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Costs and benefits of induced responses in soybean

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Herbivorous insects are known to negatively impact plant fitness, such that plants have evolved defense strategies to reduce the preference and performance of herbivores on those plants. However, a plant’s investment in defense may be costly when herbivores are absent from the environment. Defense traits that are induced only upon herbivory can mitigate costs associated with defense maintenance. Although costs and benefits of induced responses are generally assumed, empirical evidence for them is equivocal. We examined the fitness costs and benefits of jasmonic acid-induced responses by soybean (*Glycine max* L. Merr.) in the absence and presence of soybean loopers (*Chrysodeix includens* Walker) (Lepidoptera: Noctuidae), an economically important pest of soybeans in the southern United States. In a greenhouse experiment we demonstrated that induction was costly to soybeans, affecting all components of soybean fitness. Jasmonic acid-induced plants produced 10.1% fewer seeds that were 9.0% lighter, and had 19.2% lower germination rates than control plants. In contrast, induction provided only modest benefits to soybeans. In a choice experiment, soybean loopers exhibited a significantly greater preference for leaves from control plants consuming 62% more tissue than from jasmonic acid-induced plants. Soybean loopers that did feed on induced soybean plants matured at the same rate and to the same size as soybean loopers that fed on control plants. However, at high conspecific density, soybean looper survivorship was reduced by 44% on induced relative to control plants. Negative effects of induction on soybean looper preference and survivorship did not translate into fitness benefits for soybeans. Our study is the first evaluation of costs and benefits of soybean induced responses.
CHAPTER 1: INTRODUCTION

For plants, interactions with herbivorous insects are known to negatively impact fitness (Marquis 1984). Thus, plant populations are under enormous pressure to evolve defense strategies that reduce herbivory or the impact of herbivory (Fraenkel 1959, Levin 1973, Strauss and Agrawal 1999, Wittstock and Gershenzon 2002). Plant defense strategies fall into two general categories- ‘tolerance’ and ‘resistance’ (Fineblum and Rausher 1995, Núñez-Farfán et al. 2007). Plants may tolerate herbivore damage (i.e., reduce the impact of herbivory) through compensatory processes such as photosynthetic enhancement, reallocation of resources, or increased growth rates (Maschinski and Whitham 1989, Trumble et al. 1993, Strauss and Agrawal 1999). Plant resistance reduces herbivory through chemical (Fraenkel 1959, Wittstock and Gershenzon 2002) or morphological (Levin 1973, Cooper and Owen-Smith 1986, Valverde et al. 2001) traits. Resistance traits can act by deterring or repelling herbivores from plants (i.e., antixenotic traits) or by reducing the performance of herbivores on plants (i.e., antibiotic traits) (Beck 1965).

Plant resistance traits may be constitutive and expressed at all times, or may be facultatively expressed, e.g., induced by herbivory (Karban and Baldwin 1997). Induced responses to herbivory are considered a common defense strategy for plants (Karban and Baldwin 1997). Induced defenses are likely to be favored by natural selection when the risk of herbivory is variable or unpredictable because maintaining constitutive defenses in such environments could be costly relative to the benefits (Åström and Lundberg 1994). Alternative explanations for the evolution of an inducible response exist. For example, an inducible response may be favored over constitutive defenses if the induced plant response provides defense against
many types of herbivores; minimizes herbivore adaptations; or reduces plant autotoxicity (Agrawal and Karban 1999).

Costs of resistance expression for plants are generally assumed (Rhoades 1979, Karban and Baldwin 1997, Stamp 2003, Karban 2010). For constitutively expressed traits, investment in resistance is constant, even in the absence of herbivores. In contrast, induced plant responses are expected to mitigate costs of defense expression because the plant only synthesizes defenses when needed (Herms and Mattson 1992, Karban and Baldwin 1997, Karban 2010). For example, in a greenhouse study of tomato (*Lycopersicon esculentum*), induced resistance was costly in terms of the number of seeds produced per plant in the absence of herbivores (Redman et al. 2001). Costs of induced responses were also found for wild tobacco (*Nicotiana attenuata*: Baldwin et al. 1990, Baldwin 1998), but benefits of induced resistance were only seen under low rates of herbivore attack (Baldwin et al. 1998). Lastly, net fitness benefits of induced resistance have been documented in species such as wild radish (*Raphanus sativus*: Agrawal 1998), *Lepidium virginicum* (Agrawal 2000) and *Trifolium repens* (Gómez et al. 2007).

A number of agricultural plants, including tomato, soybean, wheat and rice are known to respond to herbivory (Lin and Kogan 1990, Thaler et al. 1996, Karban and Baldwin 1997, El-Wakeil et al. 2010, Hamm et al. 2010). For agricultural plants, studies of induced responses have particular economic and environmental importance (Stout et al 2002, Valland and Goodman 2004). The use of induced resistance as a crop trait can mitigate costs of resistance when chance of herbivore attack is low, benefiting yield. However, the use of induced resistance in pest management has not yet reached its full potential in part due to our limited knowledge of the fitness costs and benefits of the induced response (Thaler et al. 2001). As such, this thesis aims to
examine for the first time, the fitness costs and benefits of a soybean induced response, from the perspective of both the insect and the plant.
CHAPTER 2: COSTS AND BENEFITS OF INDUCED RESPONSES IN SOYBEANS

INTRODUCTION

Because herbivorous insects are known to negatively impact plant fitness (Bardner and Fletcher 1974, Marquis 1984), plants have evolved defense strategies to reduce herbivory or the impact of herbivory on plant fitness (Fraenkel 1959, Levin 1973, Strauss and Agrawal 1999, Wittstock and Gershenzon 2002). These defense strategies are generally assumed to incur a fitness cost to the plant (Rhoades 1979, Karban and Baldwin 1997, Stamp 2003, Karban 2010). As predicted by the resource allocation hypothesis and other prominent hypotheses proposed to explain plant-defense patterns (e.g., carbon:nutrient balance hypothesis, growth rate hypothesis; reviewed in Stamp 2003), trade-offs should exist between investment in defenses and investment in growth and reproduction. Defenses that can be expressed facultatively, e.g., induced by herbivory, are expected to mitigate costs if the plant only synthesizes defenses when needed (Herms and Mattson 1992, Karban and Baldwin 1997, Karban 2010). Under this scenario, induced defenses are expected to be favored by natural selection when the risk of herbivory is variable or unpredictable (Clark and Harvell 1992, Åström and Lundberg 1994, Karban and Nagasaka 2004).

Costs of inducible defenses have been examined for relatively few species considering the extent to which induction has been documented in plants (Karban and Baldwin 1997). Evidence for costs of inducible defenses has been equivocal. Costs have been documented for some plant species (Baldwin et al. 1990, Redman et al. 2001, Gómez et al. 2007) but not for others (Brown 1988, Karban 1993). Furthermore, no clear patterns have emerged from studies investigating the cost of inducible defenses. For example, costs of induced defenses have been found for wild and cultivated plants as well as for both annual and perennial plants. Similarly,
tests of whether induction provides reproductive benefits to the plant, in the face of subsequent exposure to herbivores, have also yielded inconsistent results. Some studies have shown a net fitness benefit of induction (Agrawal 1998, 2000, Gómez et al. 2007), others have not (Karban 1993, Thaler 1999a), and still others have demonstrated that the benefit is dependent on aspects of the plant’s environment (e.g., the density of herbivores and competitors; Baldwin 1998, Agrawal 2000). It is clear that additional studies are needed to assess the generality of costs and benefits of induced responses among plants (Agrawal 2005), an important step in understanding the distribution of plant defense strategies in nature. Furthermore, understanding how induced responses may mitigate costs of defense has obvious applications for agriculture where there is often a tradeoff between yield and insect resistance in crops (Boethel 1999).

Induced resistance has now been documented in more than 100 species of plants, including species of agricultural importance such as tomato, rice, wheat, and soybeans (Lin and Kogan 1990, Thaler et al. 1996, Karban and Baldwin 1997, El-Wakeil et al. 2010, Hamm et al. 2010). Among plants of agricultural importance, the annual plant, soybean (Glycine max L. Merr.) has emerged as a model species with which to address questions about induced resistance (e.g., Creelman et al. 1992, Underwood et al. 2000, Underwood and Rausher 2002). Induced resistance in soybean is known to negatively affect herbivore preference for plants and herbivore development (Lin and Kogan 1990, Kogan and Fischer 1991). Although the full chemistry of a soybean induced response is complex and not fully understood, soybean induced resistance is thought to be driven by changes in activity of phytoalexins and proteinase inhibitors after herbivory (reviewed in Kogan and Fischer 1991, Underwood et al. 2002) and can be elicited by multiple herbivore species (e.g., Lin and Kogan 1990, Bi et al. 1994, Srinivas et al. 2001,
Fortunato et al. 2007). To date, no published data exists regarding the allocation of resources, and lifetime costs and benefits incurred by soybean following induction.

We aimed to examine the fitness costs of a soybean induced response and benefits of that response after herbivory by soybean looper (*Chrysodeix includens* Walker) (Lepidoptera: Noctuidae), an economically important generalist defoliator of soybeans in the southern United States (Heatherly and Hodges 1999). We selected soybean variety Williams 82 because it is known to respond to feeding by soybean loopers (Lin and Kogan 1990, Srinivas et al. 2001). However, the costs and benefits associated with this response are unknown. We first tested the prediction that, in the absence of herbivores, induction of responses in soybean would incur a fitness cost relative to non-induced, control plants. Second, we performed two experiments to assess the benefits of induction to soybeans. In a host-choice experiment, we tested the prediction that induction would reduce soybean looper feeding preference for induced soybeans. Next, using induced and control soybeans in a no-choice experiment, we tested two predictions: 1) soybean looper performance (pupal mass, development time, and survivorship) would be negatively affected by antibiotic effects of soybean induction, and 2) induced soybeans would have higher fitness than control soybeans when exposed to soybean loopers. In light of our findings of a costly induced response with limited benefits to soybean, we discuss how soybean contributes to our greater understanding of induced responses and assess whether Williams 82 may be used in a pest management program for soybean.

**MATERIALS AND METHODS**

**Plants and Insects**

All soybean plants were of the Williams 82 variety and seed was obtained from the
United States Department of Agriculture’s National Plant Germplasm System (Beltsville, MD). Soybeans were grown in a greenhouse at Louisiana State University, Baton Rouge, LA, with ambient lighting. Plants were grown from seed in 13 cm square pots in a soil-less, sterile potting mix (Sunshine Mix #8, Sun Gro Horticulture, Bellevue, WA) and fertilized weekly with Jack’s Classic (J. R. Peters, Inc., Allentown, PA), water soluble fertilizer (N:P:K = 20:20:20).

Soybean looper caterpillars are generalists that may feed on 28 plant families, including wild and cultivated species, although soybean is a primary host (Herzog 1980). Soybean looper adults migrate yearly from Central America and the Caribbean to the southern United States, producing three to four generations a year (Funderburk et al. 1998). Caterpillars were obtained from Jeff Davis (Department of Entomology, Louisiana State University) from a colony that was established in 2008 from 300 soybean loopers collected from soybean fields in Winnsboro, Louisiana. Larvae were maintained on ~10 mL of artificial soybean looper diet (Southland Products, Lake Village, AR) in 30 mL closed cups, and housed in an environmental chamber set at constant 28.5° C, 50% relative humidity (RH), 12:12 L:D photoperiod, and with 1100 lux light level prior to experiments.

**Chemical Induction**

The use of herbivores as the initial agent of induction can be problematic because of the difficulty in controlling the amount of leaf tissue consumed (Baldwin 1996, Cipollini et al. 2003). To avoid this issue, we induced plants using jasmonic acid, a phytohormone important in signaling wound responses (Creelman et al. 1992, Creelman and Mullet 1997, Howe 2004). Jasmonic acid has been applied to many plant species exogenously, including soybeans (M. J. Stout, personal communication), to stimulate defensive pathways and elicit an induced response (e.g., Baldwin 1996, Thaler et al. 1996, McConn et al. 1997, Halitschke and Baldwin 2005).
Induced resistance in soybean reaches peak levels three days following herbivory, and declines thereafter (Underwood 1998).

To induce soybean plants, we used the general procedure described by Hamm et al. (2010). A 1 mM jasmonic acid solution was prepared by dissolving 31.54 mg of jasmonic acid (Sigma-Aldrich, St. Louis, MO, USA) in 1 mL of 95% ethanol and then diluting the ethanol solution with 150 mL of distilled water. Control solutions consisted of 1mL of ethanol added to 150 mL of distilled water (Hamm et al. 2010). The upper sides of all leaves were sprayed with either the jasmonic acid or control solution until run-off using a Preval® aerosol sprayer (CA Acquisition, Coal City, IL). In all experiments, chemical induction was performed when soybeans were at full bloom (the R2 stage; Fehr et al. 1971), the stage at which herbivory has the greatest effect on plant fitness (Fehr et al. 1983, Baldwin et al. 2009).

**Costs of Induction by Jasmonic Acid**

If induction is costly to soybeans, then fitness should be lower for induced than non-induced (control) plants of the same genotype or variety in the absence of herbivores (Karban and Baldwin 1997). Forty plants were grown from seed to the full bloom stage, and divided equally between a jasmonic-acid treatment (induced plants) and an untreated control. Plants were sprayed twice a week for 2.5 weeks with either the jasmonic acid or control solution, to maintain induced resistance in jasmonic acid-treated plants. This time frame is equivalent to the duration of the larval stage of a soybean looper (~2.5 weeks; Shour and Sparks 1981). Plants received approximately 9 mL of solution (~ 1.89 mg jasmonic acid) at the first treatment and this gradually increased to 15 mL (~3.15 mg of jasmonic acid) by the end of the study to accommodate the increase in soybean biomass. After the last application of jasmonic acid, plants were allowed to mature and their seeds were harvested. Components of soybean fitness,
including total seed number and mean seed mass were determined for each plant. All seeds were then subjected to warm germination tests, where seeds were contained between wet paper towels for a period of 7 days at 28.5° C, to determine the proportion of seeds that germinated (Johnson and Wax 1978, LeVan et al. 2008). The effects of induction status on components of fitness (seed number, mean seed mass, and proportion of seeds that germinated) were analyzed with two-sample $t$-tests. Sequential Bonferroni corrections were used to maintain an overall type I error rate of $\leq 0.05$ (Rice 1989).

**Benefits of Induction by Jasmonic Acid: Soybean Looper Feeding Preference**

One way that plants may benefit from induction is through its negative effects on feeding preference of insect herbivores (e.g., Barker et al. 1995, Underwood et al. 2000). Here, we conducted an experiment to determine the effect of soybean induction status (induced, non-induced control) on soybean looper feeding preference. Soybean looper caterpillars were given a choice between soybean leaf tissue from a plant that was previously induced (i.e., treated with jasmonic acid) or left untreated (control). Thirty soybeans were grown to full bloom and were divided equally and randomly between the two treatments. Each plant was sprayed with 7 mL of either the jasmonic acid solution or the control solution. Plants were given three days after the jasmonic acid application to reach maximum levels of induction (Underwood 1998). Afterward, two 2-cm diameter leaflet disks were cut from the leaf at the fifth node of each plant using a cork borer. The pair of disks from a treatment plant and a control plant were placed 1.5 cm apart in an alternating, 2x2 pattern in a Petri dish (90 x 15 mm) lined with a thin layer of cork and a moist filter paper (Whatman #1, Florham, NJ). Leaf disks were secured to the cork with a small pin. One third instar soybean looper that had been starved for 3 hours was released in the center of the 2x2 grid and the dish was placed in an environmental chamber (28.5° C, 50% RH, 3000 lux).
The feeding trial was terminated after 12 hours or when approximately 25% of the leaf disks had been consumed. Digital images of the consumed leaf disks were recorded and leaf area removed from each disk was determined using ImageJ 1.43u (National Institutes of Health, Bethesda, MD).

A feeding-preference index (PI) was calculated for each dish based on the difference in disk area consumed between control and treatment disks. Here, $PI = \frac{2T}{(T + C)}$, where $T$ and $C$ are the total areas consumed of the treatment and control leaf disks, respectively (Kogan and Goeden 1970, Kogan 1972, Underwood 1998). Values of $PI > 1$ would indicate that the caterpillar preferred the treatment disk, values of $PI < 1$ would indicate a preference for the control disks, and a $PI = 1$ would indicate no preference. We tested the specific hypothesis that $PI < 1$ using a one sample, one tailed $t$-test. All statistical analyses were performed with SYSTAT 12 (Systat Software, Inc., Chicago, IL). We note here that in two of 15 dishes, soybean loopers did not feed on any disks. These dishes were not included in the analysis.

**Benefits of Induction by Jasmonic Acid: Soybean Looper Performance and Soybean Fitness**

Another presumed benefit of induction is that herbivore performance on induced plants, i.e., growth, development time and/or survivorship, should suffer (e.g., Lin and Kogan 1990, Stout and Duffey 1996, Traw and Dawson 2002, Nykanen and Koricheva 2004). These negative effects on the herbivore should translate into reduced levels of defoliation and increased plant fitness (Karban and Baldwin 1997, Wold and Marquis 1997, Boege 2004). In the long term, herbivore population size could be reduced, resulting in lower herbivore loads later in the season or for subsequent crops (Haukioja 1980).
To determine whether there are benefits of induced resistance for soybean plants in the presence of herbivores, we subjected induced and non-induced soybeans to low and high densities of soybean loopers and determined their effects on soybean looper performance and plant fitness. Two different densities of soybean loopers were used because the effects of induction can be density dependent (Baldwin 1998). Fifty-two plants were grown from seed to the full bloom stage, and divided equally between a jasmonic acid treatment (induced plants) and an untreated control. Jasmonic acid and control solutions were applied to soybeans as outlined in the cost experiment; however each plant received only a single application of the treatment or control solution (~7 mL solution per plant). Three days following the completion of the chemical induction treatment, either four (low density) or eight (high density) first instar soybean looper caterpillars were added to each plant, resulting in 13 replicates of each induction status-herbivore density treatment combination. Herbivore density levels were chosen based on known tolerance levels of soybean and our personal observations of soybean looper damage in a greenhouse setting. At full bloom, soybean can tolerate up to 25% defoliation before experiencing losses in yield (Baldwin et al. 2009). Thus, the low herbivore density treatment was intended to produce less than 25% defoliation in individual plants, whereas the high density treatment was intended to produce defoliation levels greater than 25%. Larvae were transferred to the uppermost four leaves of the plant (one or two larvae per leaf, depending on density treatment). Each plant was confined in a white mesh sleeve for the duration of the larval stage of the soybean loopers (16-21 days). We note, however, that one plant in the induced-low density treatment was discarded because of contamination by a different caterpillar species.

Four days following herbivore release, plants were inspected daily for the presence of soybean loopers. As soybean loopers entered the pupal stage (~ 17 days), they were removed
from plants, weighed to the nearest one hundredth of a gram, and placed individually in 120 mL closed cups in an environmental chamber (28.5° C, 50% RH, 12:12 L:D, 1100 lux) until eclosion.

After all pupae were removed from each plant, mesh sleeves were removed and proportion of leaf area consumed by soybean loopers was visually estimated by comparing leaves to images of soybean leaves with known levels of defoliation ranging from 5% to 50% defoliation (Kogan and Turnipseed 1980). An estimate of defoliation for the entire plant was calculated by averaging the proportion of leaf area removed from all leaflets of each plant. All seeds were harvested from each plant at maturity. Number of seeds, mean seed mass, and proportion of seeds that germinated were quantified (see cost experiment).

Effects of induction status, herbivore density, and their interaction on variables related to larval performance (pupal mass, development time, and survivorship) were analyzed using separate two-way ANOVAs. Development time was defined as number of days from first instar to eclosion and survivorship was defined as survival to eclosion. A significant interaction term (herbivore density x induction) would indicate that the effect of induction status on larval performance varied with herbivore density. For each plant, a single estimate of the three dependent variables (mean pupal mass, mean development time, and proportion survived to eclosion) was obtained so that the potted plant was the unit of replication for all tests. Pupal mass and development time were normally distributed and had variances that were homogeneous among treatments (based on a Levene’s test). Survivorship was arcsine square-root transformed prior to analysis to meet assumptions of normality, but assumptions of homogeneity of variances were not met (Levene’s test, \( P = 0.035 \)). However, because ANOVA is robust to violations of the equal variances assumption when there is a near-balanced design such as with our experiment.
(Underwood 1997, McGuinness 2002), we do not consider this to be a problem. In the case of a significant interaction effect, post-hoc planned t-tests were conducted to determine how the induction effect varied across herbivore density levels. Sequential Bonferroni corrections were used to maintain an overall type I error rate of ≤ 0.05.

Effects of induction status, herbivore density, and their interaction on the proportion defoliated and soybean fitness components (seed number, mean seed mass, and proportion of seeds that germinated) were analyzed using the same model as for larval performance. Defoliation was arcsine square-root transformed prior to analysis to meet assumptions of normality. Sequential Bonferroni corrections were used to maintain an overall type I error rate of ≤ 0.05.

RESULTS

Cost Experiment

Induction of soybeans at full bloom was costly to soybeans, negatively affecting all components of soybean fitness. Induced plants produced 10.1% fewer seeds that were 9.0% lighter, and had 19.2% lower germination rates than control plants, differences that were statistically significant even after Bonferroni corrections (number of seeds: $t_{38} = 2.03, P = 0.05$, Figure 1A; seed mass: $t_{38} = 3.34, P = 0.002$, Figure 1B; germination: $t_{38} = 2.85, P = 0.007$, Figure 1C).

Benefit Experiment: Soybean Looper Feeding Preference

Soybean loopers preferred control over induced soybean leaf tissue. Soybean loopers consumed an average of 62.7% less tissue area from jasmonic acid treated leaf disks compared to control leaf disks (mean area removed ± SE: control: 0.53 ± 0.19, treatment: 1.41 ± 0.27). The
mean preference index, $PI$, was significantly less than one (mean $PI \pm SE = 0.66 \pm 0.18$; $t_{12} = -1.88$, $P = 0.04$).

**Benefit Experiment: Soybean Looper Performance and Soybean Fitness**

The effects of soybean induction on soybean looper performance were density-dependent. There was a marginally significant herbivore density - induction status interaction effect on soybean looper survivorship ($F_{1,47} = 5.78$, $P = 0.02$; Bonferroni-corrected alpha = 0.017; Figure 2A). In the high herbivore density treatment soybean loopers exhibited 44.2% higher survivorship on control plants compared to induced plants, a difference that was marginally significant ($t_{24} = 2.02$, $P = 0.055$). In the low herbivore density treatment, soybean loopers surprisingly exhibited 116.7% higher survivorship on induced plants compared to control plants, but the difference was not significant ($t_{23} = -1.50$, $P = 0.15$).

Increased herbivore density resulted in significantly smaller soybean looper pupae ($F_{1,27} = 16.47$, $P < 0.001$; Figure 2B). In the high-herbivore density treatment, pupae weighed 20.5% less than pupae in the low herbivore density treatment. Pupal mass was unaffected by induction status ($F_{1,27} = 0.17$, $P = 0.69$) or the interaction of induction status with herbivore density ($F_{1,27} = 2.03$, $P = 0.17$). Furthermore, neither herbivore density, induction status nor their interaction affected soybean looper development time ($F_{1,28} = 0.36$, $P = 0.55$; $F_{1,28} = 2.49$, $P = 0.13$; $F_{1,28} = 1.55$, $P = 0.22$, respectively, Figure 2C).

Increased herbivore density resulted in greater defoliation and negative effects on components of soybean fitness, but induction status had no effect on these measures of plant performance. In the high herbivore density treatment, soybean loopers removed an average of 31.3% ± 11.9% leaf area from plants, compared to an average of 11.56% ± 12.6% leaf area removed from plants in the low herbivore density treatment, differences that were statistically
significant \( (F_{1,47} = 34.26, P < 0.001, \text{Figure 3A}) \). Increased herbivore density also had significant negative effects on components of plant fitness such that plants in the high herbivore density treatment produced 13.4% fewer seeds that were 14.7% lighter and exhibited 30.8% lower germination rates than plants subjected to low herbivore densities (Figure 3B-D; Table 1). Neither induction status nor the interaction between induction status and herbivore density affected plant performance variables (Figure 3A-D; Table 1).

Table 1. ANOVA results of the benefit experiment testing the effects of herbivore density, induction status, and their interaction on proportion of leaf area removed and fitness components of soybean.

<table>
<thead>
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<th>Variable</th>
<th>Effect</th>
<th>( F_{1,47} )</th>
<th>( P )</th>
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<td>Proportion Leaf Area Removed</td>
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<td>Induction X Density</td>
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**DISCUSSION**

Our study contributes to the growing body of evidence demonstrating that induced responses to herbivory do not necessarily support theoretical predictions of both fitness costs and benefits (Brown 1988, Karban 1993, Thaler 1999a). Our finding of a significant fitness cost of induction is the first such demonstration for soybeans. Induced soybean plants produced 10% fewer and 9% lighter seeds that had 19% lower germination rates compared to control plants. Our study of the reproductive cost of soybean induction is consistent with a small number of
Figure 1. The effect of induction status (control or induced) on number of seeds (A), seed mass (B), and proportion of seeds germinated (C) of soybean plants (± 1 SE) in the absence of herbivores. Asterisks (*) denote a significant difference between means following a sequential Bonferroni correction of alpha.
Figure 2. The effect of induction status and herbivore density on survivorship of soybean loopers (A), pupal mass (B), and number of days to eclosion (C). Error bars are ± 1 SE. Asterisks (*) indicate $P < 0.05$ following a sequential Bonferroni correction of alpha.
Figure 3. The effect of induction status and herbivore density on proportion of leaf area removed from soybean (A), number of seeds (B), seed mass (C), and proportion of seeds germinated (D). Error bars are ± 1 SE. Asterisks (*) denote significance after a sequential Bonferroni correction to alpha.
studies in wild and cultivated plant species that have also found a cost of induction (Baldwin et al. 1990, Redman et al. 2001, Gómez et al. 2007). However, there are number of confounding issues inherent in studies of plant defense that make identifying patterns of defense costs among plants difficult. For example, the mode of induction (e.g., leaf tissue removal by herbivores or chemical elicitor application) and the variable(s) used to estimate reproductive success or fitness may differ among studies. Comparisons of the effects of induction and herbivory on perennial versus annual plants may also be difficult as only yearly estimates of fitness are possible for perennial plants. Lastly, although we found a reproductive tradeoff for an induced response under competition- and herbivore-free conditions in our greenhouse study, it remains to be determined what effect a less ideal environment could have on soybean induction. Factors such as physiological stressors (e.g., drought) (Bergelson and Purrington 1996, Dietrich et al. 2005), nutrient availability (Walls et al. 2005, Sampedro et al. 2011), and competition for resources (van Dam and Baldwin 1998, Baldwin and Hamilton 2000) can affect the cost of an induced response. It is clear that additional studies are needed to understand how costs of induced defenses differ among species with different life history characteristics and how the environmental context affects the cost of an induced defense.

We found only modest benefits of induction to soybeans. When given a choice, soybean loopers had a lower preference for induced leaves, consuming 62% less induced than control leaf tissue. Although we did not evaluate chemical changes in leaves directly, previous studies have attributed soybean induction’s effects on herbivores to changes in leaf phytoalexin and proteinase inhibitor content after herbivory (Kogan and Fischer 1991, Underwood et al. 2002). However, this negative effect of induction on soybean looper feeding did not translate to reduced
damage in the no-choice experiment when soybean loopers were confined to induced plants. We discuss this seemingly contradictory finding below.

Induction status significantly affected only soybean looper survivorship, but the effects were density dependent. There was a 44% reduction in survivorship for soybean loopers reared on induced plants compared to control plants in the high soybean looper density treatment (i.e., densities that caused damage to exceed the tolerance threshold level). These results are consistent with studies that have documented antibiotic effects of soybean induced resistance on soybean loopers (Hart et al. 1983, Lin and Kogan 1990). At low conspecific density (at which damage was below the tolerance threshold level), soybean looper survivorship was not significantly affected by induction status; although, surprisingly, survivorship averaged 116% higher on induced compared to control plants. We are unable to account for this unusual finding.

We suggest that reduced soybean looper survivorship only at high conspecific densities likely resulted from plant damage to previously induced plants causing further induction in plants, a pattern found in a number of species, including soybean (Karban and Baldwin 1997, Underwood 2000). When exposed to high herbivore densities, soybeans lost 31% of their leaf tissue compared to only 11% under low herbivore density conditions. Although we can assume that control plants were also induced after three days of herbivore feeding, it is probable that the greater damage caused by higher densities of herbivores on pre-induced plants elicited even greater responses from soybeans.

At high soybean looper densities, the reduced survivorship of soybean loopers on induced plants did not translate into lower defoliation levels or higher fitness for soybeans. However, reduced soybean looper survivorship on induced soybeans may have long-term benefits for the soybean plants. Soybean loopers migrate yearly from Central America and the Caribbean to the
southern United States, producing three to four generations in a year (Funderburk et al. 1998). Reduced survivorship in the first generation due to crop induced resistance could potentially translate to smaller population sizes in subsequent generations and reduced herbivore loads (Haukioja 1980). Although reduced soybean looper populations could benefit crops later in the growing season or in subsequent years, induced resistance likely cannot provide the immediate protection and direct benefits to soybeans that insecticides can provide.

There are several possible explanations for why induced soybean plants did not exhibit a reduction in defoliation or increase in fitness in our whole-plant, no-choice experiments even though at high densities soybean loopers suffered higher mortality on those plants. First, it is possible that the fewer caterpillars on induced plants compared to control plants compensated for low host quality (due to an increase in phytoalexins and other phytochemicals) by increasing the amount of leaf tissue they consumed (Slansky and Wheeler 1992, Lavoie and Oberhauser 2004). Lin and Kogan (1990) investigated induction effects on soybean loopers and suggested that compensatory feeding by the soybean loopers was the reason why induction affected development but not total food consumption. Second, even though soybeans did not benefit from induction when fed upon by soybean loopers, induction potentially may confer resistance against numerous other pests not considered in our study. Induced resistance elicited by the jasmonic acid pathway in plants is generally known to provide broad-spectrum resistance against herbivores (Howe and Jander 2008). For example, soybean induced resistance elicited by soybean loopers has been demonstrated to also confer resistance to Mexican bean beetles (Epilachna varivestis Mulsant) and bean leaf beetles (Cerotoma trifurcata Forster) (Lin and Kogan 1990, Srinivas et al. 2001). Third, our no-choice greenhouse experiment excluded any benefits that may have arisen from effects of induced responses on feeding preference if
caterpillars had alternative feeding options. This would explain why feeding damage was significantly less on induced leaf disks when soybean loopers were given a choice between induced and control leaf tissue. Lastly, the lack of benefits for soybeans could be due to low statistical power of the test. However, an effect size (proportional difference in fitness between the induced and non-induced plants) of less than 1% in our high herbivore density treatments and our ability to find significant treatment effects on soybean looper performance, suggest that our test was sufficiently powerful.

One frequent component of an induced response that we did not assess in our study was whether benefits to soybeans could also arise from the induction and emission of soybean volatiles (Rostás and Turlings 2008). Herbivore-induced plant volatiles are known to play important direct roles in plant resistance by influencing oviposition rates of herbivores (Kessler and Baldwin 2001, Horiuchi et al. 2003, Dudareva et al. 2006). Although soybeans are known to emit volatile compounds after induction (Rostás and Eggert 2008), a preliminary outdoor choice experiment of ours (Accamando and Cronin, unpublished data) suggests that volatiles may not play a direct role in soybean induced resistance to oviposition by adult soybean loopers. In contrast, there is some support for the positive, indirect role that herbivore-induced plant volatiles may play in soybean resistance through the attraction of natural enemies of herbivores (Moraes et al. 2005, Moraes et al. 2009). It has been documented with a variety of plant species that the emission of herbivore-induced plant volatiles can result in higher rates of parasitism and predation for herbivores on induced compared to non-induced plants (De Moraes et al. 1998, Thaler 1999b, Fritzsche-Hoballah and Turlings 2001). Thus, the emission of herbivore-induced volatiles in soybean represents a potentially important component of the costs and benefits of an inducible defense strategy.
A common dilemma in the development of cultivars with desirable agronomic traits (e.g., high yield, drought and disease resistance, etc.) is the tradeoff often found between insect resistance and yield (Boethel 1999, Lambert and Tyler 1999, Warrington et al. 2008). Because facultative traits can ameliorate the cost of resistance (and therefore yield losses) by not being expressed when herbivores are rare, this class of defenses has become an important issue for integrated-pest management (Stout et al. 2002, Vallad and Goodman 2004). Although only a handful of studies have investigated whether induced resistance is a viable strategy for crop protection (e.g., Black et al. 2003, Hamm et al. 2010), they provide some promising results for the future use of elicitors such as jasmonic acid and induced resistance in agriculture. However, based on our findings, induced resistance in soybean has limited potential as a candidate for integration into soybean looper management programs. In addition to finding a high cost of induced resistance for soybean, defoliation of soybean was reduced only when soybean loopers were presented with a choice of induced and non-induced soybean tissue. In a more realistic field environment, soybean loopers would be unlikely to encounter the same level of heterogeneity found in our choice experiment as soybean fields are typically only planted with a single variety. Therefore, it will be important to evaluate how the cost-to-benefit ratio of an inducible soybean variety compares to that of a variety that is constitutively resistant under a variety of field conditions when herbivores are both abundant and scarce.
CHAPTER 3: CONCLUSIONS

The mechanisms by which a plant defends itself against attack from herbivores are often complex and multifaceted (Mauricio et al. 1997, Agrawal and Fishbein 2006, Morris et al. 2006). This thesis addressed a single aspect of a plant’s total potential arsenal against herbivores - induced resistance. Although induced resistance has long been acknowledged as an important component of plant defense strategies, questions remain as to how and why plasticity in resistance evolved (Karban and Baldwin 1997, Siemens and Mitchell-Olds 1998, Agrawal and Karban 1999, Zangerl 2003). An important step toward answering questions related to the evolution of induced resistance is determining the conditions under which induced resistance is favored (Agrawal 2005). Theory predicts that trade-offs exist between a plant’s investment in defense and its investment in growth and reproduction (Stamp 2003). As such, plants are expected to initially incur a cost of an induced response, but plants should ultimately benefit from the induced response when faced with herbivory (Karban and Baldwin 1997). Although, costs and benefits have been assessed for a number of plants, both natural and agricultural species, the data remain equivocal (Brown 1988, Baldwin et al. 1990, Agrawal 1998, Thaler 1999a, Redman et al. 2001)

This thesis documents the costs and benefits of an induced response in soybean for the first time. However, the results of my experimental studies suggest that soybean induced responses do not necessarily support theoretical predictions of a costly but beneficial induced response. Rather, I demonstrate that a costly induced response does not benefit soybean fitness in the face of herbivory. Because induced responses did negatively affect soybean looper feeding preference in a no-choice experiment, I predict that fitness benefits of induced responses may only be apparent under conditions where soybean loopers have alternative feeding options.
Overall, the findings of my thesis suggest that the fitness benefits of induced responses to soybeans may be context dependent. Whether induced soybeans experienced reduced defoliation by soybean loopers depended on the presence of alternative food options. Furthermore, how induced responses affected herbivore performance was found to be density dependent, such that soybean looper survivorship was reduced on induced plants only under conditions of high conspecific density.

It is clear that no single factor will determine herbivore damage and fitness of a plant. Plant defense traits and strategies and the context in which they are employed will all likely play a role in plant damage and fitness (Karban 1997, Agrawal et al. 2006). This is of particular importance for plants that utilize an inducible defense strategy, as the expression of their defense may be influenced by the environmental context (Underwood 2000, Rodriguez-Saona and Thaler 2005, Viswanathan et al. 2005). Although, induced responses have been reported from over 35 plant families (Karban and Baldwin 1997), their importance in the context of a diverse community of plants and herbivores is still poorly understood (but see Kessler and Baldwin 2001, Rodriguez-Saona and Thaler 2005). Therefore, understanding how induced responses operate in natural environments and the relative importance of an inducible response as a defense strategy, will be important for understanding the evolution and maintenance of variation in plant defenses in nature.
LITERATURE CITED


VITA

Amanda K Accamando was born and raised in Brooklyn, New York. She attended the State University of New York at Geneseo from 1996-2000 where she majored in biology and minored in anthropology. After graduation, Amanda returned to New York City and for the next four years worked as an environmental educator for the Prospect Park Zoo and for The Butterfly Conservatory of the American Museum of Natural History. In 2004, Amanda moved to Puerto Rico to begin a position at the Caribbean Primate Research Center on Cayo Santiago Island, home to over 1000 rhesus macaques. As a research assistant for Dr. Kathlyn Robbins at the National Institutes of Health and for Dr. Melissa Gerald at the University of Puerto Rico for over three years, she conducted research on the sexual skin coloration of adult male and female macaques, and on the development of sexual behavior of juvenile female macaques. In 2007 Amanda made the move to Baton Rouge, Louisiana, to begin her master’s degree under the guidance of Dr. James Cronin, where she returned to research on plant-insect interactions. Upon graduation, Amanda intends to pursue a career in environmental education program development and management.