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# A survey of chemical elicitors and their effectiveness as promoters of plant defense against herbivory by *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae)

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A SURVEY OF CHEMICAL ELICITORS AND THEIR EFFECTIVENESS AS PROMOTERS  
OF PLANT DEFENSE AGAINST HERBIVORY BY *SPODOPTERA FRUGIPERDA* (SMITH)  
(LEPIDOPTERA: NOCTUIDAE)

A Thesis

Submitted to the Graduate Faculty of the  
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in

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John Wesley Gordy  
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## ABSTRACT

Insect herbivores can induce a range of plant defenses. Signal pathways can be activated that result in the production of secondary metabolites. Many of these compounds can reduce insect fitness, deter feeding, and attract beneficial insects. Additionally, organic and inorganic chemicals applied as a foliar spray or soil drench can activate these plant responses. Azelaic acid, benzothiadiazole (BTH), gibberellic acid (GA3), harpin, and jasmonic acid (JA) are thought to mediate plant response to pathogens and herbivores. The effects of these elicitors on the induction of plant defenses were determined by measuring the weight gain of fall armyworm (FAW), *Spodoptera frugiperda* (Smith) (Lepidoptera: Noctuidae) on four important crops, cotton, corn, rice, and soybean, treated with elicitors, under greenhouse conditions. JA consistently induced cotton and soybean resistance to FAW. In contrast, azelaic acid, BTH, and harpin treated plant material increased weight gain of FAW, suggesting negative crosstalk between the salicylic acid and JA signaling pathways. No induction of defense was observed in corn and rice, and the lack of spray mixture adhesion to those crops inspired a second experiment in which four adjuvants were co-applied with a reduced rate of JA (0.25X) to corn and cotton. Corn was more responsive to the use of an adjuvant than was cotton. The differential effectiveness of two elicitors, JA and BTH, was investigated on FAW and another noctuid species, the soybean looper (SBL), *Chrysodeixis includens* (Walker). Weight gain of FAW offered JA-treated soybean was significantly lower than FAW offered non-treated soybean in all trials, whereas growth of SBL was significantly reduced in only one trial. BTH was not effective in reducing weight gain of SBL offered plant material treated at the 1X or 5X rates. BTH reduced weight gain of FAW only in trial 2, at the 5X rate. The findings presented herein provide further support that foliar applications of JA increase resistance to arthropod herbivores, and that

this relationship between plant and herbivore could be transitioned to the field, with the ultimate goal of using elicitor-induced defense as a part of an integrated pest management program.



## 1. INTRODUCTION

Fall armyworm (FAW), *Spodoptera frugiperda* (Smith), and soybean looper (SBL), *Chrysodeixis includens* (Walker), are examples of lepidopteran larvae that can cause serious losses in crop yields due to plant defoliation and fruit injury. In 2006, approximately 10% of corn, *Zea mays* (L.), acres in Georgia required treatment for FAW, resulting in a cost of \$616,000 (Guillebeau et al. 2008). In 2012, FAW infested 763,000 acres of cotton, *Gossypium hirsutum* (L.), in the U.S., causing an estimated loss of 1,116 bales at a cost of greater than \$450,000 (Williams 2013). In soybean, *Glycine max* (L.), the armyworm complex (*Mythimna unipuncta*, *S. exigua*, *S. frugiperda*, *S. ornithogalli*) infested 2.67 million acres and caused economic losses of over \$27 million, including yield loss and treatment costs, in Alabama, Arkansas, Louisiana, Mississippi, North Carolina, Tennessee, and Virginia, during 2011 (Musser et al. 2012). Finally, in the same year and locations, SBL infested 4.66 million acres of soybean and caused economic losses of over \$59 million, including loss and treatment costs (Musser et al. 2012).

Historically, control of herbivorous insect pests has relied on broad-spectrum synthetic insecticides that exhibit several negative effects. Such effects include: development of resistance in pests, high costs of new chemistries, non-target effects, secondary pest resurgence, and deleterious effects on the environment. With the adoption of transgenic *Bacillus thuringiensis* (Bt) varieties beginning in 1996, many foliage feeding insects have been successfully managed in corn and cotton. However, there are no commercial Bt soybean varieties currently available, and there is variation in the susceptibility of FAW to Bt in the crops that are available (Adamczyk et al. 1997).

Recently, interest has increased in host plant resistance and research on chemical elicitors to stimulate host plant defense against pest insects (Boughton et al. 2006, Bruinsma et al. 2007, Dervinis et al. 2010, Hamm et al. 2010). Host plant resistance can be categorized as either constitutive or inducible. Constitutive resistance is defined as morphological or chemical attributes always present in a plant that act to deter or have negative effects on herbivores. Inducible resistance refers to a plant's response to herbivory. Both constitutive and inducible resistance can be classified as either direct, which negatively affects physiology and/or behavior of herbivores, or indirect, which increases the performance of natural enemies (Schoonhoven et al. 2005).

Constitutive and inducible plant resistance can include mechanical and/or chemical plant traits (Traw and Bergelson 2003, Schoonhoven et al. 2005). Mechanical adaptations include thorns, trichomes, and tough leaf tissues to reduce feeding. Chemical defenses are much more complex and can include hypersensitive responses such as localized cell death, the production of volatiles that attract natural enemies, and endogenously produced chemicals that decrease herbivore fitness (Fritz and Simms 1992). Typically, induced plant defense involving viruses, fungi, and bacteria is termed systemic acquired resistance whereas induced plant defense involving insect herbivory is characterized as induced resistance (Inbar 2001).

The use of plant defense elicitors should be considered as an additional approach in integrated pest management programs. Plant defense elicitors that demonstrate effectiveness against foliage and plant feeding guilds of insects could be beneficial in crops including field corn, cotton, sorghum, *Sorghum bicolor* (L.), and rice, *Oryza sativa* (L.), as an alternative to conventional insecticides. This could reduce early season chemical control needs by boosting plant resistance to herbivores, and decreasing losses to secondary pests by minimizing non-target

beneficial insect mortality. Additionally, their use could extend the life of transgenic *Bt* technologies by being included as an additional tactic with a different mode of action thereby aiding in insecticide resistance management. This potential, coupled with the ability to “piggyback” their application with a planned herbicide application, encourages consideration for the use of plant defense elicitors in an integrated pest management program.

Due to the dynamics of the pest arthropod complexes for most crops throughout the U.S., there is a need to continuously re-evaluate existing management strategies. The purpose of this project is to screen potential elicitors for their potential use in inducing resistance to herbivorous insects in field trials of major agricultural commodities, evaluate the effectiveness of adjuvants in increasing elicitor activity, and examine differential effects of elicitors on selected crop plants and herbivorous insects.

## 2. REVIEW OF LITERATURE

### 2.1 Identification and Biology of Target Insects

#### 2.1.1 Fall Armyworm

Fall armyworm (FAW), *Spodoptera frugiperda* (Smith), is in the family Noctuidae in the order Lepidoptera (Anonymous 2012). Larvae can vary greatly in color, from light tan to green to nearly black, with stripes running the length of the body. Later instars lack primary setae and tend to be smooth (Oliver and Chapin 1981, Capinera 1999). Larvae of FAW can be distinguished from other members of the family by the presence of an inverted “Y” on the front of the head capsule (Oliver and Chapin 1981, Drees 1998). Adult FAW possess dark gray mottled forewings with a distinctive lighter colored spot near the tip, and whitish hindwings. The wingspan measures approximately 3.8 centimeters from tip to tip (Oliver and Chapin 1981, Capinera 1999).

The larval stage of FAW is polyphagous, feeding on foliage and occasionally fruit. FAW is a pest of many crops, including corn, *Zea mays* (L.), cotton, *Gossypium hirsutum* (L.), rice, *Oryza sativa* (L.), soybean, *Glycine max* (L.), turf grass, pastures, and vegetables (Luttrell and Mink 1999). Adult females oviposit in large masses on the abaxial leaf surface (Cranshaw 2004). Larvae are the damaging stage and cause defoliation resulting in yield loss (Leigh et al. 1996). The larval stage of FAW consume 80 percent of their total feeding intake during the last two days of the larval stages; therefore, it is beneficial to eliminate early instars or to affect larval fitness such that populations of successive generations are reduced (Knutson 2008).

Overwintering of FAW usually occurs in the pupal stage. However, in very mild winters it is not uncommon to see all life stages. The tropical nature of FAW does not allow this species to survive winter temperatures in midwestern and northern U.S. states. However, FAW do

overwinter across southern U.S., specifically in Florida and South Texas (Knutson 2008). In addition, each year FAW adults migrate north from Mexico and the Caribbean islands (Cranshaw 2004). Females may oviposit as many as 400 or more eggs in a large mass (Luginbill 1928, Drees 1998). Typical development time for larvae is two to three weeks, depending on temperature, as well as availability and quality of food. Late instars leave the host plant and pupate in the soil. The pupal stage often lasts 10 to 14 days, after which the adult emerges and mates (Luginbill 1928, Knutson 2008). Southern states can have as many as ten (typically three to four) generations per year, while the northern states have only one or two generations per year (Capinera 1999, Cranshaw 2004).

Two strains of FAW that are morphologically identical, but differ in host specificity, occur in Louisiana (Pashley 1986). They are commonly referred to as the corn-strain and the rice-strain; the former preferring corn and the latter preferring rice and bermudagrass, *Cynodon dactylon* (L.) (Quisenberry 1991, Nagoshi and Meagher 2004). Additionally, the corn-strain develops in greater numbers on cotton, compared to the rice-strain (Nagoshi et al. 2007). Inter-strain mating can occur with rice-strain females accepting corn-strain males; however, corn-strain females and rice-strain males appear to be reproductively incompatible (Whitford 1988, Quisenberry 1991).

### 2.1.2 Soybean Looper

Soybean looper (SBL), *Chrysodeixis includens* (Walker), is in the family Noctuidae in the order Lepidoptera (Anonymous 2012). SBL larvae are green and usually have lighter-colored longitudinal stripes and small dark spots on the abdomen. The body of the larvae tapers slightly from posterior to anterior and they possess three pairs of true legs and three pairs of prolegs. Adult SBL possess brown, mottled forewings with a silvery spot near the center, lighter-colored

hindwings and fore- and hindwing margins, and a wingspan that measures approximately 3.3 centimeters (Higley and Boethel 1994, Stewart et al. 2010).

The larval stage of SBL is a polyphagous foliage feeder and rare fruit feeder that is known to feed on soybean, cotton, peanut, *Arachis hypogaea* L., sweet potato, *Ipomoea batatas* (L.), tomato, *Lycopersicon esculentum* Miller, and many other hosts (Herzog 1980). Higher SBL populations occur when soybean and cotton are grown in close proximity (Herzog 1980, Funderburk et al 1999). Adult females oviposit single eggs on the abaxial leaf surface, where they hatch after two to three days and begin to feed on foliage, causing defoliation that can result in yield loss (Herzog 1980, Higley and Boethel 1994, Stewart et al. 2010). SBL larvae consume approximately 80 percent of their total feeding intake in the last (sixth) instar; therefore, as with FAW, it can be beneficial to eliminate early instars to mitigate yield loss (Boldt et al. 1975).

Overwintering of SBL occurs in the United States across southern Florida and southern Texas. Generally, moths migrate north from these areas, Mexico and Central America, and the Caribbean islands (Herzog 1980). Females may oviposit as many as 600 or more eggs after mating (Higley and Boethel 1994, Stewart et al. 2010). Typical development time for larvae is two to three weeks, again depending on temperature, as well as availability and quality of food. Late instars spin a loose cocoon and pupate on the abaxial leaf surface. The pupal stage often lasts 7 to 10 days, at which time the adult emerges and mates. In Louisiana, there are three to four SBL generations on soybeans annually (Burleigh 1972, Higley and Boethel 1994).

## **2.2 Insect – Host Relationships**

Grasses are preferred by FAW larvae, but they have been observed feeding on over 80 host species of plants, including both monocots and dicots (Pashley 1988, Leigh et al. 1996, Knutson 2008). Early instars feed on the abaxial leaf surface; usually not chewing completely

through the leaf, allowing them to go unnoticed (Knutson 2008). Second and later instars begin eating holes and feeding inward from the margin of the leaf, causing defoliation (Barlow and Kuhar 2009).

In corn, FAW larvae will feed on the whorl, resulting in stunted growth and misshapen leaves, and later on the tassels and the ear, causing grain loss (Herbert and Malone, 2005). In cotton, first and second instars typically feed on foliage, while third instars and older larvae tend to prefer squares, blooms, and bolls (Luttrell and Mink 1999). In soybean, FAW may feed on leaves, stems, pods, and beans (Stewart et al. 2011), and in rice, FAW can cause serious yield loss with early-season feeding on seedling rice that results in stand reduction and significant defoliation (Shipp 2002).

Adult SBL oviposit on the abaxial leaf surface and emerging larvae begin to feed on leaves in the middle canopy of soybean plants. As they defoliate the plant, larvae move higher in the canopy and consume upper leaves as they move (Herzog 1980). Feeding by SBL larvae is most damaging when it occurs during the pod-fill stage (R1-R6) and causes a reduction in canopy, decrease in photosynthesis, and yield loss. After plants reach the R8 development stage, SBL feeding has little or no effect on yield (Funderburk et al. 1999).

### **2.3 Plant Hormones, Elicitors of Plant Defense, and Adjuvants**

Induced resistance against plant pathogens was recognized over a century ago while induced resistance against herbivorous arthropod pests was discovered only in the last half-century (Karban and Kuć 1999). To date, over 30 plant species have demonstrated induced resistance against pathogens and over 100 plant species have demonstrated induced resistance against herbivores (Karban and Kuć 1999). Plant hormones most commonly identified as mediating plant responses to pathogens and herbivores are salicylic acid and jasmonic acid (JA),

respectively (Smith et al. 2009). However, azelaic acid, benzothiadiazole, harpin, gibberellins, and several other organic and inorganic chemicals can affect plant response to pathogen and/or herbivorous arthropod attack (Kahl et al. 2000, Traw and Bergelson 2003, Nombela et al. 2005, Yang et al. 2005, Jung et al. 2009, Hamm et al. 2010).

### 2.3.1 Azelaic Acid

Azelaic acid is a naturally occurring saturated dicarboxylic fatty acid that has demonstrated anti-inflammatory and antibacterial properties (Garelnabi et al. 2010), as well as inducing local and systemic resistance to the plant pathogen, *Pseudomonas syringae*, in *Arabidopsis* (Jung et al. 2009). Research involving the effects of azelaic acid as a primer in systemic acquired resistance is very limited. Its role in induced resistance has not been investigated.

### 2.3.2 Benzothiadiazole and Salicylic Acid

Benzothiadiazole (BTH) is labeled as a promoter of systemic acquired resistance for plant protection from bacterial and fungal pathogens including *Pseudomonas syringae*, *Xanthomonas campestris*, and *Peronospora destructor* (Anonymous 2011). BTH is labeled under the trade name Actigard® by Syngenta Crop Protection. BTH is a salicylic acid mimic and has shown promise in systemic reduction of infections by plant pathogens, but has limited effects on reducing damage or affecting host preference for insects. Salicylic acid is the primary signaling hormone for induced plant defense to pathogenic infection, especially biotrophic pathogens (Glazebrook 2005, Smith et al. 2009).

Infection frequency of the plant pathogen *Uromyces pisi* was shown to be significantly decreased in *Pisum sativum* (L.) with the application of BTH (Barilli et al. 2010). Additionally, BTH induces local, but not systemic, resistance in tomato (*Solanum lycopersicum*) to *Bemisia*



*tabaci* (Gennadius) (Hemiptera: Aleyrodidae), causing a decrease in the number of eggs and resulting pupae (Nombela et al. 2005). Boughton et al. (2006) demonstrated that application of BTH reduces growth of populations of green peach aphid, *Myzus persica*, on tomato. However, Inbar et al. (2001) showed that BTH had no significant effect on host preference of *B. tabaci* or feeding efficiency of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on cotton.

### 2.3.3 Gibberellins

Gibberellins belong to a group of plant compounds called terpenoids (Naqvi 1995). Gibberellins are at highest concentrations in immature seed and lower concentrations in root and, especially, shoot tissue, comparatively (Naqvi 1995). Currently, 126 gibberellins have been identified in 128 plants, 7 fungi, and 7 bacteria (Macmillan 2002). They are biologically active in plants and cause the elongation of cells, breaking of seed and bud dormancy, and the mobilization of nutrients including the synthesis of hydrolytic enzymes in barley, *Hordeum vulgare* (L.), wheat, *Triticum spp.*, and wild oat, *Avena fatua* (L.) (Naqvi 1995).

Plant damage from insect feeding can be reduced by gibberellins. They promote morphological changes resulting in physical defense strategies. When used alone or in conjunction with fenclorfenuron, a synthetic cytokinin, as a pretreatment for the black pecan aphid, *Melanocallis caryaefoliae* (Davis) (Hemiptera: Aphididae), on pecan, *Carya illinoensis* (Wangenh), gibberellic acid significantly reduced leaf chlorosis (Cottrell et al. 2010). Additionally, gibberellic acid acted synergistically with JA to increase the number and density of leaf trichomes in *Arabidopsis* (Traw and Bergelson 2003).

### 2.3.4 Harpin

According to the Plant Health Care, Inc. federal registration label, Harpin  $\alpha\beta$  protein is a biochemical pesticide that suppresses nematode egg production and enhances plant growth,

stamina, and vigor (Anonymous 2011). Harpin is produced by the bacterium *Erwinia amylovora* (Baker et al. 1993). It promotes resistance to plant pathogens including fungi and bacteria (Yang et al. 2005, Dong et al. 1999). Harpin is registered under the trade name Employ® H&T (Plant Health Care, Incorporated, Pittsburgh, PA, USA).

Harpin significantly decreased lesion diameter of *Trichothecium roseum* in certain varieties of harvested hami melons, *Cucumis melo* (L.) var. *inodorus* (Jacq.), without a significant effect on mycelial growth (Yang et al. 2005). Additionally, harpin induced resistance to the fungal pathogen, *Peronospora parasitica*, and the bacterial pathogen, *Pseudomonas syringae*, in *Arabidopsis spp.*, but did not decrease green peach aphid (*Myzus persicae*) populations when applied exogenously to tomato (Dong et al. 1999, Boughton et al. 2006).

### 2.3.5 Jasmonic Acid

In plants, JA acts as a signaling molecule in the octadecanoid pathway (Staswick 1995). JA is involved in the inhibition of seed germination and plant growth, and promotes leaf senescence, fruit abscission, tuber formation, flower and fruit development, pigment formation, and tendril coiling (Davies, 1995, Staswick 1995).

As a major signaling molecule, JA is responsible for mediating plant responses to herbivorous insect attack. Levels of endogenous JA increase following attack and, in response, secondary metabolites are produced *in vivo*. These metabolites deter insect feeding, inhibit digestion of plant material, or attract natural enemies (Smith et al. 2009). Omer et al. (2001) showed that the application of a 1mM solution of methyl ester of JA significantly decreased preference of cotton aphids, *Aphis gossypii* (Glover), two-spotted spider mites, *Tetranychus urticae* (Koch), and western flower thrips, *Frankliniella occidentalis* (Pergande), on cotton, compared to non-treated cotton. Survivorship and reproduction of cotton aphid were reduced by

40% and 75%, respectively. Egg production was reduced by more than 75% in two-spotted spider mite, and leaf feeding was reduced by more than 80% in western flower thrips. Additionally, Hamm et al. (2010) showed that rice treated with JA had fewer rice water weevil, *Lissorhoptrus oryzophilus* (Kuschel), eggs ranging from 23% to 69% and 54% to 85% for 1mM and 5mM concentrations, respectively, compared to a non-treated control. Thaler (1999) showed that tomato plants treated with JA had twice as many *Hyposoter exiguae* (Viereck) parasitized *Spodoptera exigua* (Hübner) (both naturally occurring in the field) compared to control plants. Additionally, there were 37% more parasitized *S. exigua* on treated plants, compared with control plants. Additionally, trichome number is increased by artificial wounding, application of JA alone, or JA with gibberellin (Traw and Bergelson 2003).

#### 2.3.6 Adjuvants

In an attempt to maximize pesticide efficacy, adjuvants have been used in agriculture as dispersants, stickers, emulsifiers, penetrants, and for other various purposes, since the onset of modern pesticide use (Stevens 1993, Witt 2012). Following the development and advancement of pesticides, adjuvants have been improved and their use is considered standard practice in agriculture. Holloway et al. (2000) showed that the use of organosilicone and methylated vegetable oil surfactants on pea, *Pisum sativum*, resulted in leaf coverage of 93% and 34% coverage, respectively, compared to the 0.3% coverage achieved with only water. Dyne-Amic, an organosilicone-oil surfactant, decreases surface tension of water by 30% at concentrations as low as 0.01% (Singh and Mack 1993). The modes of action for adjuvants most relevant to this study include increasing stomatal infiltration via reduction in spray mixture surface tension, and enhancing penetration of the plant cuticle.

### 3. OBJECTIVES AND HYPOTHESES

The following objectives and hypotheses were developed for this work.

Objective 1: Determine the effects of application of suspected elicitors of plant resistance

- azelaic acid, benzothiadiazole (BTH), harpin, gibberellic acid, and jasmonic acid (JA) - on the resistance of cotton, *Gossypium hirsutum* (L.), corn, *Zea mays* (L.), rice, *Oryza sativa* (L.), soybean, *Glycine max* (L.), as measured by weight gain of larval fall armyworm (FAW), *Spodoptera frugiperda* (Smith), offered treated excised leaves.

$H_0$  = No significant difference in weight gain between FAW larvae offered elicitor/hormone treated plant material will be detected, compared to FAW larvae offered non-treated plant material.

$H_A$  = Weight gain of FAW larvae offered elicitor/hormone treated plant material will be significantly lower compared to FAW larvae offered untreated plant material.

Objective 2: Determine if the use of adjuvants enhance the response of corn and cotton to JA as measured by weight gain of FAW larvae offered treated excised leaves.

$H_0$  = No significant difference of weight gain between FAW larvae offered plant material treated with JA + adjuvant compared to FAW larvae offered plant material treated with JA alone.

$H_A$  = Weight gain of FAW larvae offered JA + adjuvant-treated plant material will be significantly lower than FAW larvae offered on plant material treated with JA alone.

Objective 3: Determine if applications of BTH or JA to soybean differentially affect soybean resistance to FAW and soybean looper (SBL), *Chrysodeixis includens* (Walker) fed excised leaf tissue.

$H_0$  = No significant difference in weight gain of FAW or SBL larvae offered excised leaf tissue of soybean treated with JA or BTH will be detected, compared to those offered non-treated soybean..

$H_A$  = Weight gain of FAW and SBL larvae offered non-treated soybean or soybean treated with JA or BTH will be differentially affected.

This study served as an initial screen of elicitors to evaluate their potential use in field trials on major agronomic crops and against economically important target species of insects. This research compared the effect of selected elicitors on the weight gain of FAW larvae across four major agronomic crops: corn, cotton, rice, and soybean. The elicitors used had previously been shown to increase resistance of one or more plants to arthropods or pathogens (Omer et al. 2001, Dong et al. 2004, Nombela et al. 2005, Jung et al. 2009, Cottrell et al. 2010, Hamm et al. 2010). This work also evaluated adjuvants in increasing the effectiveness or activity of JA applied to two agronomic crops, corn and cotton. Results will provide a better understanding of overall elicitor effectiveness, differences in effectiveness between monocots and dicots, and will offer insight into the effect of adjuvants on the activity of JA. Finally, this research serves as a basis for further field experiments using elicitor-mediated induction of plant defense as a potential tactic in integrated pest management.

## **4. MATERIALS AND METHODS**

### **4.1 Effects of Putative Elicitors on Induced Plant Resistance to Fall Armyworm**

Cotton (cv LA1110017, LSU AgCenter, Agronomy Department Cotton Breeding Program), field corn (cv Trucker's Favorite Yellow, River Valley Heirloom Seeds, Glenwood, AR, USA.), rice (cv CL131, BASF, Research Triangle Park, NC, USA), and soybean (cv Clifford, LSU AgCenter, Agronomy Department Soybean Breeding Program) were grown in 1.6 liter round (15 cm diameter) plastic pots using Clegg's potting soil (peat - aged pine bark - perlite, 50-40-10; Sun Gro Horticulture, Bellevue, WA, USA). Plants were maintained in the greenhouse under natural lighting with temperatures ranging from 20 °C to 35 °C (Table A1). After plant emergence, granular fertilizer (13.5 g, N-P-K, 13-13-13; Meherrin Fertilizer Inc., Severn, NC, USA) was applied. The plants were watered to maintain adequate soil moisture. Cotton, corn, and rice were grown to the 3-4 leaf stages, and soybean was grown to the V1-V2 stage.

Fall armyworm (FAW) larvae were obtained from a fallow rice field at the Rice Research Station in Crowley, Louisiana in 2011. This collection is presumed to be rice-strain and will be referred to as such in this paper. Another FAW colony was established from larvae collected from a cotton field at the Macon Ridge Research Station in Winnsboro, Louisiana in 2005, and supplemented in 2006 and 2008. The FAW populations sampled in Winnsboro have been genetically confirmed as being the corn-strain and will be referred to as such in this paper (Hardke 2011). The rice-strain and corn-strain colonies were maintained in the laboratory on meridic diet (Fall Armyworm Diet (Southland Products Incorporated, Lake Village, AR, USA) and Stonefly Heliiothis Diet (Ward's Natural Science, Rochester, NY) for rice -strain and corn-strain FAW, respectively) using the methods as described by Hardke (2011). Pupae were placed in

buckets covered with cheese cloth and provided with fresh water and a mixture of honey, beer, water, and ascorbic acid (150ml-150ml-300ml-12g). After emergence, adults mated and females oviposited eggs onto the cheesecloth, which was collected daily and placed in a plastic bag, labeled, and set aside. When neonates began to emerge, they were placed in 8-cell trays (Bio-Serv, Frenchtown, NJ, USA), 20-30 per cell, and supplied with meridic diet (Fall Armyworm Diet, Southland Products Incorporated, Lake Village, AR, USA). The FAW larvae were kept on diet until third instars (approximately 6-7 days), and were synchronized by selecting larvae that were about to molt (noticeable gap behind head associated with slippage of the head capsule). The larvae were then placed individually into cells of 32-cell trays (Bio-Serv, Frenchtown, NJ, USA) and starved for 18-24 h to ensure that the gut was evacuated before mass was measured. Larval mass were determined using a microbalance (model XS105, Mettler-Toledo LLC, Columbus, OH, USA). For every 10 larvae needed for the experiment, at least 15 were evaluated and only newly molted larvae with the most similar masses (mean  $\pm$  1 standard deviation) were used in the experiment. See Appendix for FAW strain used by experiment. After the feeding assay was completed (approximately 72 h), the larvae were returned to individual cells for an additional 6-24 h starvation period to ensure that the larval gut was evacuated before final mass was measured and recorded.

Azelaic acid, benzothiadiazole, gibberellic acid, harpin, and jasmonic acid (JA) were measured at a 3X rate (Table 1) for trial one, and at the 1X rate for trials two, three, and four (if applicable). The 1X rates for benzothiadiazole, gibberellic acid, and harpin were equivalent to label rates whereas azelaic acid and JA were mixed at concentrations of 1.0 and 2.0 mM, respectively (Jung et al. 2009, Hamm et al. 2010). The elicitors were thoroughly mixed into

**Table 1.** Putative Elicitors of Plant Defense Used in this Study

Chemical	Trade Name (if applicable)	Manufacturer	1X rate (g/100ml) unless otherwise noted	Maximum amount per plant
Azelaic Acid	n/a	Sigma Aldrich	0.019 (1 mM)	1.9 mg
Benzothiadiazole	Actigard® 50wg	Syngenta Crop Protection	0.005	0.5 mg
Gibberellin (GA <sub>3</sub> )	ProGibb® 40%	Valent BioSciences	0.01	1.0 mg
Harpin	Employ® H&T	Plant Health Care, Inc.	0.03	3.0 mg
Jasmonic Acid	n/a	Tokyo Chemical Industry Co., Inc.	0.042 (2 mM)	4.2 mg
Organosilicone surfactant	Dyne-Amic®	Helena Chemical Co.	50µl/100ml	5 µl
Nonionic oil surfactant	Penetrator Plus®	BASF	50µl/100ml	5 µl
Polyethylene glycol	Triton X100	Sigma Aldrich	50µl/100ml	5 µl
Polyoxyethylene (20) sorbitan monolaurate	Tween 20	Sigma-Aldrich	50µl/100ml	5 µl



100 ml of deionized water and applied using a gas (propane, butane, dimethylether) propellant-powered hand sprayer (Preval, Coal City, IL, USA). The JA was first dissolved in 1 ml of ethanol; the spray jar containing azelaic acid and water was placed in a sonicator for approximately five minutes to aid in mixing. The control treatment was 1.0% (v/v) ethanol in deionized water. To prevent cross-treatment exposure, each group of plants was removed from the greenhouse bench, placed in front of an exhaust fan for treatment, and allowed to dry before being returned to common greenhouse area.

Approximately 48 h after elicitor treatment, all leaves were removed from plants, using scissors, and were placed on ice for transport to the lab. Excised leaf material was placed in 9cm plastic petri dishes (for rice) or 32-cell trays (for cotton, corn, and soybean) containing four layers of cotton batting saturated with deionized water. Selected FAW larvae were placed on excised leaves for feeding and the petri dish or cell was labeled with the identification number of the larva. Larvae were allowed to feed for 72 hours and were checked daily to ensure they were not food-limited.

Data Analysis: Analysis of covariance (ANCOVA) was performed with final weight as the response variable, initial larva weight as the covariate, and treatment (elicitor) as a fixed effect. The ANCOVA was performed using PROC MIXED in SAS 9.3 (SAS Institute 2010) (Stout et al. 2009). Each experiment was tested for a treatment by covariate interaction using PROC MIXED. If a significant interaction was found, a contrast was performed comparing treatments at the mean of the covariate. Means were separated using Dunnett's method for multiple comparisons to a control. Least squares means for estimated final weight are reported in results.

#### **4.2 Adjuvant Effects on the Response of Corn and Cotton to Jasmonic Acid and Induced Resistance to Fall Armyworm**

Corn and cotton plants were managed as described in Objective 1. They were grown under greenhouse conditions and lighted with 400 watt metal halide lights placed 1.25 meters above the pots, on a 14:10 hour (light: dark) schedule. Corn-strain FAW were reared and treated as described in Objective 1. Tween 20, Triton X100, Penetrator Plus®, and Dyne-Amic® were mixed at 50µl per 100ml of solution (0.05% v/v), half the concentration of Tween 20 used in application of JA in previous studies by Bruinsma et al. (2007) and Xin et al. (2012), and one-fifth the label rate of Penetrator Plus® and Dyne-Amic®. This rate was selected to minimize phytotoxic effects of JA previously reported (Boughton et al. 2006), while improving penetration and increasing spray coverage (Sengh and Mack 1993). The treatments included: adjuvant with 0.5 mM JA (0.25X rate) (four treatments), adjuvant alone (four treatments), 0.5 mM JA (0.25X rate), 2.0 mM JA (1X rate), and 1.0% ethanol (v/v) in deionized water (control). Elicitor application, feeding assay, and data analyses were performed as described in Objective 1.

#### **4.3 Effects of Benzothiadiazole and Jasmonic Acid on Induced Resistance of Soybean to Fall Armyworm and Soybean Looper**

Soybean plants were managed as described in Objective 1. They were grown under greenhouse conditions and lighted with 400 watt metal halide lights placed 1.25 meters above the pots, on a 14:10 hour (light: dark) schedule.

Corn-strain FAW larvae were reared as described in objective 1. Soybean looper (SBL) larvae were obtained from the soybean entomology research laboratory at Louisiana State University. The colony was originally collected in 2008 from a soybean field at the Macon Ridge Research Station near Winnsboro, Louisiana. The colony was maintained in the laboratory following methods described by Brown (2012). The laboratory growth room was kept at

approximately 27 °C and 80 % humidity, under a 14:10 hour photoperiod (light:dark). Pupae were placed in covered buckets lined with paper towels and provided with a mixture of 10% v/v honey-water mixture. After emergence, adults mated and females oviposited onto the paper towels, which were collected and placed in a plastic bag, labeled, and set aside. When neonates emerged from eggs, they were placed in one ounce solo cups containing SBL diet (Southland Products Incorporated, Lake Village, AR, USA). For trials one and two, third instars were used; for trial three, second instars were used. SBL and FAW larvae were treated comparably in preparation for the feeding assay.

Benzothiadiazole treatments were applied at 1x, 5X, and 5X for trials one, two, and three, respectively. The control treatment and JA treatment (1X) were prepared as explained in Objective 1 and all treatments were applied as described in Objective 1. The feeding assay was performed as explained in Objective 1. Data analysis was performed as explained in Objective 1, with comparisons made only within the same insect species for each trial.

## 5. RESULTS

### 5.1 Effects of Putative Elicitors on Induced Plant Resistance to Fall Armyworm

For corn, elicitor treatment had a significant effect on final weight of fall armyworm (FAW) larvae in trial 1 ( $F_{5,51} = 3.80$ ,  $P = 0.0053$ ) and trial 2 ( $F_{5,53} = 2.51$ ,  $P = 0.0410$ ), but not in trial 3 ( $F_{5,53} = 0.26$ ,  $P = 0.9311$ ) (Table 2). In trial 1, only the weight gain of larvae offered benzothiadiazole treated corn was different from that of the larvae fed non-treated corn, being significantly greater ( $P = 0.0322$ ). While there was a significant treatment effect in Trial 2, no treatments differed significantly from the control treatment. There was a significant initial weight (covariate) effect for trial 1 ( $P < 0.0001$ ), trial 2 ( $P = 0.0001$ ), and trial 3 ( $P = 0.0041$ ).

**Table 2.** Least Squares Means Estimate<sup>1</sup> for Final Larval Weight (mg) of Fall Armyworm Offered Elicitor-Treated Corn.

Treatment	Trial Number; Treatment Date <sup>1</sup>		
	1; 4/11/2012 <sup>2</sup>	2; 4/27/2012	3; 7/11/2012
Control	58.0 ± 6.2	107.5 ± 5.8	71.1 ± 6.0
Azelaic Acid	75.2 ± 6.0	109.3 ± 5.9	68.7 ± 6.0
Benzothiadiazole	81.6 ± 5.9 *	115.8 ± 5.8	76.1 ± 6.0
Gibberellic Acid	69.4 ± 5.9	115.6 ± 5.8	73.1 ± 6.0
Harpin	66.7 ± 5.9	113.5 ± 5.8	67.6 ± 6.0
Jasmonic Acid	48.3 ± 6.3	91.3 ± 5.8	72.5 ± 6.0
Treatment p-value	0.0053	0.0410	0.9311
Initial weight p-value	<0.0001	0.0001	0.0041

<sup>1</sup> Estimate based on LS means from analysis of covariance performed in SAS 9.3.

<sup>2</sup> Treatment using 3X elicitor rates.

\* Indicates final weight is significantly different from the control treatment ( $P < 0.05$ ) as determined by Dunnett's test for multiple comparisons to a single control.

For cotton, elicitor treatment had a significant effect on final weight of FAW larvae in trial 1 ( $F_{5,50} = 5.94$ ,  $P = 0.0002$ ), trial 2 ( $F_{5,53} = 7.31$ ,  $P < 0.0001$ ), trial 3 ( $F_{5,50} = 11.01$ ,  $P = < 0.0001$ ), and trial 4 ( $F_{5,48} = 7.72$ ,  $P < 0.0001$ ) (Table 3). In three trials, final weights of larvae reared on jasmonic acid (JA) treated leaves were significantly lower than that of larvae reared on

non-treated leaves. For larvae offered leaves treated with benzothiadiazole ( $P = 0.0226$ ) and harpin ( $P = 0.0328$ ) in trial 2, and azelaic acid ( $P = 0.0145$ ) in trial 4, weight gain was significantly higher compared to larvae fed non-treated cotton. There was a significant initial weight (covariate) effect for trial 1 ( $P < 0.0001$ ), trial 2 ( $P < 0.0001$ ), and trial 3 ( $P < 0.0001$ ).

**Table 3.** Least Squares Means Estimate<sup>1</sup> for Final Larval Weight (mg) of Fall Armyworm Offered Elicitor-Treated Cotton.

Treatment	Trial Number; Treatment Date <sup>1</sup>			
	1; 3/7/2012 <sup>2</sup>	2; 7/11/2012	3; 7/18/2012	4; 8/1/2012
Control	56.5 ± 4.7	34.0 ± 3.9	30.7 ± 1.9	20.0 ± 1.8
Azelaic Acid	49.1 ± 4.7	39.4 ± 3.9	35.8 ± 1.8	27.7 ± 1.7 *
Benzothiadiazole	45.9 ± 4.4	50.7 ± 4.2 *	34.7 ± 1.8	22.6 ± 1.7
Gibberellic Acid	43.5 ± 5.4	40.3 ± 4.0	31.5 ± 1.9	20.0 ± 1.7
Harpin	46.3 ± 4.4	50.0 ± 4.2 *	33.0 ± 1.8	23.8 ± 1.7
Jasmonic Acid	22.3 ± 4.7 *	20.2 ± 4.0	16.5 ± 2.2 *	11.4 ± 2.2 *
Treatment p-value	0.0002	<0.0001	<0.0001	<0.0001
Initial weight p-value	0.0111	0.0694	0.3184	<0.0001

<sup>1</sup> Estimate based on LS means from analysis of covariance performed in SAS 9.3.

<sup>2</sup> Treatment using 3X elicitor rates.

\* Indicates final weight is significantly different from the control treatment ( $P < 0.05$ ) as determined by Dunnett's test for multiple comparisons to a single control.

For rice, elicitor treatment had a significant effect on the growth of FAW in trial 1 ( $F_{5,45} = 4.26$ ,  $P = 0.0029$ ), but not in trial 2 ( $F_{5,51} = 0.46$ ,  $P = 0.8021$ ), trial 3 ( $F_{5,52} = 0.79$ ,  $P = 0.5645$ ), or trial 4 ( $F_{5,52} = 1.70$ ,  $P = 0.1500$ ) (Table 4). In trial 1, final weights of FAW larvae offered azelaic acid-treated rice were higher than the control ( $P = 0.0141$ ). There was a significant initial weight (covariate) effect for trial 1 ( $P < 0.0001$ ), trial 2 ( $P < 0.0001$ ), trial 3 ( $P = 0.0014$ ), and trial 4 ( $P < 0.0001$ ).

**Table 4.** Least Squares Means Estimate<sup>1</sup> for Final Larval Weight (mg) of Fall Armyworm Offered Elicitor-Treated Rice.

Treatment	Trial Number; Treatment Date <sup>1</sup>			
	1; 4/12/2012 <sup>2</sup>	2; 6/6/2012	3; 7/11/2012	4; 12/05/2012
Control	61.0 ± 4.7	59.5 ± 4.2	50.0 ± 3.7	47.2 ± 4.4
Azelaic Acid	81.6 ± 4.7 *	58.9 ± 4.4	52.1 ± 3.5	40.1 ± 4.6
Benzothiadiazole	62.1 ± 5.5	60.4 ± 4.2	50.0 ± 3.6	36.4 ± 4.4
Gibberellic Acid	63.9 ± 4.7	56.7 ± 4.2	55.1 ± 3.5	35.4 ± 4.4
Harpin	63.9 ± 4.5	65.4 ± 4.2	56.5 ± 3.5	42.7 ± 4.4
Jasmonic Acid	50.4 ± 5.0	59.8 ± 4.4	48.6 ± 3.6	31.2 ± 4.4
Treatment p-value	0.0029	0.8021	0.5645	0.1500
Initial weight p-value	<0.0001	<0.0001	0.0014	<0.0001

<sup>1</sup> Estimate based on LS means from analysis of covariance performed in SAS 9.3.

<sup>2</sup> Treatment using 3X elicitor rates.

\* Indicates final weight is significantly different from the control treatment (P < 0.05) as determined by Dunnet's test for multiple comparisons to a single control.

In soybean, elicitor treatment had a significant effect on weight gain of FAW in trial 1 ( $F_{5,53} = 9.09$ ,  $P < 0.0001$ ), trial 2 ( $F_{5,51} = 10.18$ ,  $P < 0.0001$ ), trial 3 ( $F_{5,52} = 9.32$ ,  $P < 0.0001$ ), and trial 4 ( $F_{5,53} = 3.98$ ,  $P = 0.0038$ ) (Table 5). Weight gains of FAW offered soybean leaves treated

**Table 5.** Least Squares Means Estimate<sup>1</sup> for Final Larval Weight (mg) of Fall Armyworm Offered Elicitor-Treated Soybean.

Treatment	Trial Number; Treatment Date <sup>1</sup>			
	1; 4/12/2012 <sup>2</sup>	2; 5/16/2012	3; 7/25/2012	4; 12/05/2012
Control	79.4 ± 7.2	76.2 ± 4.9	14.6 ± 0.8	34.3 ± 3.7
Azelaic Acid	94.3 ± 7.4	79.3 ± 4.4	14.6 ± 0.8	38.7 ± 3.7
Benzothiadiazole	92.5 ± 7.2	73.1 ± 4.4	17.0 ± 0.8	33.0 ± 3.7
Gibberellic Acid	94.1 ± 7.2	66.7 ± 4.4	15.5 ± 0.8	29.0 ± 3.7
Harpin	95.8 ± 7.4	84.5 ± 4.6	15.2 ± 0.8	44.3 ± 3.7
Jasmonic Acid	40.4 ± 7.2 *	44.6 ± 4.4 *	9.9 ± 0.8 *	23.3 ± 3.7
Treatment p-value	<0.0001	<0.0001	<0.0001	0.0038
Initial weight p-value	0.0073	<0.0001	<0.0001	0.0330

<sup>1</sup> Estimate based on LS means from analysis of covariance performed in SAS 9.3.

<sup>2</sup> Treatment using 3X elicitor rates.

\* Indicates final weight is significantly different from the control treatment (P < 0.05) as determined by Dunnet's test for multiple comparisons to a single control.

with JA were significantly lower than larvae offered control plants in trial 1 ( $P = 0.0015$ ), trial 2 ( $P < 0.0001$ ), and trial 3 ( $P = 0.0005$ ). While there was a significant treatment effect in trial 4, none of the treatments were significantly different from the control. There was a significant initial weight (covariate) effect for trial 1 ( $P = 0.0073$ ), trial 2 ( $P < 0.0001$ ), trial 3 ( $P < 0.0001$ ), and trial 4 ( $P = 0.0330$ ).

## 5.2 Adjuvant Effects on the Response of Corn and Cotton to Jasmonic Acid and Induced Resistance to Fall Armyworm

For corn, treatment had a significant effect on weight gain of FAW in trial 1, ( $F_{10,85} = 4.49$ ,  $P < 0.0001$ ), and trial 3 ( $F_{10,96} = 2.13$ ,  $P = 0.0289$ ), but not in trial 2 ( $F_{10,96} = 1.87$ ,  $P = 0.0595$ ) (Table 6). In trial 3, weight gain of FAW fed plants treated with 0.5 mM JA + Penetrator Plus was significantly lower than FAW offered plants treated with 2.0 mM JA in trial

**Table 6.** Least Squares Means Estimate<sup>1</sup> for Final Larval Weight (mg) of Fall Armyworm Offered Treated Corn.

Treatment	Trial Number; Treatment Date <sup>2</sup>		
	1; 09/28/2012	2; 10/24/2012	3; 11/06/2012
Control	111.3 ± 7.1 a	124.6 ± 4.6 a	141.5 ± 7.6 a
Triton X100	77.9 ± 7.9 ab	113.8 ± 4.6 a	127.7 ± 7.1 ab
Tween 20	81.0 ± 7.7 ab	125.4 ± 4.6 a	132.4 ± 7.2 ab
Penetrator Plus	85.2 ± 11.2 ab	120.1 ± 4.6 a	124.8 ± 7.2 ab
Dyne-Amic	68.8 ± 7.1 b	121.4 ± 4.6 a	130.8 ± 7.2 ab
0.5 mM JA	71.1 ± 7.5 b	109.7 ± 4.6 a	124.4 ± 7.2 ab
2.0 mM JA	72.7 ± 7.5 b	109.3 ± 4.6 a	138.5 ± 7.2 a
0.5 mM JA + Triton X100	63.4 ± 7.1 b	124.5 ± 4.8 a	125.7 ± 7.2 ab
0.5 mM JA + Tween 20	76.1 ± 8.3 ab	120.5 ± 4.6 a	113.0 ± 7.3 ab
0.5 mM JA + Penetrator Plus	59.3 ± 7.1 b	128.1 ± 4.6 a	104.2 ± 7.2 b
0.5 mM JA + Dyne-Amic	54.2 ± 7.1 b	121.4 ± 4.8 a	128.3 ± 7.5 ab
Treatment p-value	<0.0001	0.0595	0.0289
Initial weight p-value	<0.0001	<0.0001	<0.0001

<sup>1</sup> Estimate based on LS means from analysis of covariance performed in SAS 9.3.

<sup>2</sup> Values in the same column followed by the same letter not significantly different as determined by Tukey-Kramer method for multiple comparisons.

3 ( $P = 0.0389$ ). Additionally, FAW reared on corn treated with 0.5 mM JA + Penetrator Plus gained significantly less weight than those offered non-treated corn in trial 1 ( $P < 0.0001$ ) and trial 3 ( $P = 0.0228$ ). For Trial 1, weight gain of FAW reared on all JA treatments except 0.5 mM JA + Tween 20 were significantly lower than the control. There was a significant initial weight (covariate) effect for trial 1 ( $P < 0.0001$ ), trial 2 ( $P < 0.0001$ ), and trial 3 ( $P < 0.0001$ ).

For cotton, treatment had a significant effect on the growth of FAW in trial 1 ( $F_{10,98} = 7.98$ ,  $P < 0.0001$ ), trial 2 ( $F_{10,82} = 5.49$ ,  $P < 0.0001$ ), and trial 3 ( $F_{10,81} = 4.63$ ,  $P < 0.0001$ ) (Table 7). Only FAW larvae offered 2.0 mM JA-treated cotton in trial 1 showed significantly different weight gain compared to the control ( $P = 0.0003$ ). There was a significant initial weight

**Table 7.** Least Squares Means Estimate<sup>1</sup> for Final Larval Weight (mg) of Fall Armyworm Offered Treated Cotton.

Treatment	Trial Number; Treatment Date <sup>2</sup>		
	1; 09/07/2012	2; 10/12/2012	3; 11/09/2012
Control	40.9 ± 3.1 abc	21.6 ± 2.0 ab	42.2 ± 4.2 ab
Triton X100	45.9 ± 3.1 ab	30.0 ± 2.0 a	39.3 ± 2.9 ab
Tween 20	50.0 ± 3.1 a	25.6 ± 2.2 ab	43.4 ± 2.8 a
Penetrator Plus	41.6 ± 3.1 abc	22.7 ± 2.2 ab	48.0 ± 3.6 a
Dyne-Amic	40.3 ± 3.1 abc	19.7 ± 1.7b	45.5 ± 3.6 a
0.5 mM JA	36.1 ± 3.1 abc	23.2 ± 1.7 ab	33.7 ± 2.9 ab
2.0 mM JA	20.4 ± 3.1 d	17.4 ± 1.9 b	29.1 ± 2.9 b
0.5 mM JA + Triton X100	31.7 ± 3.1 bcd	29.5 ± 1.7 a	34.7 ± 3.2 ab
0.5 mM JA + Tween 20	28.6 ± 3.1 cd	30.6 ± 1.8 a	29.3 ± 3.0 b
0.5 mM JA + Penetrator Plus	29.5 ± 3.1 cd	23.8 ± 1.8 ab	35.8 ± 2.8 ab
0.5 mM JA + Dyne-Amic	30.2 ± 3.1 cd	19.4 ± 2.2 b	29.1 ± 2.9 b
Treatment p-value	<0.0001	<0.0001	<0.0001 <sup>3</sup>
Initial weight p-value	<0.0001	<0.0001	<0.0001

<sup>1</sup> Estimate based on LS means from analysis of covariance performed in SAS 9.3.

<sup>2</sup> Values in the same column followed by the same letter not significantly different as determined by Tukey-Kramer method for multiple comparisons.

<sup>3</sup> Because trial 3 showed a significant treatment by initial weight interaction ( $F_{10,81} = 2.76$ ,  $P = 0.0056$ ), a contrast was performed to determine significant differences at the mean of the covariate of each treatment.



(covariate) effect for trial 1 ( $P < 0.0001$ ), trial 2 ( $P < 0.0001$ ), and trial 3 ( $P < 0.0001$ ). In trial 3, there was a significant treatment by initial weight (covariate) interaction and a contrast statement was used to compare the final weight at the mean of initial weight. In trial 3, no treatments were different from the control and there was no difference among JA treatments.

### **5.3 Effects of Benzothiadiazole and Jasmonic Acid on Induced Resistance of Soybean to Fall Armyworm and Soybean Looper.**

Elicitor treatment had a significant effect on growth of FAW in trial 1 ( $F_{2,35} = 11.17$ ,  $P = 0.0002$ ), trial 2 ( $F_{2,37} = 5.49$ ,  $P = 0.0082$ ) and trial 3 ( $F_{2,40} = 31.76$ ,  $P < 0.0001$ ) and soybean looper (SBL) in trial 1 ( $F_{2,39} = 3.31$ ,  $P = 0.0472$ ) and trial 3 ( $F_{2,41} = 12.54$ ,  $P < 0.0001$ ), but not in trial 2 ( $F_{2,41} = 0.10$ ,  $P = 0.9062$ ) (Table 8). In trial 1, 2, and 3, weight gain of FAW offered JA-treated plants was lower than the control ( $P = 0.0001$ ,  $P = 0.0043$ ,  $P < 0.0001$ , respectively). Additionally, weight gain of FAW reared BTH-treated soybean was lower than the control, only in trial 3 ( $P = 0.0043$ ). In trials 1 and 3, SBL fed soybean treated with JA gained less weight than SBL fed the ethanol-water treatment ( $P = 0.0497$  and  $P < 0.0001$ , respectively). There was a significant initial weight (covariate) effect for FAW in trial 1 ( $P < 0.0001$ ) and trial 3 ( $P = 0.0111$ ), but not in trial 2 ( $P = 0.7416$ ), and trial 3 ( $P < 0.0001$ ). For SBL, there was a significant covariate effect in trial 2 ( $P = 0.0002$ ) and trial 3 ( $P = 0.0231$ ), but not in trial 1 ( $P = 0.0932$ ).

**Table 8.** Least Squares Means Estimate<sup>1</sup> for Final Larval Weight (mg) of Fall Armyworm and Soybean Looper Offered Elicitor-Treated Soybean.

Treatment	Trial Number; Treatment Date <sup>1</sup>					
	1; 11/06/2012 <sup>2</sup>		2; 12/07/2012 <sup>3</sup>		3; 01/15/2013 <sup>3</sup>	
	SBL	FAW	SBL	FAW	SBL	FAW
Control	204.2 ± 4.1	39.9 ± 1.4	221.8 ± 6.0	49.1 ± 3.4	130.1 ± 4.9	75.7 ± 3.9
Benzothiadiazole	203.4 ± 4.0	38.4 ± 1.5	225.5 ± 5.9	43.0 ± 3.5	120.2 ± 4.9	58.1 ± 3.7 *
Jasmonic Acid	190.9 ± 4.1 *	29.8 ± 1.7 *	222.84 ± 6.0	32.9 ± 3.4 *	96.5 ± 4.9 *	33.1 ± 3.7 *
Treatment p-value	0.0472	0.0002	0.9026	0.0082	<0.0001	<0.0001
Initial weight p-value	0.0932	<0.0001	0.0002	0.8448	0.0231	0.0111

<sup>1</sup> Estimate based on LS means from analysis of covariance performed in SAS 9.3.

<sup>2</sup> Larvae for treatment date 11/06/2012 were only on tissue for 48 h.

<sup>3</sup> Treatment using 5X elicitor rate for benzothiadiazole.

\* Indicates final weight is significantly different from the control treatment ( $P < 0.05$ ) as determined by Dunnett's test for multiple comparisons to a single control.

## 6. DISCUSSION

Host plant resistance can be categorized as either constitutive, referring to morphological or chemical attributes that are always present, or inducible, referring to a plant's response to herbivory or chemical elicitors. Plant defense can be subsequently categorized as direct, negatively affecting the physiology or behavior of herbivores, or indirect, increasing the performance of natural enemies (Schoonhoven et al. 2005). Induced resistance against herbivorous arthropod pests was discovered only in the last half-century and, to date, over 100 plant species have demonstrated inducible resistance against herbivores (Karban and Kuć 1999). The plant hormones most responsible for mediating plant responses to pathogens and herbivores are salicylic acid and jasmonic acid (JA), respectively; however, azelaic acid, benzothiadiazole (BTH), harpin, gibberellins, and several other organic and inorganic chemicals have been shown to alter plant resistance to pathogens or herbivores when applied exogenously (Kahl et al. 2000, Traw and Bergelson 2003, Nombela et al. 2005, Yang et al. 2005, Smith et al. 2009, Jung et al. 2009, Hamm et al. 2010). For this study, we were interested in elicitor-mediated induction of direct defenses, specifically those that could reduce growth and, consequently, herbivore fitness. Ultimately, the goal is to incorporate the use of chemical defense elicitors into an integrated pest management program.

Although there is a large body of research regarding elicitor-mediated plant defense responses to insect herbivores (Smith et al. 2009), much of the research in this area has been performed on a limited number of plants including tomato, *Arabidopsis*, and cotton (Dong et al. 1999, Stout et al. 1999, Thaler 1999, Omer et al. 2001, Thaler et al. 2001, Boughton et al. 2005, Boughton et al. 2006). Additionally, most studies focus on a single plant species and a limited number of elicitors (Bi et al. 1997, Black et al. 2003, Traw and Bergelson 2003, Nombela et al.

2005, Weston 2008). A comprehensive examination of several elicitors across multiple agronomic crops in a single study is lacking in the scientific literature. The goal of this research was to screen several elicitors on four agronomic crops, two monocots and two dicots (Objective 1), investigate the possibility of increasing the effectiveness of a confirmed elicitor by combining it with an adjuvant (Objective 2) and, examine differential effects of elicitors on two species of lepidopteran larvae fed treated soybean tissue (Objective 3).

The results of experiments for the first objective support previous findings that JA induces a defense response when applied exogenously (Thaler 1999, Omer et al. 2001, Boughton et al. 2006, Hamm et al. 2010). In this study, that response was stronger and more consistent in the dicotyledonous crops, cotton and soybean, than the monocotyledonous crops, corn and rice. Interestingly, in the second experiment, corn was more responsive to the application of an adjuvant with JA than was cotton, indicating that the ineffectiveness of JA alone on corn may have been due to poor spray adhesion or reduced penetration, both as a result of the plant cuticular barrier. Finally, the results of experiment three demonstrate a differential effect of elicitor-induced resistance on two chewing insect herbivores in soybean: *Spodoptera frugiperda* showed greater sensitivity to induced resistance from elicitor application, than did to *Chrysodeixis includens*.

Of the five putative elicitors, JA was the only one to consistently reduce weight gain of fall armyworm (FAW) when larvae consumed treated leaf tissue, especially from cotton and soybean. Weight gains of FAW fed JA-treated cotton and soybean were significantly lower than those of FAW offered non-treated cotton and soybean in six of eight trials. For corn and rice, significant differences in weight gain of FAW between JA-treated and control leaves were not observed in any of the seven trials. There are three possible explanations for these findings: 1)

there was a difference in penetration of exogenous JA due to inherent differences in plant cuticle between the selected monocots and dicots, 2) the selected monocots and dicots differed in their inherent sensitivity to JA and thus in the ability of exogenous JA to induce a defense response, and 3) FAW larvae are more tolerant of the defense response elicited in the monocots, corn and rice, than in the dicots.

In support of the first hypothesis, spray mixtures adhered better to cotton and soybean foliage than corn and rice foliage, spreading better and resulting in decreased run-off. On corn and rice, the mixture appeared to simply form beads and roll off the plant. It is widely accepted that there is considerable variation in structure and permeability of plant cuticles, which could account for the observed differences in water adherence in monocots and dicots in this study (Schönherr and Baur 1994). As a result of decreased adherence, less elicitor could make contact with the leaf surface and be available for plant uptake, thus reducing activation of defense responses.

Alternatively, different plant species employ different defense strategies, and corn, rice, and cotton have been shown to produce volatiles that may act as attractants to parasitoids and other natural enemies (Rodriguez-Saona et al. 2003, Schoonoven et al. 2005, Rostás and Turlings 2008, Yuan et al. 2008, Köllner et al. 2009). It is possible that the primary defense response in corn and rice is an indirect defense mechanism to attract natural enemies pests, which these experiments were not designed to test, as opposed to a direct induced chemical defense mechanism. Lastly, the preference by FAW for grasses as a primary host suggests that it has developed tolerance of, or resistance to, the direct defenses produced by common host plants, specifically field corn and rice.

Experiment 2 was designed to investigate the possibility that the lack of difference in growth of FAW on corn and rice, compared to cotton and soybean, was a result of decreased adherence of spray mixtures to corn and cotton previously observed. These results demonstrate that the use of some spray adjuvants co-applied with JA decreased the final weights of FAW offered treated corn, but not cotton. In the first experiment, there was a significant decrease, compared to the control, in weight gain FAW offered cotton treated with the 3X JA rate (6.0mM) and in two of the three 1X JA rate (2.0mM) treatments, but no difference in any of the corn trials. These results may have occurred due to the use of a spray adjuvant increasing spray adhesion to and penetration of the leaf surface, which in turn aids in elicitation of a defense response in corn. The use of adjuvants appeared to increase elicitation of a defense response by exogenous JA application. The differential response between corn and cotton treated with a co-application of JA (0.5mM) and an adjuvant is supported by the concept of variability in cuticle structure and water adherence among plant species (Schönherr and Baur 1994). In the corn experiment, the 0.5mM JA + Penetrator Plus® treatment reduced growth of FAW compared to the 0.5mM JA and control treatments in one of three and two of three trials, respectively. Additionally, the 2.0mM JA only treatment reduced FAW weight compared to the control in only one of three trials. In the cotton experiment, only one trial demonstrated a significant effect on FAW from JA treatment. The lack of significant treatment differences in cotton between the first and second series of experiments is likely due to a combination of two factors. First, the addition of adjuvants does not dramatically affect the adhesion and spreading of water on the already receptive leaf surface of cotton, and second, the use mean separation by Tukey's method in experiment 2, compared to Dunnett's method in experiment 1, increases discrimination of

significance. The findings in these experiments indicate that field corn responds to exogenous application of JA, and that FAW is susceptible to direct induced defenses of corn elicited by JA.

Also notable in a few trials and crops, FAW offered leaf material treated with azelaic acid, BTH, and harpin demonstrated greater larval weights compared to the control. These elicitors have previously been shown to promote resistance to plant pathogens, probably by activating the salicylic acid pathway (Dong et al. 1999, Yang et al. 2005, Jung 2009, Barilli 2010). Increased weight gain of FAW larvae fed leaves treated with these specific elicitors is consistent with other research showing crosstalk between the salicylic acid (SA) and JA signaling pathways. The activation of the SA pathway, responsible for resistance to some pathogens and piercing-sucking insects, can have a suppressive effect on the JA pathway and induced defenses, especially against chewing insect herbivores (Thaler et al. 1999, Stout et al. 1999, Felton and Korth 2000, Leon-Reyes et al. 2010).

The results presented here agree with those in previous studies that demonstrate induction of plant defenses by JA (Thaler 1999, Omer et al. 2001, Boughton et al. 2005, Hamm et al. 2010) but not SA (Bi et al. 1997, Inbar et al. 2001). The results with harpin are supported by Boughton et al. (2005), who showed that harpin was not effective in reducing growth of insect populations on treated plants. Our findings indicated no significant negative effect of BTH-treated tissue on FAW weight gain. These results are similar those from Bi et al. (1997) and Inbar et al. (2001) who found that BTH- treated cotton had no effect on growth of corn earworm, *Helicoverpa zea*, or cotton bollworm, *Helicoverpa armigera*. However, Boughton et al. (2005) demonstrated that BTH did decrease development of green peach aphid populations on tomato. The SA pathway is believed to be more responsive to and instrumental in plant defense against piercing-sucking arthropods and biotrophic pathogens (Glazebrook 2005, Leitner et al. 2005, Smith et al. 2009),

and can have a suppressive effect on the JA pathway. The findings presented here, in conjunction with prior literature, further support the suggested roles of SA and JA in defense signaling in piercing-sucking versus chewing insect herbivores.

Finally, whereas soybean looper (SBL) and FAW are known to be polyphagous, with each known to feed on over 40 host species, it is not surprising that there is a differential effect of elicitors on larval weights using soybean as the treated host. FAW larvae prefer grasses (Luttrell and Mink 1999) and SBL larvae prefer dicots, especially legumes such as soybean (Herzog 1980). The preference of grasses by FAW could explain the tolerance of secondary defensive chemistry presumably produced by grasses following elicitor treatment. The SBL, in contrast, prefers legumes, especially soybeans (Martin et al. 1976, Herzog 1980, Jost and Pitre 2002), and may be more tolerant to direct defenses produced by those plants. In the third experiment, there were only minor indications of elicitor effectiveness. SBL weights were reduced by JA application to soybean in two of three trials, but did not respond to BTH applications in the 1X trial or either of the 5X rate trials. Second and third SBL instars are approximately two and ten times larger than FAW, respectively, which could have influenced these results. Additionally, one trial of FAW offered soybean treated with the 5X rate of BTH resulted in decreased weight, compared to the control ( $P = 0.0043$ ). Schmelz et al. (2009) demonstrated that there is considerable variation in response across several species of plants (soybean included), in terms of the variety and amounts of phytohormones produced in response to elicitors from insect secretions. It is possible that soybean produces a mixture of secondary metabolites at different concentrations in response to specific elicitors. This variability could differentiate between the two target species.



The findings presented here support previous research suggesting that JA induces defenses against insect herbivores. For the sum of all results, there are important implications for the potential use of elicitors, specifically JA, as an additional tool in an integrated pest management program. JA-treated cotton and soybean consistently reduced weight gain of FAW. The co-application of an adjuvant with a reduced-rate JA treatment on field corn also decreased FAW weights. The next logical step is to investigate specific plant-arthropod herbivore interactions, especially in cotton and soybean, which could be manipulated using JA as a defense elicitor, and to transition this experimentation to field trials against native populations. As indicated in the studies presented herein, 48 h is sufficient time for some plants to initiate defense characters and provide positive results reducing the success of insect herbivores. An important follow-up study would be to determine the duration of defense traits elicited in plant species to have a better understanding of the residual effects of the induced state. There is a lag time in the induction of defenses, and a prophylactic or pre-infestation application may be most useful against a pest with a predictable pattern of infestation. Similarly, additional laboratory and greenhouse research is warranted to clarify the interactions and influence of co-applications of adjuvants on JA-induced defense characters. As demonstrated, corn and cotton respond differently to JA applied with an adjuvant. The optimal combination of adjuvant, adjuvant rate, JA concentration, and application timing to maximize induction of defenses and minimize plant injury is needed.

These results will add to the current literature on chemically induced herbivore resistance in selected crops and will serve as the foundation for ongoing and future research involving elicitor-induced defenses, and the potential value for co-application with adjuvants. Currently, field studies are being conducted to assess the effectiveness of JA in reducing population growth

of two-spotted spider mite (*Tetranychus urticae* Koch) in cotton. Preliminary results suggest that JA treatments provide population reductions equivalent to that of Zeal® (Etoxazole), an acaricide (spider mite growth regulator), at 15 days after treatment (J. Gordy, unpublished data). Eventually, the strategy of inducing herbivore resistance with elicitors may be incorporated into an integrated pest management program.

## 7. SUMMARY AND CONCLUSIONS

Integrated pest management is a multifaceted approach to mitigate damage by herbivorous insect pests which usually relies heavily on broad-spectrum synthetic insecticides that exhibit several negative effects. The use of elicitors for the induction of plant defenses that result in decreased herbivore fitness should be considered as an additional approach in integrated pest management. The purpose of this project was to screen potential elicitors for their use in inducing resistance to herbivorous insects in field trials of major agricultural commodities, to evaluate the effectiveness of adjuvants in increasing uptake of jasmonic acid (JA), and to examine possible differential effects of JA and benzothiadiazole on soybean looper (SBL) and fall armyworm (FAW) fed treated soybean.

In objective one, JA was the only elicitor to consistently reduce weight gain of FAW. In the two dicotyledonous crops, cotton and soybean, JA was most effective as an elicitor. In cotton and soybean, six of eight trials resulted in reduced weight gain of larvae fed JA treated plant tissue. This supports previous findings that exogenous application of JA can cause changes in plant chemistry that negatively affect the growth or fitness of arthropod herbivores (Thaler 1999, Omer et al. 2001, Boughton et al. 2005, Hamm et al. 2010). Additionally, weight gain of FAW fed azelaic acid, benzothiadiazole, and harpin-treated plant tissue tended to be higher than FAW fed control-treated plant material, although only five treatments over four trials exhibited significant differences. These three elicitors had previously been shown to induce resistance to plant pathogens (Yang et al. 2005, Jung et al. 2009, Barilli et al. 2010), which may explain the resulting increased growth of FAW and further support the proposal that activation of the salicylic acid signaling pathway against plant pathogens suppresses the JA signaling pathway (Thaler et al. 1999, Stout et al. 1999, Felton and Korth 2000, Leon-Reyes et al. 2010).

In performing objective one, it was noted that the spray mixtures appeared to be less adherent to the monocotyledonous crops, corn and rice, and so objective two was designed to investigate the use of adjuvants to increase plant contact and/or cuticle penetration as a means of increasing the effectiveness of JA. Interestingly, in objective two, corn showed a greater response to the use of an adjuvant compared to cotton, even with a reduced JA concentration and an adjuvant rate below the label recommendation. This suggests that the lack of response in objective one was a result of inadequate spray coverage limiting the amount of elicitor available for plant uptake. Additionally, this finding indicates that grasses, specifically corn, are capable of producing a direct defense response that negatively affects growth of FAW, and that FAW may not be tolerant of or resistant to that response, despite grasses being the preferred host for FAW.

In objective three, the two lepidopteran foliage feeders, FAW and SBL, were differentially affected by elicitor application. FAW larvae fed JA-treated plant tissue gained less weight than FAW fed control-treated plant material in trials one, two, and three, in which weight gain reduced by 25%, 33%, and 56%, respectively. For SBL, only trials one and three showed reduced weight gain of 6.3% and 26%, respectively, for larvae fed JA treated plant material, compared to the control. The application of BTH showed no effect on SBL and was effective at reducing growth of FAW in only one trial, at the 5X rate. It is hypothesized that the SBL host preference of soybean (Martin et al. 1976, Herzog 1980, Jost and Pitre 2002), and FAW host preference of grasses (Luttrell and Mink 1999), is responsible for the differential effect observed here. SBL may be more tolerant than FAW to the induced response of soybean resulting from the application of JA, compared to FAW. Additionally, it is possible that SBL was less sensitive to the induced plant defenses because of the larger size, compared to FAW.

Results from this study provide support for the investigation of JA as an elicitor of plant defense in field studies. The addition of an adjuvant increased activity of JA in corn, and that relationship should be studied more extensively. There was differential growth of FAW and SBL fed soybean treated with JA and BTH, with SBL being less affected. All of these results indicate that JA may be effective as a tool in an integrated pest management program. Currently, there are plans to continue field trials investigating how JA affects populations of two-spotted spider mite in cotton, and to begin field trials investigating the effect of JA on populations of thrips in seedling cotton.

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## APPENDIX: TRIAL INFORMATION

Information for planting date, treatment date, temperature, and fall armyworm (FAW) strain by crop and objective.

<b>Objective</b>	<b>Crop</b>	<b>Plant Date</b>	<b>Treat Date</b>	<b>Temp. Range (°F: °C)</b>	<b>FAW Strain</b>
Objective 1	Corn	9-Mar-12	11-Apr-12	65-90: 18-32	Rice
		12-Apr-12	27-Apr-12	70-90: 21-32	Corn
		28-Jun-12	11-Jul-12	75-95: 24-35	Corn
	Cotton	7-Feb-12	7-Mar-12	65-85: 18-29	Rice
		28-Jun-12	11-Jul-12	75-95: 24-35	Corn
		5-Jul-12	18-Jul-12	75-95: 24-35	Corn
		11-Jul-12	1-Aug-12	75-95: 24-35	Corn
	Rice	14-Mar-12	12-Apr-12	65-90: 18-32	Rice
		15-May-12	6-Jun-12	70-95: 21-35	Rice
		28-Jun-12	11-Jul-12	75-95: 24-35	Rice
		14-Nov-12	5-Dec-12	65-85: 18-29	Rice
	Soybean	14-Mar-12	12-Apr-12	65-90: 18-32	Rice
		2-May-12	16-May-12	70-90: 21-35	Corn
		11-Jul-12	25-Jul-12	75-95: 24-35	Corn
18-Nov-12		5-Dec-12	65-85: 18-29	Corn	
Objective 2	Corn	14-Sep-12	28-Sep-12	70-95: 21-35	Corn
		8-Oct-12	24-Oct-12	65-90: 18-32	Corn
		19-Oct-12	6-Nov-12	65-85: 18-29	Corn
	Cotton	17-Aug-12	7-Sep-12	70-95: 21-35	Corn
		21-Sep-12	12-Oct-12	65-90: 18-32	Corn
		17-Oct-12	9-Nov-12	65-85: 18-29	Corn
Objective 3	Soybean	21-Oct-12	6-Nov-12	65-85: 18-29	Corn
		20-Nov-12	7-Dec-12	65-85: 18-29	Corn
		21-Dec-12	15-Jan-13	65-85: 18-29	Corn

## **VITA**

John Wesley Gordy is the son of Leslie Ettredge, Jack Claiborne, Jr., and Joe Gordy. He was born in Bastrop, Texas. John graduated from Bastrop High School in May of 2001. In December of 2005, he received a Bachelor of Science degree in Agronomy and Entomology from Texas Agricultural and Mechanical University. John began his graduate studies in June 2011 at Louisiana State University and Agricultural and Mechanical College under the supervision of Drs. Rogers Leonard and Mike Stout. He currently resides in Baton Rouge, Louisiana; is married to Jennifer Reck-Gordy, and has a son, Mason Jacob. John is currently a candidate for the Masters of Science degree in the Department of Entomology at Louisiana State University.