of nodules and the efficacy of nitrogen fixation in soybean.

### **1.7 Effects of K Nutrition on Insect Development in Plant**

Changes of nutrient availability may influence herbivore population size and/or herbivore development. Previous studies have shown that potassium is a critical component in the synthesis and allocation of primary metabolites in plants, and such physiological traits influence metabolic, hormonal and signaling pathways in plants. These changes can have strong impacts on plant susceptibility and attractiveness to insects and diseases (Amtmann et al. 2008). More than 2000 studies have been reviewed by the International Potash Institute (Perrenoud 1990) to compile the effects of K nutrition on pest incidence, with 63% demonstrating that application of K fertilizer reduced insects and mites in plants (Amtmann et al. 2008). But it is also true that K fertilizer sometimes has no effect on insect pest development.

In general, both plant nutrition levels and insect pest attack could change plant metabolic, hormonal and signaling pathways, and these pathways could affect each other and have significant effects on plant susceptibility against insect pests (Amtmann et al. 2008). For example, some K-deficient plants prefer to accumulate low-molecular weight organic compounds like sugars, organic acids, and nitrate, while failing to synthesize high-molecular weight compounds such as starch, proteins, and cellulose. Such low-molecular weight organic compounds in both leaves and roots make plants more susceptible and attractive to some insect pests (Marschner 1995, Amtman et al. 2008).

K-deficiency can increase plant defenses. It induces various genes related to JA that are important plant hormones related to signaling transduction pathways responding to mechanical wounding and damage by some herbivores (Armengaud et al. 2004, Staswick and Lehman

1999). It is clear that there is a relationship between K nutrition levels and plant resistance due to the interactions of signaling pathways when plants are subject to both nutritional and insect pest stress. Furthermore, increased susceptibility and attractiveness to insect pests caused by physical and metabolic changes can be counteracted by increased plant resistance in plants (Amtman et al. 2008). More detailed information is needed to document the relationship between K fertilization and specific herbivores in the field.

### **1.8 K Fertilizer and Soybean Looper in Soybean**

N is the most limiting to plant feeding insects and the most important for cellular growth. However, not all crops receive supplemental nitrogen. Soybean fixes its own nitrogen via rhizobacteria and N fertilization can inhibit nodulation. Potassium and phosphorus are often limiting in Louisiana rice/soybean rotations and supplemental fertilizer is added. High K availability in soybean fields may affect soybean looper populations. Previous work on soybean has shown that K can increase velvetbean caterpillar, *Anticarsis gemmatilis* (Hubner), but not southern green stink bug, *Nezara viridula* (L.), population densities (Funderburk et al. 1991). Studies should be conducted to estimate the interactions of K fertilization and soybean looper development in soybean because excessive K fertilization in soybean may increase soybean looper pressure in the field.

### **1.9 Objectives**

The objectives of this research were to 1) determine the effects of induced resistance by exogenous application of Actigard 50 WG, MeJA, and Regalia on soybean looper development in soybean plants, 2) evaluate the effect of potassium fertilization rates on soybean yield and soybean looper development.

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## **CHAPTER 2**

# **EFFECTS OF INDUCED PLANT RESISTANCE IN SOYBEAN TO SOYBEAN LOOPER (*CHRYSODEIXIS INCLUDENS*) BY EXOGENOUS ELICITORS**

### **2.1 Introduction**

Soybean, *Glycine max* (L.) Merrill, is the second most planted field crop in the United States with a total of 77.2 million acres grown in 2013 (NASS 2013). Like other agricultural crops, soybean plants can be damaged by a variety of insect pests feeding on leaves, stems, roots, nodules, and pods during different plant stages (Turnipseed and Kogan 1987, Turnipseed 1972). The soybean looper, *Chrysodeixis includens* (Walker), is an important defoliating pest of soybean in the southern U.S. with soybean being its preferred host for feeding and oviposition (Herzog 1980). Adults of soybean looper migrate annually from overwintering areas in Florida, southern Texas, and the Caribbean Islands to build economically damaging populations in Gulf Coast States (Mitchell et al. 1975, Herzog 1980). Three to four generations can occur in the Mid-South in one soybean growing season (Funderburk et al. 1999). Prior to R3 (Fehr et al. 1971), soybeans can tolerate up to 30% defoliation without yield loss (Turnipseed and Kogan 1987). However, during R3 to R5, defoliation greater than 20% can cause significant yield loss to soybean plants (Turnipseed and Kogan 1987).

Traditionally, insecticidal control strategies using organophosphates, pyrethroids, and carbamates were the main tools for soybean looper control (Boethel et al. 1992). However, the efficacy of insecticides has significantly decreased and soybean looper has become a difficult pest to manage in recent years (Temple et al. 2008). Widespread use of these insecticides has caused soybean looper to develop high insecticidal resistance (Felland et al. 1990, Boethel et al. 1992, Leonard et al. 1990). In addition, application of these insecticides has resulted in negative effects on non-target natural enemies and reduced their populations (Temple et al. 2008).

Induced plant resistance is an alternative way to manage soybean looper. Plants have evolved numerous strategies to defend themselves against insect attacks. These strategies can be classified as constitutive and induced defenses (Kempel et al. 2011). Constitutive defenses are always present regardless of herbivory, whereas induced resistance are generated by previous damage of herbivory or mechanical injury in plants (Smith 1989). Plant responses induced by previous herbivores are generally assumed to benefit plants by reducing the suitability or palatability of plants during current or subsequent attack (Stout et al. 2009). Induced resistance can reduce the preference and/or performance of herbivores by negatively affecting behavior and growth, which has been studied and reported in more than 100 plant species (Karban and Baldwin 1997). Induced resistance in soybean has also been studied and stated previously (Kogan and Fischer 1991, Chiang et al. 1986, Bi et al. 1994). Chiang et al. (1986) reported that Mexican bean beetles, *Epilachna varivestis* (Mulsant), were subjected to delayed development resulting from previous Mexican bean beetle herbivory. Bi et al. (1994) also observed that corn earworm, *Helicoverpa zea* (Boddie), larvae induced resistance in soybean plants, resulting in reduced larval growth rates.

Elicitors, natural or synthetic chemicals, can trigger plant resistance against herbivores and provide a tool to investigate the effect of induced resistance on herbivory without natural herbivores (Stout et al. 2002, Wittstock and Gershenson 2002). Induced responses by application of elicitors have the similar or even the same roles in inducing plant responses by natural herbivores (Karban and Kuć 1999, Stout et al. 2002). Stout et al. (2009) showed induced resistance produced by exogenous application of JA to fall armyworm *Spodoptera frugiperda* in rice. JA is one of plant hormones in response to mechanical wounding and some herbivore injury that activate the expression of resistance-related compounds to protect plants from

herbivores (Thaler et al. 1996, Korth and Thompson, 2006). Franceschi et al. (2002) also documented that exogenous use of methyl ester of jasmonic acid (MeJA) to spruce trees caused induced defense response against bark-beetle attack. MeJA is a volatile organic compound for activating plants' defenses against insect-driven wounding and various pathogens (Wasternack and Parthier 1997, Franceschi et al. 2002).

Because soybean can tolerate significant defoliation without affecting yield and high caterpillar infestations in soybean in the southern United States occur in late August and September when most soybeans are in the R2 to R5 stage (Herzog et al. 1980), elicitor induced resistance may be an alternate means of controlling soybean looper populations. In this study, three different elicitors, Actigard 50WG (acibenzolar-S-methyl), Regalia (extract of *Reynoutria sachalinensis*) and MeJA (methyl jasmonate), were applied to soybean plants at two different growth stages, R2 (full flowering) and R5 (pod filling) respectively, to evaluate the efficacy of induced plant resistance on soybean looper. Actigard 50 WG (Syngenta Crop Protection, Greenboro, NC) has been registered as a plant fungal and bacterial pathogen protectant for tomatoes, spinach and tobacco; and has been documented to induce resistance against pathogen in tomato, pepper, tobacco, rice, cotton and soybean (Mondal et al. 2005, Faessel et al. 2007, LaMondia 2008, Cole 1999, Babu et al. 2003). Regalia (Marrone Bio Innovations, Davis, CA) is a commercial product of extracts from leaves of *Reynoutria sachalinensis* and exogenous treatment with Regalia can induce resistance in long English cucumber, tomato, apple and grape against powdery mildew and grey mold (Schmitt 2002). The objectives of this study were to determine the effects of a single field application of Actigard 50WG, Regalia, or MeJA on field grown soybean on mortality, leaf consumption, pupal weight, egg hatch rate, and development time of soybean looper.

## 2.2 Materials and Methods

**2.2.1 Insect Colony.** The soybean looper colony (MR08) used in this study was collected from soybean fields using a standard cloth sweep net with a 38.1 cm opening at Macon Ridge Research Station in Winnsboro, LA in 2008 (Brown 2012). The colony is maintained as follows. Larvae were reared in 30-ml plastic cups (2 larvae/ cup) with about 10 ml artificial soybean looper diet (Southland Products, Lake Village, AR). The larval-rearing cups were placed in a rearing room with 28.5 °C, 50% RH and 14:10 (L:D) photoperiod until pupal stage (Mascarenhas and Boethel 2000). Pupae collected from larval-rearing cups were placed into plastic rearing containers containing about 30 g of vermiculite (Sun Gro, Bellevue, WA). Two 30-ml plastic cups containing towel paper saturated with approximate 10% honey solution were placed in the center of the container as supplementary nutrition for the adults (Mascarenhas and Boethel 2000). The inside of the container was covered with strips (8 x23 cm) of paper towel as oviposition sheets and the container was sealed with a muslin cover (Jensen et al. 1974). The 10% honey solution and oviposition sheets were changed every 2 days and egg sheets were placed into plastic bags until eclosion (Brown 2012).

**2.2.2 Field Planting and Chemical Induction.** Soybean variety Pioneer 94Y90 (Pioneer Hi-Bred, Johnston, IA) was used in the field experiments at Ben Hur Research Station, LSU AgCenter, Baton Rouge and Dean Lee Research Station, LSU AgCenter, Alexandria, LA in 2011 (experiment A), and at Ben Hur Research Station and Burden Research Station, LSU AgCenter, Baton Rouge, LA in 2012 (experiment B). In each experiment, seeds were sown at 8 seeds per 30 cm with 76 cm centers. In experiment A, the plots were 7.6 m long by 4 rows wide and two treatments, Actigard 50 WG (Syngenta Crop Protection, Greenboro, NC) at 21 mg/m<sup>2</sup> (Actigard 3X) and negative control with water (UTC) were applied to each two rows at R2 (full

flower) stage. In experiment B, a total of six treatments were used: water (negative control; UTC), Belt SC (positive control; Bayer Crop Science, Research Triangle Park, NC) at 21.0  $\mu\text{l}/\text{m}^2$ , Actigard 50WG at 7.0  $\text{mg}/\text{m}^2$  (Actigard 1X), Actigard 50 WG at 21.0  $\text{mg}/\text{m}^2$  (Actigard 3X), Regalia (Marrone Bio Innovations, Davis, CA) at 0.47  $\text{ml}/\text{m}^2$ , and MeJA (Bedoukian Research, Danbury, CT) at 5.49  $\text{ml}/\text{m}^2$ . In soybean, R2 (full flower) and R5 (pod filling) stages play a very important role in soybean yield, and soybean plants are less tolerant for defoliation during the period between R2 to R5 stages (McWilliams et al. 1999, Turnipseed and Kogan 1987). Therefore, treatments were sprayed at R2 and R5 using a  $\text{CO}_2$  backpack sprayer equipped with Teejet 8006 flat spray nozzles, delivering 14.03  $\text{ml}/\text{m}^2$  at 2.1  $\text{kg}/\text{cm}^2$ . Soybean plants were sprayed thoroughly wet by each treatment. All treatments were arranged in a randomized complete block design (RCBD) with four and three replications in the experiment A and experiment B, respectively.

**2.2.3 Laboratory Bioassays.** Two days after treatment, fifty leaf samples per treatment were randomly selected and brought back to laboratory. Each leaf was cut using a #149 Arch Punch (Osborne and Co., Harrison, NJ) to produce an 11.34  $\text{cm}^2$  leaf core. Soybean looper neonates previously placed on artificial diet for 24 hr to reduce handling mortality were transferred to leaf cores, one per core, and placed into a sterile petri dish (100×15 mm) with Whatman 90 mm (#1) filter paper wetted for moisture. Petri dishes were placed in a growth chamber at 26 °C, 75% RH and a 14:10 (L: D) photoperiod. Leaf cores were changed daily after the 4<sup>th</sup> day, while filter paper was wetted daily and changed when needed. Leaf core defoliation ( $\text{cm}^2$ ), mortality, and stadia of soybean looper were recorded daily. To estimate defoliation of each larva, defoliation percentage of each leaf core consumed by each larva was visually estimated and then calculated

based on the total area of each leaf core with 11.34 cm<sup>2</sup>. Pupae of soybean looper from each treatment were individually weighted and sexed according to Shorey et al. (1962).

At the end of feeding, developmental time from the 1<sup>st</sup> day of laboratory bioassay to pupation was calculated. Ten pairs (male and female) of soybean looper pupae were randomly selected from each treatment for mating and oviposition. Pupae were then placed into 3.8-L paper rearing containers (Huhtamaki Foodservice, De Soto, KS) containing about 30 g of vermiculite (Sun Gro, Bellevue, WA). A 30-ml plastic cup containing a towel paper saturated with approximate 10% honey solution was placed in the center of the container as supplementary nutrition for the adults. The inside of the container was covered with a strip (8×23 cm) of paper towel as oviposition sheets and the container was sealed with one single layer of pored poly plastic paper (23×31cm) (Jensen et al. 1974). The containers were maintained in a rearing room at 26 °C, 50% RH and a 14:10 (L: D) photoperiod. The 10% honey solution and oviposition sheets were changed daily. Oviposition sheets for each treatment were placed into a poly bag (23cm x 31cm) until hatching. The number of eggs deposited by soybean looper females on the oviposition sheets was counted using a head magnifying glass, and the number of neonates hatched from these eggs was also recorded. Percentage egg hatch was then calculated by dividing the number of hatched neonates by the number of eggs.

**2.2.4 Data Analysis.** Life tables were conducted to estimate population parameters of soybean looper following the methods of Birch (1948) in the experiment A. Age (x), age-specific female survival ( $l_x$ ), days to each stadia, and number of offspring produced by females at age x ( $m_x$ ) was recorded, and age-specific fecundity ( $l_x m_x$ ) was calculated. Intrinsic rate of increase ( $r_m$ ) was calculated as  $\sum e^{-rx} l_x m_x = 1$ , and net reproductive rate ( $R_0$ ) was calculated as:  $\sum l_x m_x$ . In the experiment A, data on intrinsic rate of increase ( $r_m$ ), net reproductive rate ( $R_0$ ), leaf

defoliation ( $\text{cm}^2$ ), pupal weight (g), and development time (day) were transformed to  $\log(x + 1)$  scale first, while mortality and egg hatch rate were converted to arcsine square-root scale for normal distribution. Transformed data were then analyzed by one-way analysis of variance (ANOVA) using PROC MIXED to determine the differences among the treatments. Means were separated using Turkey's honestly significant difference (HSD) tests at  $\alpha = 0.05$  level.

In experiment B, all soybean looper larvae were killed with Belt SC within 3 days when placed on leaf cores. Data collected from this treatment were excluded from statistical analysis. Data collected from the experiment B were net reproductive rate ( $R_0$ ), leaf defoliation, mortality, pupal weight, egg hatch rate, and development time of soybean looper. Similarly, the original data were first transformed using  $\log(x + 1)$  scale or arcsine square-root scale as described in the experiment A for normal distribution, and the transformed data were then analyzed by two-way analysis of variance (ANOVA) using PROC MIXED with elicitors and plant stages as two main factors. Means were separated using Turkey's honestly significant difference (HSD) tests at  $\alpha = 0.05$  level.

### **2.3 Results**

In experiment A, compared to the UTC, treatment effects for application of Actigard 3X on net reproductive rate ( $R_0$ ) ( $F = 14.68$ ;  $df = 1, 3$ ;  $P = 0.0313$ ), leaf defoliation ( $F = 6.20$ ;  $df = 1, 344$ ;  $P = 0.0133$ ), pupal weight ( $F = 166.30$ ;  $df = 1, 344$ ;  $P < 0.0001$ ), mortality ( $F = 20.57$ ;  $df = 1, 3$ ;  $P = 0.0201$ ), and egg hatch rate ( $F = 684.00$ ;  $df = 1, 3$ ;  $P = 0.0001$ ) were all significant. However, there were no significant differences for intrinsic rate of increase ( $F = 0.12$ ;  $df = 1, 3$ ;  $P = 0.7521$ ) and developmental time of soybean looper ( $F = 0.36$ ;  $df = 1, 343$ ;  $P = 0.5482$ ) between the two treatments. Soybean looper defoliation on Actigard 3X treated leaf was  $62.1 \text{ cm}^2$ , which was significantly reduced relative to  $63.5 \text{ cm}^2$  on UTC (Table 2.1). In addition, pupal weight



Table 2.1. Induction effects (mean  $\pm$  se) of Actigard 50 WG on soybean looper in 2011 (experiment A)\*

Parameters	UTC	Actigard 3X	<i>F</i> -value	<i>P</i> -value
Intrinsic rate of increase (rm)	1.126 $\pm$ 0.061 a	1.106 $\pm$ 0.051 a	$F_{1,3} = 0.12$	0.7521
Net reproductive ( $R_0$ )	514.5 $\pm$ 53.5 a	305.8 $\pm$ 41.3 b	$F_{1,3} = 14.68$	0.0313
Defoliation (cm <sup>2</sup> )	63.5 $\pm$ 1.1 a	62.1 $\pm$ 1.2 b	$F_{1,344} = 6.20$	0.0133
Pupal weight (g)	0.173 $\pm$ 0.001 a	0.147 $\pm$ 0.001 b	$F_{1,344} = 166.30$	<0.0001
Mortality (%)	2.50 $\pm$ 1.26 b	23.00 $\pm$ 6.56 a	$F_{1,3} = 20.57$	0.0201
Egg hatch rate (%)	93.6 $\pm$ 0.7 a	82.9 $\pm$ 1.2 b	$F_{1,3} = 684.00$	0.0001
Development time (day)	13.91 $\pm$ 0.12 a	13.90 $\pm$ 0.13 a	$F_{1,344} = 0.36$	0.5482

\*Means within a row followed by the same letter are not significantly different ( $P > 0.05$ ; Tukey's HSD).

obtained from Actigard 3X treated leaves was only 0.147 g, which was significantly lower than that 0.173 g from UTC (Table 2.1). Overall, results from the experiment A conducted in 2011 suggested that application of Actigard 3X on soybean plants induced resistance and caused negative impacts on the development and performance of soybean looper.

In experiment B, all soybean looper larvae were killed with Belt SC within the average of 3 days of being placed on leaf cores. The results from the remaining treatments showed that the main effects of both elicitor and plant stage as well as their interactions were not significant on the net reproductive rate ( $F = 2.33$ ;  $df = 4, 18$ ;  $P = 0.0955$ ,  $F = 2.36$ ;  $df = 1, 18$ ;  $P = 0.1419$ , and  $F = 0.79$ ;  $df = 4, 18$ ;  $P = 0.5466$ , respectively), mortality ( $F = 2.66$ ;  $df = 4, 18$ ;  $P = 0.0667$ ,  $F = 0.21$ ;  $df = 1, 18$ ;  $P = 0.6550$ , and  $F = 0.38$ ;  $df = 4, 18$ ;  $P = 0.8232$ , respectively) and egg hatch rate ( $F = 1.91$ ;  $df = 4, 18$ ;  $P = 0.1530$ ,  $F = 10.48$ ;  $df = 1, 18$ ;  $P = 0.0500$ , and  $F = 0.03$ ;  $df = 4, 18$ ;  $P = 0.9979$ , respectively). However, both plant elicitor and plant stage significantly affected soybean looper defoliation ( $F = 41.58$ ;  $df = 4, 1295$ ;  $P < 0.0001$  for elicitors,  $F = 1039.25$ ;  $df = 1, 1295$ ;  $P < 0.0001$  for plant stages), pupal weight ( $F = 61.72$ ;  $df = 4, 1295$ ;  $P < 0.0001$  for elicitors,  $F = 493.74$ ;  $df = 1, 1295$ ;  $P < 0.0001$  for plant stages) and developmental time ( $F = 124.06$ ;  $df = 4,$

Table 2.2. Induction effects (mean  $\pm$  se) of three different elicitors on soybean looper in 2012 (experiment B)\*

Plant stage	Elicitors	Net reproductive ( $R_0$ )	Defoliation ( $\text{cm}^2$ )	Pupal weight (g)	Mortality (%)	Egg hatch rate (%)	Developmental time (day)
R2	Actigard 1X	1333.2 $\pm$ 72.8 a	70.8 $\pm$ 1.2 b	0.250 $\pm$ 0.002 a	12.67 $\pm$ 3.33 a	51.99 $\pm$ 4.07 a	14.66 $\pm$ 0.10 b
	Actigard 3X	1541.8 $\pm$ 180.2 a	68.5 $\pm$ 1.0 b	0.251 $\pm$ 0.002 a	18.00 $\pm$ 12.22 a	48.48 $\pm$ 0.68 a	14.95 $\pm$ 0.10 b
	MeJA	1218.9 $\pm$ 244.5 a	76.4 $\pm$ 0.9 a	0.223 $\pm$ 0.003 c	19.33 $\pm$ 5.21 a	51.07 $\pm$ 2.01 a	16.81 $\pm$ 0.12 a
	Regalia	1050.2 $\pm$ 85.9 a	71.5 $\pm$ 0.9 b	0.238 $\pm$ 0.003 b	7.33 $\pm$ 1.76 a	53.88 $\pm$ 1.67 a	14.64 $\pm$ 0.10 bc
	UTC	1505.3 $\pm$ 179.2 a	72.0 $\pm$ 0.8 b	0.251 $\pm$ 0.002 a	8.67 $\pm$ 2.91 a	55.48 $\pm$ 2.42 a	14.93 $\pm$ 0.09 b
R5	Actigard 1X	1039.1 $\pm$ 262.1 a	52.1 $\pm$ 0.8 e	0.213 $\pm$ 0.003 cd	16.67 $\pm$ 7.69 a	56.98 $\pm$ 3.07 a	14.60 $\pm$ 0.13 bc
	Actigard 3X	1514.9 $\pm$ 123.2 a	48.9 $\pm$ 0.7 e	0.210 $\pm$ 0.003 d	23.33 $\pm$ 5.81 a	54.31 $\pm$ 5.64 a	14.67 $\pm$ 0.14 b
	MeJA	846.2 $\pm$ 106.6 a	61.1 $\pm$ 0.9 c	0.184 $\pm$ 0.002 e	16.67 $\pm$ 5.46 a	57.35 $\pm$ 1.77 a	16.50 $\pm$ 0.17 a
	Regalia	1400.9 $\pm$ 477.6 a	55.8 $\pm$ 0.8 d	0.223 $\pm$ 0.002 c	8.00 $\pm$ 1.15 a	59.71 $\pm$ 3.04 a	14.54 $\pm$ 0.09 bc
	UTC	1243.1 $\pm$ 241.9 a	58.5 $\pm$ 0.7 dc	0.220 $\pm$ 0.002 c	6.67 $\pm$ 0.67 a	62.40 $\pm$ 1.33 a	14.20 $\pm$ 0.08 c
F-test	Elicitor	$F_{4, 18} = 2.33$ 0.0955	$F_{4, 1295} = 41.58$ < 0.0001	$F_{4, 1295} = 61.72$ < 0.0001	$F_{4, 18} = 2.66$ 0.0667	$F_{4, 18} = 1.91$ 0.1530	$F_{4, 1295} = 124.06$ < 0.0001
	Stage	$F_{1, 18} = 2.36$ 0.1419	$F_{1, 1295} = 1039.35$ < 0.0001	$F_{1, 1295} = 493.74$ < 0.0001	$F_{1, 18} = 0.21$ 0.6550	$F_{1, 18} = 10.48$ 0.0500	$F_{1, 1295} = 19.70$ < 0.0001
	Elicitor*Stage	$F_{4, 18} = 0.79$ 0.5466	$F_{4, 1295} = 8.52$ < 0.0001	$F_{4, 1295} = 11.23$ < 0.0001	$F_{4, 18} = 0.38$ 0.8232	$F_{4, 18} = 0.03$ 0.9979	$F_{4, 1295} = 3.46$ 0.0081

\*Means within a column followed by the same letter are not significantly different ( $P > 0.05$ ; Tukey's HSD).

1295;  $P < 0.0001$  for elicitors,  $F = 19.70$ ;  $df = 1, 1295$ ;  $P < 0.0001$  for plant stages). In addition, the interaction between plant stage and elicitor also showed significant differences on these three parameters ( $F = 8.52$ ;  $df = 4, 1295$ ;  $P < 0.0001$  for defoliation,  $F = 11.23$ ;  $df = 4, 1295$ ;  $P < 0.0001$  for pupal weight,  $F = 3.46$ ;  $df = 4, 1295$ ;  $P = 0.0081$  for development time). Overall, the average defoliation of soybean looper during R2 was  $71.8 \pm 1.3 \text{ cm}^2$ , which was significantly more than  $55.3 \pm 2.2 \text{ cm}^2$  during R5. Within each plant stage, the defoliation of elicitor treated leaf was either numerically or significantly reduced compared to the UTC, except for MeJA leaf, in which the leaf consumption was more than that on the UTC (Table 2.2). Treatment effects of elicitors were also significant on the pupal weight during these two stages. Pupal weight obtained from leaves sprayed with MeJA ( $0.223 \pm 0.003 \text{ g}$ ) and Regalia ( $0.238 \pm 0.003 \text{ g}$ ) were significantly reduced relative to the UTC ( $0.251 \pm 0.002 \text{ g}$ ) during R2; while significant effects of pupal weight, compared to the UTC ( $0.220 \pm 0.002 \text{ g}$ ), were observed on leaves treated with Actigard 1X ( $0.213 \pm 0.003 \text{ g}$ ), Actigard 3X ( $0.210 \pm 0.003 \text{ g}$ ), and MeJA ( $0.184 \pm 0.002 \text{ g}$ ) during the R5 (Table 2.2). As observed in the defoliation, the overall pupal weight during R5 was  $0.210 \pm 0.007 \text{ g}$ , which was significantly less than  $0.243 \pm 0.005 \text{ g}$  compared to R2. Delayed development of soybean looper was also found on the MeJA leaf during R2 ( $16.81 \pm 0.12 \text{ d}$ ) and R5 ( $16.50 \pm 0.17 \text{ d}$ ), which was significantly longer than that observed on the UTC of R2 ( $14.93 \pm 0.09 \text{ d}$ ) and R5 ( $14.20 \pm 0.08 \text{ d}$ ), respectively (Table 2.2). However, significant treatment effects on developmental time for the other three elicitors were not found in this experiment. Overall, results of experiment B suggest that exogenous application of elicitors had adverse effects on the development and performance of soybean looper.

## 2.4 Discussion

The results of this study showed that inducible responses of soybean plants by exogenous elicitors such as Actigard 50WG, MeJA, and Regalia in the field had adverse effects on the development and performance of soybean looper. Effects of Regalia induction on soybean looper were very limited compared to Actigard 50WG and MeJA either during both the R2 and R5 stages. In the experiment A, defoliation was significantly decreased on Actigard 50WG than on UTC leaves. Although the differences were statistically significant, the defoliation on Actigard 50WG was numerically close to UTC for a single soybean looper. Unlike lab conditions, in the fields a soybean plant could be damaged by numerous soybean loopers, which could make the defoliation differences between Actigard 50WG and UTC more obvious. In this study, MeJA showed most significant induced effects on soybean looper during both R2 and R5 stages. Several other studies have indicated that induced responses to MeJA-treatment than induced responses to Actigard treatment (Boughton et al. 2005, Buzi et al. 2004). In this study, MeJA caused a significant reduction in pupal weight with a 11.2% and 17.9% lower pupal weights relative to those in UTC leaf at R2 and R5 plant stages, respectively. Moreover, development of soybean looper on MeJA treated leaves was also significantly delayed with a 12.8% and 16.2% developmental time reduction compared to soybean loopers feeding on UTC leaves, on R2 and R5 growth stages, respectively. Previous studies also showed that application of exogenous JA could induce resistance to insects by reducing their performance and/or slowing down their development (Stout et al. 2009, Mészáros et al. 2011, Omer et al. 2001). Lin and Kogan (1990) found that soybean looper fed on leaves of previously injured soybean plants had an 8.5% longer developmental time and a 10.4% lower pupal weight compared to larvae fed on leaves of uninjured plants. Our results showed the retarding effects on soybean looper

development are greater than those found by Lin and Kogan (1990), further demonstrating that MeJA could induce defensive responses against herbivores, and showed the potential use of elicitors in field applications. Overall, defoliation, pupal weight and developmental time of soybean looper were significantly affected by feeding on elicitor treated leaves compared to the UTC in both experiments in this study. The results confirm that exogenous elicitors can trigger plant resistance against herbivores and provide a means for pest population control in the field.

Elicitor concentrations play a critical role in the process of triggering plant defense response (Rijhwani and shanks 1998). In this study, two different concentrations of Actigard 50WG were used. However, treatment effects of induced resistance by these three concentrations were different. In the experiment A conducted in 2011, Actigard 3X exhibited significant effects on net reproductive, defoliation, pupal weight, egg hatch rate and mortality of soybean looper, but no significant effects on intrinsic rate of increase and developmental time. In contrast, application of Actigard 1X and Actigard 3X in the experiment B showed significant effects only on defoliation and pupal weight. Srinivas and Danielson (2001) showed that the highest level of induction by Actigard against bean leaf beetle, *Cerotoma trifurcata* (Forster) feeding was produced at 25 ppm concentration with the treatments ranging from 0, 15, 25, 40, 60, to 80 ppm. In addition, Plažek et al. (2003) found defense responses of winter oilseed rape callus to different concentrations of elicitors were different and not positively correlated with the concentration. Similarly, Namdeo et al. (2002) also reported higher concentration (5.0 %) of elicitor extracts induced higher ajmalicine in plant cells compared to lower concentration (0.5%). However, the accumulation of ajmalicine was negatively impacted when the concentration was increased to 10.0% (Namdeo et al. 2002). All of these studies suggested the importance of using an appropriate concentration of elicitors to maximize the defense response of the plants. Because

our study was conducted in different years and plant stages, it is possible that Actigard 50WG induced response interacted with the environments and plant stages, and further confirmation of detailed correlations between concentration of Actigard 50WG and intensities of defense responses is needed. Moreover, Dann et al. (1998) suggested that multiple applications of Actigard were necessary in field and greenhouse soybean to reduce fungal disease incidence and severity. However, other studies indicated a single application of Actigard was sufficient to induce resistance in soybean against the pests (Gorlach et al. 1996, Srinivas and Danielson 2001). Our study demonstrated that a single application of Actigard could induce resistance in soybean against soybean looper either at R2 or R5 stages.

In this study, total defoliation by soybean looper was numerically or significantly greater on MeJA treated leaves than on UTC leaves. These results are consistent with the reports from Lin and Kogan (1990) who also found induced resistance increased the total leaf consumption of soybean looper. Studies have shown that the activity of phytoalexins and proteinase inhibitors was changed by induced resistance after subject to herbivory and jasmonic acid in soybean plants (Kogan and Fischer 1991, Accamando and Cronin 2012). These changes would decrease the nutritional quality of soybean leaves (Lin and Kogan 1990). When soybean looper consumes these low nutritional leaves, they may increase their total consumption in order to get equivalent nutrition (Lin and Kogan 1990). In addition, the low nutritional quality food could have some toxic impacts on insects, resulting in a delayed development and lower weight gain (Tabashnik and Slansky 1987, Lin and Kogan 1990). These results were further confirmed by our study; soybean looper development was significantly delayed and pupal weight was significantly lower on MeJA-treated leaves than on UTC leaves.

Plant development stage plays an important role in induced plant responses, and the level of induction is often higher in early plant stages or young plants (Cipollini and Redman 1999, Omer et al. 2001). Mészáros et al. (2011) reported that large plants produced higher levels of induced resistance than small plants to fall armyworm after plants were applied exogenously with JA. Similarly, Quintero and Bowers (2011) also showed that the ability of plant to stimulate defensive reaction was stronger at juvenile plant stages rather other mature plant stages. In our study, treatment effects on defoliation, pupal weight and developmental time were all significant between R2 and R5 stages, suggesting induced response by elicitors were different among plant stages. However, contrary to the previous findings that induced response is most pronounced in young stages, we found induced responses were stronger on R5 stages than on R2 stages for both Actigard and MeJA when compared UTC. In the field, environment is more complicated and likely to affect induced resistance expression (Walters et al. 2005). Dietrich et al. (2005) demonstrated that induction response on seed production was related to environmental conditions like nitrogen supply, nutrient and water stress, and weed competition. Therefore, application of elicitors at appropriate plant stages is critical to maximize the impact of resistance induction. Future research should also focus on the optimal concentration of elicitors in the field against soybean loopers and explore the possibilities of using elicitors like MeJA in combination with other managements for insect pest management in soybean.

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# **CHAPTER 3**

## **EFFECTS OF POTASSIUM FERTILIZER RATES ON SOYBEAN YIELD AND SOYBEAN LOOPER (*CHRYSODEIXIS INCLUDENS*) DEVELOPMENT**

### **3.1 Introduction**

Fertilizers are important inputs used to increase agricultural productivity (Yardtm and Edwards 2003). Inorganic fertilizers, especially nitrogen (N), phosphorus (P) and potassium (K) are critical nutrients in maintaining or improving crop yields (Belay et al. 2002). K is one of the primary crop nutrients and plays an important role in crop physiological growth processes and yield development (Pettigrew 2008). Previous studies have reported that K had an impact on the distribution of primary metabolites in plant tissues. Evans and Sorger (1966) demonstrated that K activated some enzymes that were involved in sugar and nitrogen metabolism. Blatt (1988) stated that K influenced the transport rate of nutrients in xylem and phloem through regulating cell opening and closing. Furthermore, K was also recognized as key in maintaining pH, voltage, charge balance, and osmotic gradient in plant cells (Streeter and Barta 1984, Mengel and Haeder 1977, Marschner 1995).

However, fertilizer supplement in crops may influence herbivore populations and/or their development. Plant nutritional changes could have an impact on metabolism and hormonal and signaling pathways, which may result in changes in plant susceptibility and attractiveness to insects and diseases (Amtmann et al. 2008). More than 2000 studies have been compiled by the International Potash Institute to summarize K supplement/pest interactions in the field and greenhouse (Amtmann et al. 2008, Perrenoud 1990). Approximate 63% of these studies reported that K applications reduce insect and mite populations in crops (Amtmann et al. 2008). Nevertheless, K fertilization in some cases may also simulate insect outbreaks and/or improve insect performance, while in other cases K fertilizer had no effects. Funderburk et al. (1991)

showed that application of K fertilizer on soybean plants increased population densities of velvetbean caterpillar, *Anticarsis gemmatilis* (Hubner), but had no effects on southern green stink bug, *Nezara viridula* (L.), population densities. Therefore, the interactions for insect performance and K fertilizer are dependent on specific insect species and plants.

Soybean looper, *Chrysodeixis includens* (Walker), is an important defoliator of soybean, *Glycine max* (L.) Merrill, in the southern U.S. (Herzog 1980). As a polyphagous insect, soybean looper has 73 known host-plant species in 29 families including several cultivated hosts, but soybean is the most preferred host for its feeding and oviposition (Herzog 1980). In 2012, 95% of the soybean acreage in Louisiana was treated for this pest with a yield loss plus cost of insecticide treatments reaching \$26 million (Musser et al. 2013). Furthermore, soybean looper is highly resistant to most insecticides and few products exist for control. The problem Louisiana is now facing is methoxyfenozide resistant soybean looper. Resistance ratios (resistance relative to a susceptible laboratory colony) of soybean looper to methoxyfenozide have been documented up to 24X in Louisiana (Brown 2012). Alternative control strategies (such as cultural control) are needed to extend insecticide life while reducing pest populations.

Soybean is one of major crops in the world, and it is an important food source in providing protein, oil, and minerals for humans and livestock. About one-third of the world's edible oils and two-thirds of its proteins are derived from soybeans (Maxwell and Hauppauge 2011). In the U.S., soybean is the second most planted crop and it requires high K availability for optimal yield because utilization of K in plants is critical for the vegetative growth, yield production and crop quality. Moreover, K has also been reported to affect nitrogen accumulation and carbon assimilation in plants, and a correlative relationship between N and K has been found in various crops such as maize, rice, and soybean (Barta 1982, Osaki 1995, Wojcieszka and Kocouř 1997). It

is well known that N is an essential ingredient of protein and chlorophyll in plants and the availability of N in host plants impacts phytophagous insect feeding and development (Wier and Boethel 1995; Scriber, 1984). Wier and Boethel (1995) demonstrated that soybean looper fed on leaves from soybean plants supplied with high rates of N fertilizer had less number of stadia and short larval development period. Similarly, development and performance of soybean looper on soybean plants could also be influenced by external application of K fertilizers. Understanding the effects K fertilization has on soybean looper population densities is an important step in controlling this pest.

The objectives of this study were to evaluate 1) the effects of K fertilizer rates on soybean yield and seed quality, 2) the relationship between N concentration and K fertilizer rates in soybean plants, and 3) the effects of K fertilizer rates on soybean looper development.

### **3.2 Materials and Methods**

**3.2.1 Field planting.** Soybean commercial variety DynaGro 31RY45 (Dyna-Gro, Richmond, CA) was planted in a field at the LSU AgCenter's Rice Research Station in Crowley, Acadia Parish, Louisiana on April 8, 2013. Seeds were planted at a rate of 321,236 seeds per hectare, and rows spacing was approximate 76 cm. Six different rates of K fertilizer; 0, 33.6, 67.3, 100.9, 134.5, and 168.1 kg/ha were applied at planting as  $K_2O$ . Different rates of K fertilizer was achieved by mixing  $K_2O$  with water and sprayed to the soil based on a spray volume of 200 L per hectare. These six different K rates were six different treatments in the experiment. Each treatment was applied into a plot of 4 rows wide by 7.0 m long. Treatments were arranged in a completely block design (CBD) with four replications.

**3.2.2 Insect colony.** The soybean looper colony (MR08) used in this study was collected from soybean fields using a standard cloth sweep net with a 38.1 cm opening at Macon Ridge

Research Station in Winnsboro, LA in 2008 (Brown 2012). The colony was maintained as follows. Larvae were reared in 30-ml plastic cups (2 larvae/ cup) with about 10 ml artificial soybean looper diet (Southland Products, Lake Village, AR). The larval-rearing cups were placed in a rearing room with 28.5 °C, 50% RH and 14:10 (L:D) photoperiod until pupal stage (Mascarenhas and Boethel 2000). Pupae collected from larval-rearing cups were placed into plastic rearing containers containing about 30 g of vermiculite (Sun Gro, Bellevue, WA). Two 30-ml plastic cups containing a towel paper saturated with approximate 10% honey solution were placed in the center of the container as supplementary nutrition for the adults (Mascarenhas and Boethel 2000). The inside of the container was covered with strips (8 x23 cm) of paper towel as oviposition sheets and the container was sealed with a muslin cover (Jensen et al. 1974). The 10% honey solution and oviposition sheets were changed every 2 days and egg sheets were placed into plastic bags until eclosion (Brown 2012).

**3.2.3 Leaf and seed analysis.** Whole soybean plants were destructively sampled when plants reached R1 and R3 (five plants), and R5 and R6 (two plants) growth stages, respectively (Fehr et al. 1971). All leaf tissues were removed from these plants and placed into paper bags by treatment with a total of 24 paper bags; four replicates per each treatment. Leaf tissues were maintained in a cooler box with ice packs and submitted to the Soil Testing & Plant Analysis Lab of Louisiana State University, Baton Rouge, LA, for nutritional analysis of K and N content. Soybean plants were harvested on 18 September 2013. Four rows of each plot were harvested in the field and seeds of each plot were put into paper bags and brought back to the laboratory. Total weight of soybean seeds from each plot was recorded. Soybean seed moisture was tested using a moisture meter (Model GAC®500 XT). Three samples of 100 soybean seeds were randomly selected from each plot and weighed and recorded. 275g seeds were randomly

selected from each plot and were sent to Analab (Division of Agri-King, Inc.) for nutritional analysis including the content of protein, oil, fiber, and K percentage with a total of 24 samples.

**3.2.4 Laboratory bioassays.** To reduce mortality due to handling, newly hatched neonates were maintained on 30-ml cups (2 larvae per cup) with 10-ml soybean looper artificial diet (Southland Products, Lake Village, AR) for 24 hours before laboratory bioassays. Fifty leaf samples per treatment were randomly selected at R1, R3, R5, and R6, respectively, and brought back to the laboratory. Leaves were cut using a #149 Arch Punch (Osborne and Co., Harrison, NJ) to produce leaf cores (11.34 cm<sup>2</sup>). A single soybean looper neonate and a leaf core were placed onto a sterile petri dish (100 X 15 mm) covered with a moistened Whatman 90 mm filter paper using a fine camel hair paintbrush. All Sterile petri dishes were maintained in a growth chamber at 26 °C, 75% RH and a 14:10 (L: D) photoperiod. A completely randomized design (CRD) was used in the laboratory bioassay with 4 replications and 50 neonates per replication for each treatment. New, fresh leaf cores and clean moistened filter paper were changed when needed. Soybean looper development in sterile petri dishes was checked daily until pupal stage. Leaf defoliation (cm<sup>2</sup>), mortality, and stadia of soybean looper were recorded daily. Larval developmental time was determined based on the number of days needed from neonate to pupae. Pupae were weighted individually to the nearest 1.0 mg and sexed according to the methodology described by Shorey et al. (1962). Ten pairs (male and female) of soybean looper pupae were randomly chosen from each treatment and were confined in a 3.8-L paper-rearing container (Huhtamaki Foodservice, De Soto, KS) with about 30g of vermiculite (Sun Gro, Bellevue, WA) for mating and oviposition. The containers were maintained in the same conditions as described above. The number of eggs deposited on the oviposition sheets was counted using a head magnifying glass, and the number of larvae hatched from these eggs was counted with a fine



camel hair paint brush. The egg hatch ratio was calculated by dividing the total number of hatched larvae by the total number of eggs.

**3.2.5 Data analysis.** Data on soybean seed yield (bu/A), 100 seed weight (g), soybean looper leaf defoliation (cm<sup>2</sup>), pupal weight (g), no. eggs/10 female, and development time (day) were transformed to log (x + 1) scale first, while soybean seed moisture (%), seed nutritional parameters including protein (%), oil (%), fiber (%), and K content (%), soybean looper mortality (%) and egg hatch rate (%) were converted to arcsine square root scale for normal distribution. Transformed data were then analyzed by one-way analysis of variance (ANOVA) using PROC MIXED to determine the differences among the treatments (SAS Institute 2011). In addition, data on K and N content (%) in leaf tissues were transformed with arcsine square root scale and analyzed by two-way ANOVA using PROC MIXED in SAS with K fertilizer rates and plant stage as two main factors (SAS Institute 2011). Means were separated using Turkey's honestly significant difference (HSD) tests at  $\alpha=0.05$  level.

### 3.3 Results

Treatment effects of K fertilizer rates on soybean seed moisture were not significant ( $F = 0.62$ ;  $df = 5, 15$ ;  $P = 0.6854$ ). The average moisture for soybean seed was 11.66% (Fig. 3.1). However, there were significant differences for soybean yield ( $F = 5.39$ ;  $df = 5, 14$ ;  $P = 0.0057$ ) and 100 seed weight ( $F = 3.15$ ;  $df = 5, 63$ ;  $P = 0.0134$ ). In general, a positive correlation was observed for K fertilizer rates and soybean yield as well as 100 seed weight (Fig. 3.2, Fig. 3.3). Increasing application of K fertilizer rates also enhanced soybean yield and 100 seed weight (Fig. 3.3). Soybean yield significantly increased at the highest K rate 168.1 kg/ha with 46.3 bu/A compared with other K rates (Fig. 3.2).

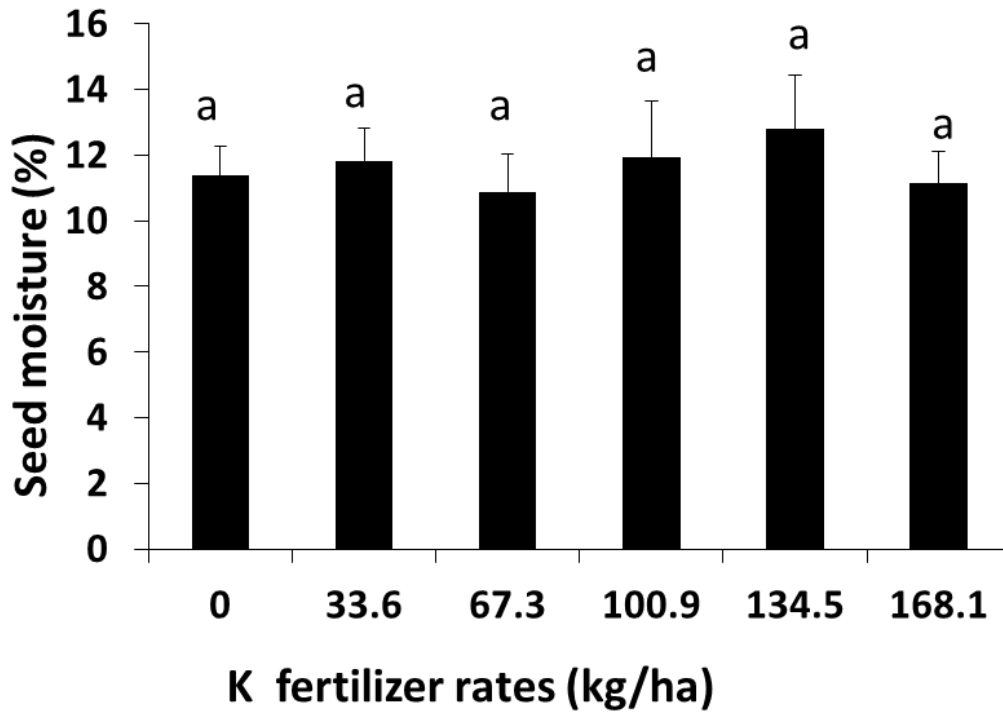


Figure 3.1. Soybean seed moisture (%) ( $\pm$ se) at different K fertilizer rates. Bars accompanied by the same letter indicate means do not differ significantly ( $P = 0.6854$ ).

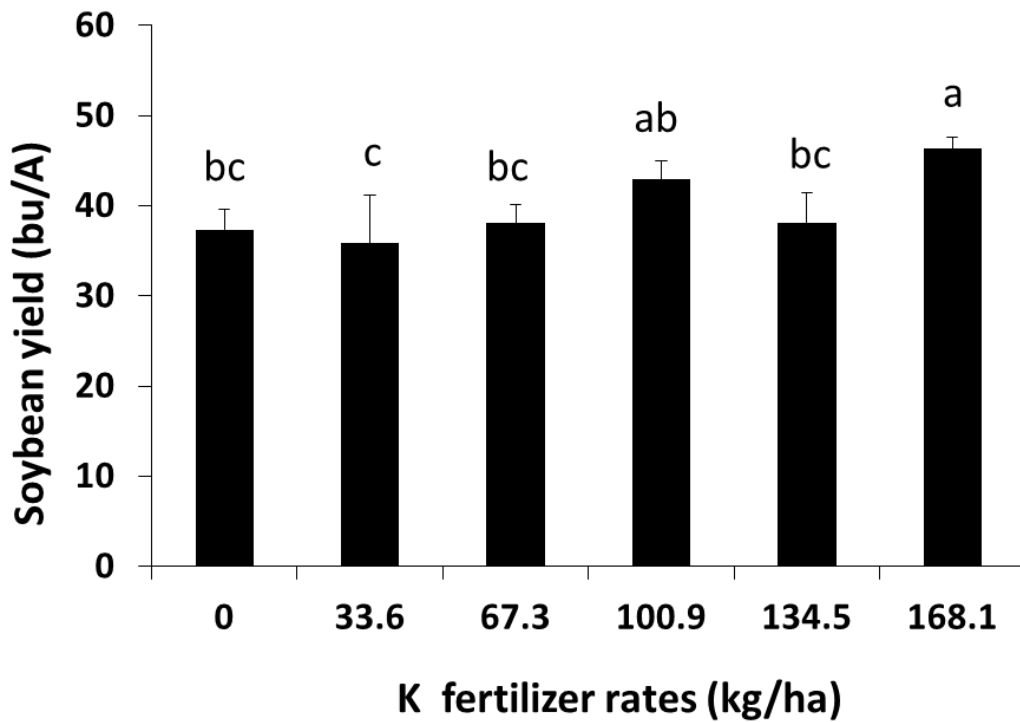


Figure 3.2. Soybean yield (bu/A) ( $\pm$ se) at different K fertilizer rates. Bars accompanied by different letters indicate means differ significantly ( $P = 0.0057$ ).

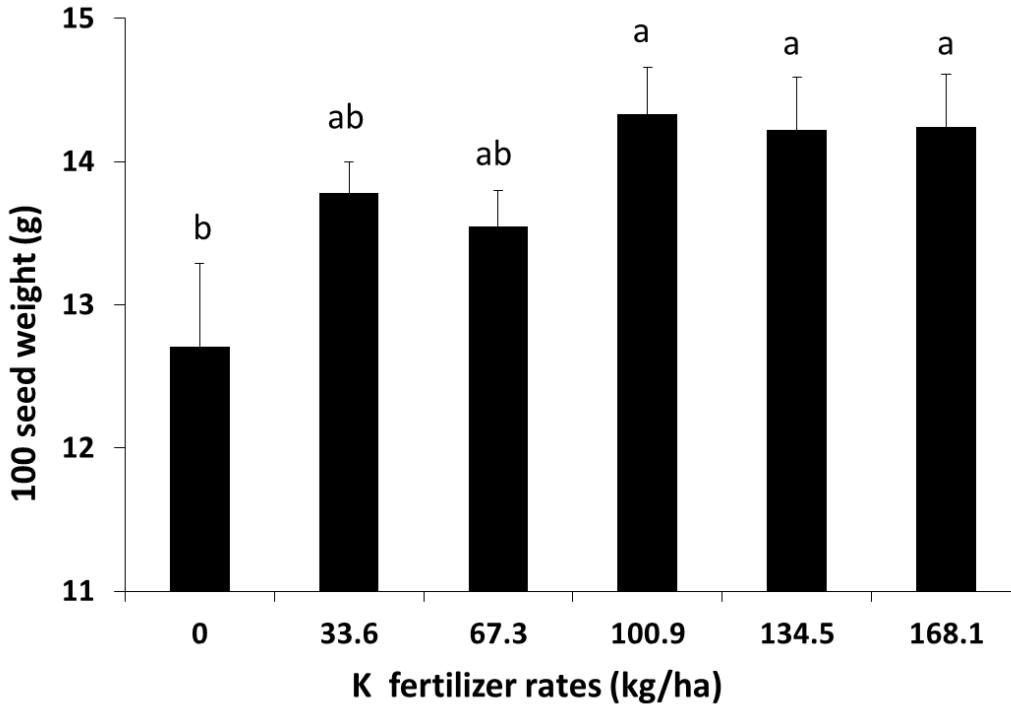


Figure 3.3. Soybean 100 seed weight (g) ( $\pm$  se) at different K fertilizer rates. Bars accompanied by different letters indicate means differ significantly ( $P = 0.0134$ ).

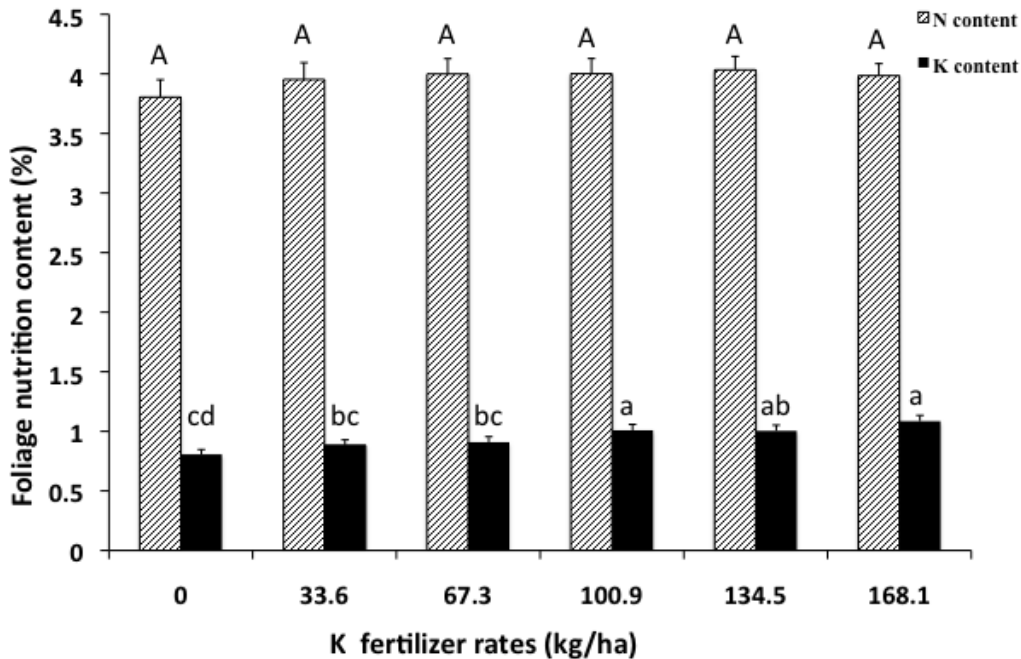


Figure 3.4. Foliage mineral content (%) ( $\pm$  se) of N and K at different K fertilizer rates. Bars accompanied by different *lower case* letters indicate means differ significantly for foliage K content ( $P < 0.0001$ ) while bars accompanied by the same *upper case* letter indicates means do not differ significantly for foliage N content ( $P = 0.3380$ ).

K fertilizer rates did not significantly affect soybean seed nutritional value in protein ( $F = 0.86$ ;  $df = 5, 15$ ;  $P = 0.5322$ ), fiber ( $F = 0.74$ ;  $df = 5, 15$ ;  $P = 0.6084$ ), and oil ( $F = 2.36$ ;  $df = 5, 15$ ;  $P = 0.0909$ ), but significant impact on K content ( $F = 4.60$ ;  $df = 5, 15$ ;  $P = 0.0096$ ). The average values for protein, fiber, and oil were 39.46 %, 8.81 %, and 22.05 %, respectively (Table 3.1). Increasing K fertilizer rates also improved soybean seed K content (Table 3.1). The highest seed K content was 1.82 %, which was observed on the highest K rate 168.1 kg/ha.

Table 3.1. Effects of K fertilizer rates on soybean seed nutritional value (mean  $\pm$  se)\*

Treatment	Protein (%)	Oil (%)	Fiber (%)	K content (%)
0 kg/ha	39.94 $\pm$ 0.66 a	22.14 $\pm$ 0.38 a	8.52 $\pm$ 0.43 a	1.69 $\pm$ 0.04 b
33.6 kg/ha	39.45 $\pm$ 0.42 a	22.37 $\pm$ 0.06 a	8.93 $\pm$ 0.30 a	1.69 $\pm$ 0.03 b
67.3 kg/ha	39.00 $\pm$ 0.29 a	22.30 $\pm$ 0.22 a	9.12 $\pm$ 0.28 a	1.73 $\pm$ 0.04 ab
100.9 kg/ha	39.62 $\pm$ 0.28 a	21.75 $\pm$ 0.22 a	8.50 $\pm$ 0.64 a	1.79 $\pm$ 0.03 ab
134.5 kg/ha	39.72 $\pm$ 0.35 a	21.80 $\pm$ 0.23 a	8.67 $\pm$ 0.26 a	1.78 $\pm$ 0.05 ab
168.1 kg/ha	39.01 $\pm$ 0.37 a	21.93 $\pm$ 0.40 a	9.12 $\pm$ 0.40 a	1.82 $\pm$ 0.03 a
<i>F</i> - value	$F_{5,15} = 0.86$	$F_{5,15} = 2.36$	$F_{5,15} = 0.74$	$F_{5,15} = 4.60$
<i>P</i> - value	0.5322	0.0909	0.6084	0.0096

\*Means within a column followed by the same letter are not significantly different ( $P > 0.05$ ; Tukey's HSD).

Two-way ANOVA results showed that the main effects of K fertilizer rates were not significant on the foliage N content ( $F = 1.16$ ;  $df = 5, 69$ ;  $P = 0.3380$ ), but plant stages significantly affected N content in soybean leaf tissues ( $F = 46.65$ ;  $df = 3, 69$ ;  $P < 0.0001$ ). The average N content in leaf tissues for the six K fertilizer rates was 3.963 % (Fig. 3.4). The highest N content (4.569 %) in leaf tissues was observed on R5 stage, while the lowest (3.447 %) was on R6 stage (Fig. 3.5). The interactions between K fertilizer rates and plant stages were not significant ( $F = 0.57$ ;  $df = 15, 69$ ;  $P = 0.8855$ ). The main factors of K fertilizer rates and plant stages all significantly affected the K content in soybean plant leaf tissues ( $F = 18.24$ ;  $df = 5, 69$ ;  $P < 0.0001$  for K fertilizer rates; and  $F = 84.00$ ;  $df = 3, 69$ ;  $P < 0.0001$  for plant stages). Overall, increasing K fertilizer rates also improved K content in plant leaf tissues. But the interactions

between K fertilizer rates and plant stages were not significant ( $F = 0.64$ ;  $df = 15, 69$ ;  $P = 0.8339$ ). Average leaf K content was 0.807, 0.883, 0.909, 1.009, 1.004, and 1.085 % for the treatments 0, 33.6, 67.3, 100.9, 134.5, and 168.1kg /ha, respectively (Fig. 3.4). The lowest (0.772 %) and highest (1.132 %) leaf K content were observed on the R6 and R3 stages, respectively, which were significantly different from other plant stages (Fig. 3.5).

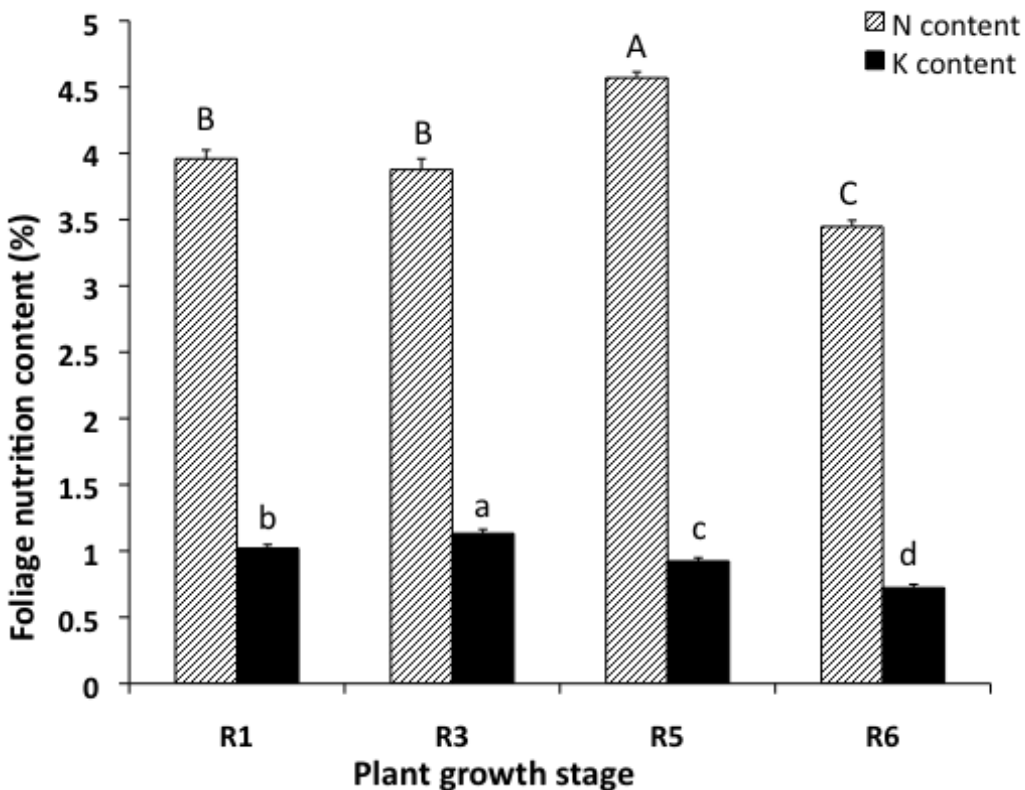


Figure 3.5. Foliage mineral content (%) ( $\pm$  se) of N and K at different soybean growth stages. Bars accompanied by different *lower case* indicate means differ significantly for foliage K content ( $P < 0.0001$ ) while bars accompanied by different *upper case* letters indicate means differ significantly for foliage N content ( $P < 0.0001$ ).

Laboratory bioassay showed that treatment effects for K fertilizer rates on soybean looper mortality ( $F = 0.23$ ;  $df = 5, 15$ ;  $P = 0.9940$ ), no. eggs/10 females ( $F = 1.88$ ;  $df = 5, 15$ ;  $P = 0.1583$ ), and egg hatch rate ( $F = 0.74$ ;  $df = 5, 15$ ;  $P = 0.6070$ ) were not significant. The average values for mortality, number of eggs/10 females, and egg hatch rate were 5.1%, 14,517.0, 58.6%, respectively (Table 3.2). However, K fertilizer rates significantly affected soybean looper

Table 3.2. Effects of K fertilizer rates on soybean looper (mean  $\pm$  se) \*

Treatment	% Mortality	Defoliation (cm <sup>2</sup> )	Pupal wt. (g)	No. egg/10 females	% Egg hatch	Development time (day)
0 kg/ha	0.030 $\pm$ 0.006 a	64.23 $\pm$ 0.52 b	0.209 $\pm$ 0.002 c	15918.75 $\pm$ 1207.12 a	0.602 $\pm$ 0.036 a	13.86 $\pm$ 0.09 a
33.6 kg/ha	0.055 $\pm$ 0.015 a	64.75 $\pm$ 0.46 b	0.212 $\pm$ 0.002 bc	13880.75 $\pm$ 2552.19 a	0.604 $\pm$ 0.035 a	13.68 $\pm$ 0.08 ab
67.3 kg/ha	0.070 $\pm$ 0.040 a	65.76 $\pm$ 0.45 ab	0.214 $\pm$ 0.001 bc	13262.00 $\pm$ 2834.22 a	0.578 $\pm$ 0.038 a	13.54 $\pm$ 0.08 bc
100.9 kg/ha	0.040 $\pm$ 0.014 a	64.50 $\pm$ 0.47 b	0.212 $\pm$ 0.002 bc	13775.50 $\pm$ 1626.90 a	0.583 $\pm$ 0.035 a	13.28 $\pm$ 0.08 c
134.5 kg/ha	0.050 $\pm$ 0.024 a	65.95 $\pm$ 0.49 ab	0.215 $\pm$ 0.001 ab	13772.50 $\pm$ 1898.95 a	0.556 $\pm$ 0.028 a	13.27 $\pm$ 0.09 c
168.1 kg/ha	0.060 $\pm$ 0.022 a	66.68 $\pm$ 0.48 a	0.221 $\pm$ 0.002 a	16492.75 $\pm$ 2628.78 a	0.590 $\pm$ 0.039 a	12.95 $\pm$ 0.07 d
<i>F</i> - value	F <sub>5,15</sub> = 0.23	F <sub>5,1131</sub> = 4.52	F <sub>5,1131</sub> = 8.18	F <sub>5,15</sub> = 1.88	F <sub>5,15</sub> = 0.74	F <sub>5,1131</sub> = 24.91
<i>P</i> - value	0.9440	0.0004	< 0.0001	0.1583	0.607	< 0.0001

\*Means within a column followed by the same letter are not significantly different ( $P > 0.05$ ; Tukey's HSD).

defoliation ( $F = 4.52$ ;  $df = 5, 1131$ ;  $P = 0.0004$ ), pupal weight ( $F = 8.18$ ;  $df = 5, 1131$ ;  $P < 0.0001$ ), and developmental time ( $F = 24.91$ ;  $df = 5, 1131$ ;  $P < 0.0001$ ). In general, soybean looper defoliation increased as K fertilizer rates increased. The highest defoliation by soybean looper was  $66.68 \text{ cm}^2$ , which was observed on the highest K rates  $168.1 \text{ kg/ha}$ . Larval development time of soybean looper was also significantly affected by K fertilizer rates. The higher K fertilizer rates, the less larval development time. Soybean looper neonates took only 12.95 days to become pupae at the highest K rate  $168.1 \text{ kg/ha}$ , which was significantly shortened compared with other K rates. Overall, soybean looper consumed more leaf tissues and spent less time completing larval development when fed on foliage from treatments of  $134.5$  and  $168.1 \text{ kg/ha}$ . Pupal weight was also significantly affected by the K rates. The higher the K rates, the more the pupal weight. The highest pupal weight was  $0.221 \text{ g}$  obtained from the highest K rate  $168.1 \text{ kg/ha}$  (Table 3.2).

### **3.4 Discussion**

Crop yield and quality are the most important factors impacting producer profits. Previous research has documented that K fertilization benefited crop yields in maize, wheat, cotton, and soybean (Pettigrew 2008, Bharati et al. 1986). Bharati et al. (1986) reported that high K fertility increased soybean yield due to increased number of pods per plant and the weight of individual seeds. In our study, soybean yield was significantly enhanced by the highest K fertilization rate. Moreover, 100 seed weight was also increased with higher K rates. Seed moisture is an indicator of plant maturity and K content could influence crop maturity. In our study, the seed moistures were identical among the different K rates, suggesting the maturity was not affected by the K rates. Soybean seed quality is also of great economic value. Protein and oil content are the two important parameters for the soybean seed quality and they were found to be affected by the K

fertilization (Weber 1985). Data from our study showed soybean seed protein and seed oil content did not increase with higher K fertilizer rates, which was consistent with the results shown by Haq and Mallarino (2005) and Seguin and Zheng (2006), but inconsistent with those found by Gaydou and Arrivets (1983) and Yin and Vyn (2003) who stated that K fertilization increased seed oil content while decreased seed protein content.

N and K are critical nutritional components for plants. It was reported that K promoted the incorporation of N into proteins, resulting in higher levels of proteins in crops such as tobacco, rice, and barley (Koch and Mengel 1974, Mengel et al. 1976, Helal and Mengel 1979). In addition, K has been reported to enhance specific nitrogenase activation and nitrogen fixation in the family Leguminosae (Cowie 1951, Parthipan and Kulasoorya 1989). However, in this study, N content in leaf tissues was not significantly different among the K rates. Similarly, protein content in soybean seeds was also identical for the six K fertilizer rates. The failure of a correlative relationship between N and K content in leaf tissues might be due to physiological and genotypic differences among soybean varieties. Soybean genotypic differences could influence its root systems such as root morphology and root hair, which lead to variability of K utilization efficiency (Rengel and Damon 2008). Thus, it is highly possible that soybean varieties result in different K utilization levels, which affected the relationship between K and N fixation in soybean. Furthermore, our results also showed soybean plants accumulated the most amount of K at R3 stage when soybean began podding, which was consistent with the results showed by Batchelor and Scott (1979) that the majority of total K uptake occurred after flowering (R1-R2) in soybean plants.

Most previous research has focused on the effects of nitrogen on insect pest. N is the most limiting to plant feeding insects and the most critical nutrient for cellular growth. However,



soybean can fix its own nitrogen via rhizobacteria, and supplemental nitrogen can inhibit nodulation and specific nitrogenase activity in soybean (Hardarson et al. 1984). Generally, N fertilizer is not recommended for cultivation of soybean (Hardarson et al. 1984). But K and P are limiting when rice and soybean are rotated in Louisiana and supplemental fertilizer is needed. In this study, the effects of K fertilizer rates on soybean looper development were evaluated. The results showed positive interactions between K fertilizer rates and soybean looper development and performance. Soybean looper consumed more leaf tissues and developed faster on higher rates of K fertilizer, which suggested that foliage from plants treated with higher K fertilizer is more suitable for soybean looper development. Funderburk et al. (1990) also demonstrated that population of velvetbean caterpillar, *Anticarsia gemmatilis* (Hübner), were increased under higher soil level of K in soybean. Studies indicate fertilizer applications influence pest injury because fertilizers change plant growth rates, size, time of maturity, and defense (Altieri and Nicholls 2003). Furthermore, K nutrition is involved in chemical signals and hormonal pathways that are relevant to defense mechanisms (Amtmann et al. 2008). Jasmonic acid (JA) is one of most important plant hormones in response to mechanical and herbivory injury that stimulate to express resistance-related metabolites to defend herbivory (Korth and Thompson 2006). Interactions between K nutrition and JA response have been studied in *Arabidopsis* (Amtmann et al. 2008). K nutrition regulated plant defense hormonal pathways at several levels (Amtmann et al. 2008). Shin and Schachtman (2004) showed that ethylene (ET) concentration was associated with JA and JA increased when *Arabidopsis* planted under K deficient conditions (Lorenzo et al. 2003). Armengard et al. (2004) found that JA played a vital role in K nutrition signaling. Numerous JA responsive genes were strongly stimulated in K-deficient *Arabidopsis*, while the expression of these genes was rapidly decreased

after K resupplied (Armengard et al. 2004). *Arabidopsis thaliana* has been the model plant to study the interactions between potassium nutrition levels and pest development (Amtmann et al. 2008). The model plant presented the basic studies to establish causal relationships between metabolite profile and hormonal pathways and pest susceptibility (Amtmann et al. 2008). This knowledge can further extend to other agricultural crops to improve crop performance by balancing crop nutrition and pest pressure (Amtmann et al. 2008). In our study, K fertilizer improved soybean plant performance but made it more susceptible and preferred for soybean looper. It is possible that increased K fertilizer rates reduced the levels of plant resistance and insect-deterrent metabolites in soybean.

In conclusion, understanding the complex interactions between host/herbivore/nutrient inputs is key to creating action thresholds and management plants as well as maintaining a balance between yield (productivity) and control strategies. Based on our results, K fertilizer rates will need to be balanced between increasing yields and reducing population growth.

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## SUMMARY AND CONCLUSION

Soybean is the second most agronomic crop in the U.S. Strategies are required to simultaneously manage herbivores populations while optimizing fertilization to obtain optimal yield. In order to achieve an economically and environmentally sustainable agroecosystem, Integrated Pest Management (IPM) is widely recommended. Soybean looper in soybean has become the most expensive insect in Louisiana due to rapidly developed insecticide resistance. Induced host plant resistance has been sought as an important strategy to reduce herbivore populations and/or lower its fitness. Commercial elicitors could be used to induce host plant resistance to regulate herbivore populations. Fertilizer supplement in crops could increase yield but also influence insect pest populations. In this study, Actigard 50WG, Regalia, and MeJA were used as elicitors to evaluate if induce resistance would lower soybean looper fitness on soybean plants; and different K fertilizer rates were supplied to estimate their effects on soybean yield and quality as well as on soybean looper development.

In the first objective, exogenous application of Actigard 50WG, MeJA and Regalia in soybean plants induced resistance and had adverse effects on soybean looper development and performance. The induced effects by Regalia were very limited compared to the other two elicitors. Pupal weight of soybean looper was significantly reduced by 11.2% and 17.9% on MeJA treated leaves than on control leaves on R2 and R5 stages, respectively. Moreover, larval development of soybean looper on MeJA treated leaves was elongated by 12.8% and 16.2% on R2 and R5 stages, respectively compared with that on control leaves. The induced effects by MeJA were consistent at both R2 and R5 stages. In this project, MeJA was the most effective elicitor. MeJA could be potentially considered as an alternative tactic to be compatible with

other control strategies in the field. However, use of MeJA as an independent approach to control herbivores in the field still requires future research.

In the second objective, six different K fertilizer rates were applied to soybean at planting. Soybean yield and soybean quality were positively related to K rates. At the rate of 168.1 kg/ha soybean yield reached 46.33 bushel per acre, which was increased by 19.38% and 22.66% compared to those at rates of 0 and 33.6 kg/ha. Additionally, 100 seed weight was also increased significantly from treatment of 168.1 kg/ha. However, the two important nutritional values, protein and oil, were not increased with increasing K fertilizer rates. Soybean looper development and performance were enhanced due to K supplement in soybean. Soybean mortality and fecundity were not significantly affected by K fertilization. However, soybean looper defoliation and pupal weight were significantly increased with increasing K fertilizer rates, while larval development time was shortened. Larval development time at 168.1 kg/ha rate was decreased up to 6.57% and 5.34% in contrast to those at the rates of 0 and 33.6 kg/ha, respectively, which could be a contribution to complete the 4<sup>th</sup> generation for soybean looper in Louisiana. In this project, soybean plants with high K levels were more susceptible and suitable to soybean looper. K fertilization improved host plant nutritional quality, which had a direct impact on herbivore development and performance. Moreover, high nutrition levels could reduce host plant defense mechanism such as reduction of deterrent metabolites, which could increase herbivore development and performance. Therefore, understanding these complex interactions between host/herbivore/nutrient inputs is an important step to maintain a balance between crop productivity and herbivore management.

## **VITA**

Xuan Chen is the daughter of Shoufang Yan and Rongcai Chen. She was born in PeiXian, Jiangsu, China. Xuan graduated from High School in June 2004. She received a Bachelor of Science degree in Ecology from Yangzhou University of China in June of 2008. Xuan has been at Louisiana State University Agriculture and Mechanical College under Dr. Jeffrey A. Davis since May 2012. She is married to Fei Yang, and has a son Louis Yang. Xuan is currently a candidate for the Master of Science degree in the Department of Entomology at Louisiana State University.