Costs and Benefits of Jasmonic Acid Induced Responses in Soybean

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ABSTRACT In response to herbivory, plants have evolved defense strategies to reduce herbivore preference and performance. A strategy whereby defenses are induced only upon herbivory can mitigate costs of defense when herbivores are scarce. Although costs and benefits of induced responses are generally assumed, empirical evidence for many species is lacking. Soybean (*Glycine max* L. Merr.) has emerged as a model species with which to address questions about induced responses. To our knowledge, this is the first study to examine the fitness costs and benefits of jasmonic acid-induced responses by soybean in the absence and presence of soybean loopers (*Chrysodeix includens* Walker) (Lepidoptera: Noctuidae). In a greenhouse experiment we demonstrated that soybean induction was costly. Induced plants produced 10.1% fewer seeds that were 9.0% lighter, and had 19.2% lower germination rates than noninduced plants. However, induction provided only modest benefits to soybeans. In a choice experiment, soybean loopers significantly preferred leaves from noninduced plants, consuming 62% more tissue than from induced plants. Soybean loopers that fed on plants that were previously subjected to treatment with jasmonic acid matured at the same rate and to the same size as those that fed on control plants. However, at high conspecific density, soybean looper survivorship was reduced by 44% on previously induced relative to control plants. Reduced soybean looper preference and survivorship did not translate into fitness benefits for soybeans. Our findings support theoretical predictions of costly induced defenses and highlight the importance of considering the environmental context in studies of plant defense.

KEY WORDS jasmonic acid, induced resistance, *Glycine max*, *Chrysodeix includes*

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 (Wittstock and Gershenzon 2002). These defense strategies are generally assumed to incur a fitness cost to the plant (e.g., Stamp 2003, Karban 2011). 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 synthesizes defenses only when needed (Herms and Mattson 1992, Cipollini and Heil 2010, Karban 2011). Under this scenario, induced defenses are expected to be favored by natural selection when the risk of herbivory is variable or unpredictable (Åström and Lundberg 1994, Karban and Nagasaka 2004).

Despite the fact that induction has been found commonly in plants, fitness costs of induced responses have been examined for relatively few plant species (Karban and Baldwin 1997). To date, the majority of studies (e.g., Baldwin et al. 1990, Redman et al. 2001, Cipollini 2002, Gómez et al. 2007, Björkman et al. 2008), but not all (Brown 1988, Karban 1993), have found evidence for costs of induced responses. Tests of whether induction provides reproductive benefits to the plant, in the face of subsequent exposure to herbivores, are more rare than tests of the cost of induction, but have yielded inconsistent results. Some studies have shown a net fitness benefit of induction (Agrawal 1998, 2000; Heil 2004; Gómez et al. 2007), others have not (Karban 1993, Thaler 1999), and still others have demonstrated that the benefit is dependent on aspects of the plant's environment (e.g., the density and identity 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, Cipollini and Heil 2010), 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 >100 species of plants, including species of agricul-

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tural importance such as tomato (*Solanum lycopersicum* L.), rice, and soybeans (*Glycine max* L. Merr.) (e.g., Lin and Kogan 1990, Thaler et al. 1996, Hamm et al. 2010). Among plants of agricultural importance, the annual plant, soybean has emerged as a model species with which to address questions about induced resistance (e.g., Creelman et al. 1992, Underwood et al. 2000). 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 chemical basis 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, Srinivas et al. 2001, Fortunato et al. 2007) and jasmonic acid (M. J. Stout, personal communication. To date, no published data exists regarding the allocation of resources, and lifetime costs and benefits incurred by soybean after induction.

Using jasmonic acid to promote induction, we examined the fitness costs of a soybean induced response and benefits of that response after herbivory by the soybean looper (*Chrysodeix includens* Walker) (Lepidoptera: Noctuidae), an economically important generalist defoliator of soybeans in the southern United States (Heatherly and Hodges 1999). We also tested whether the benefits of an induced response to soybean were dependent on the density of herbivores, as demonstrated in a previous study (Baldwin 1998). We selected soybean variety Williams 82 because it is known to exhibit an induced response 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 noninduced, control plants. Second, we performed two experiments to assess the benefits of induction to soybeans as it could relate to the evaluation of soybean pest management programs that use artificial induction. In a host-choice experiment, we tested the prediction that soybean loopers would exhibit reduced feeding preference for induced as compared with control soybeans. Next, using induced and control soybeans in a no-choice experiment, we tested two predictions: 1) soybean looper performance (pupal mass, egg-to-pupa and pupa-to-adult development time, and survivorship) would be negatively affected by prior soybean induction, and 2) induced soybeans would have higher fitness than control soybeans when exposed to soybean loopers, and this response would vary with the density of 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 useful 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). Soybean loopers are known as late-season attackers of soybean, with damaging infestations occurring in Louisiana from early August to September when soybean plants are in full bloom (Baldwin et al. 2011).

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, LA. Larvae were maintained on ≈10 ml of artificial soybean looper diet (Southland Products, Lake Village, AR) in 30-ml closed cups (2–3 larvae per cup), and housed in an environmental chamber set at constant 28.5°C, 50% RH, a photoperiod of 12:12 (L:D) h, and with 1100 lux light level before 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, Halitschke and Baldwin 2005). Induced resistance in soybean reaches peak levels three days after 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 31.5 mg of jasmonic acid (Sigma-Aldrich, St. Louis, MO) in 1 ml of 95% ethanol and then diluting the ethanol solution with 150 ml of distilled water. Control solutions consisted of 1 ml 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 by using a Preval aerosol sprayer (CA Acquisition, Coal City, IL) so that each plant received an approximately equal concentration of jasmonic acid per unit of leaf area. To achieve similar levels of coverage among plants, the volume of solution sprayed had to be increased with increasing plant size. In all experiments, chemical induction was performed when soybeans were at full bloom (the R2 stage; Fehr et al. 1971). The full bloom stage was chosen because it is the stage at which herbivory has the greatest effect on plant fitness (Fehr et al. 1983), and it is the stage present when soybean loopers begin colonizing soybean fields in Louisiana (Baldwin et al. 2011).

Costs of Induction by Jasmonic Acid. If induction is costly to soybeans, then fitness should be lower for induced than noninduced (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 wk 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 wk; Shour and Sparks 1981). Plants received ≈9 ml of solution (≈ 1.9-mg jasmonic acid) at the first treatment and this gradually increased to 15 ml (≈3.2 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 mass per seed 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 d 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 ≤ 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, noninduced 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 sprayed with a control solution (control). Thirty soybean plants were grown to full bloom and 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, 2 by 2 pattern in a petri dish (90 by 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 h was released in the center of the 2 by 2 grid and the dish was placed in an environmental chamber (28.5°C, 50% RH, 3,000 lux). The feeding trial was terminated after 12 h or when ≈25% of the leaf disks had been consumed. Digital images of the consumed leaf disks were recorded and leaf area removed (cm^2) 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, $PI = 2T/(T + C)$, 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, 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).

Induced resistance as a management tool is likely to involve artificial treatment of crops with an elicitor before the colonization of target pests (Vallad and Goodman 2004). With this in mind, we conducted an experiment to determine whether an induced response by soybean plants, before exposure to soybean loopers, is beneficial to the plant and detrimental to the herbivores. Here, we subjected induced and noninduced 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 (prefeeding induction treatment) and an untreated control (prefeeding 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 (≈9 ml solution per plant). Three days after the jasmonic-acid (or control solution) application, either four (low density) or eight (high density) first instar soybean looper caterpillars were added to each plant. The result was 13 replicates of each prefeeding induction status (induction or control) and herbivore density treatment combination. Herbivore density levels were chosen based on known tolerance threshold levels of soybean and our unpublished data 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. 2011). Thus, the low herbivore density treatment was intended to produce <25% defoliation in individual plants, whereas the high density treatment was intended to produce defoliation levels >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 d). We note, however, that one plant in the induced-low density treatment was discarded because of contamination by a different caterpillar species.

Four days after herbivore release, plants were inspected daily for the presence of soybean loopers. As soybean loopers entered the pupal stage ($\approx$ 17 d), 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, a photoperiod of 12:12 [L:D] h, and 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 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 prefeeding induction status, herbivore density, and their interaction on variables related to juvenile looper performance (pupal mass, larval development time, pupal development time, and survivorship) were analyzed using separate two-way ANOVAs. Larval and pupal development times were defined as number of days from first instar to pupation, and pupation to adult eclosion, respectively. Survivorship was defined as survival to adult eclosion. A significant interaction term (herbivore density x induction) would indicate that the effect of prefeeding induction status on larval performance varied with herbivore density. For each plant, a single estimate of the four dependent variables (mean pupal mass, mean larval and pupal development times, and proportion survived to eclosion) was obtained so that the potted plant was the unit of replication for all tests. If no pupae were produced on a single plant, the plant was eliminated from the analyses of pupal mass and development times. We also note that in the case of one plant, we were unable to measure the mass of the single pupa produced before eclosion.

Pupal mass was normally distributed and had a variance that was homogeneous among treatments (based on a Levene's test). Both development times had variances that were homogenous among treatments, but were not normally distributed. Because no transformation improved normality, we used the raw data in our analyses below. Survivorship was arcsine square-root transformed before analysis to meet assumptions of normality, but assumptions of homogeneity of variances were not met (Levene's test, $P = 0.035$). Because analysis of variance (ANOVA) is robust to violations of normality and the equal variances assumption when there is a near-balanced design such as ours (Underwood 1997, McGuinness 2002), we do not consider these issues to be a problem in our subsequent analyses. In the case of a significant interaction effect, posthoc planned *t*-tests were conducted to determine how the prefeeding induction effect varied across herbivore density levels. Sequential Bonferroni corrections were used to maintain an overall type I error rate of $\leq$0.05.

Effects of prefeeding 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 before analysis to meet assumptions of normality. Sequential Bonferroni corrections were used to maintain an overall type I error rate of $\leq$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: $t_{38} = 2.03$, $P = 0.05$, Fig. 1A; seed mass: $t_{38} = 3.34$, $P = 0.002$, Fig. 1B; germination: $t_{38} = 2.85$, $P = 0.007$, Fig. 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 with control leaf disks (mean area removed ± SE: treatment: 0.53 ± 0.19 cm^2, control: 1.41 ± 0.27 cm^2). The mean preference index, *PI*, was significantly less than one (mean $PI \pm$ SE = 0.66 ± 0.18; $t_{12} = -1.88$, $P = 0.04$) indicating that soybean loopers preferred control over induced soybean tissue.

Benefit Experiment: Soybean Looper Performance and Soybean Fitness. The effects of soybean prefeeding induction on soybean looper performance were density-dependent. On their own, neither herbivore density ($F_{1,47} = 3.19$, $P = 0.080$) nor prefeeding in-

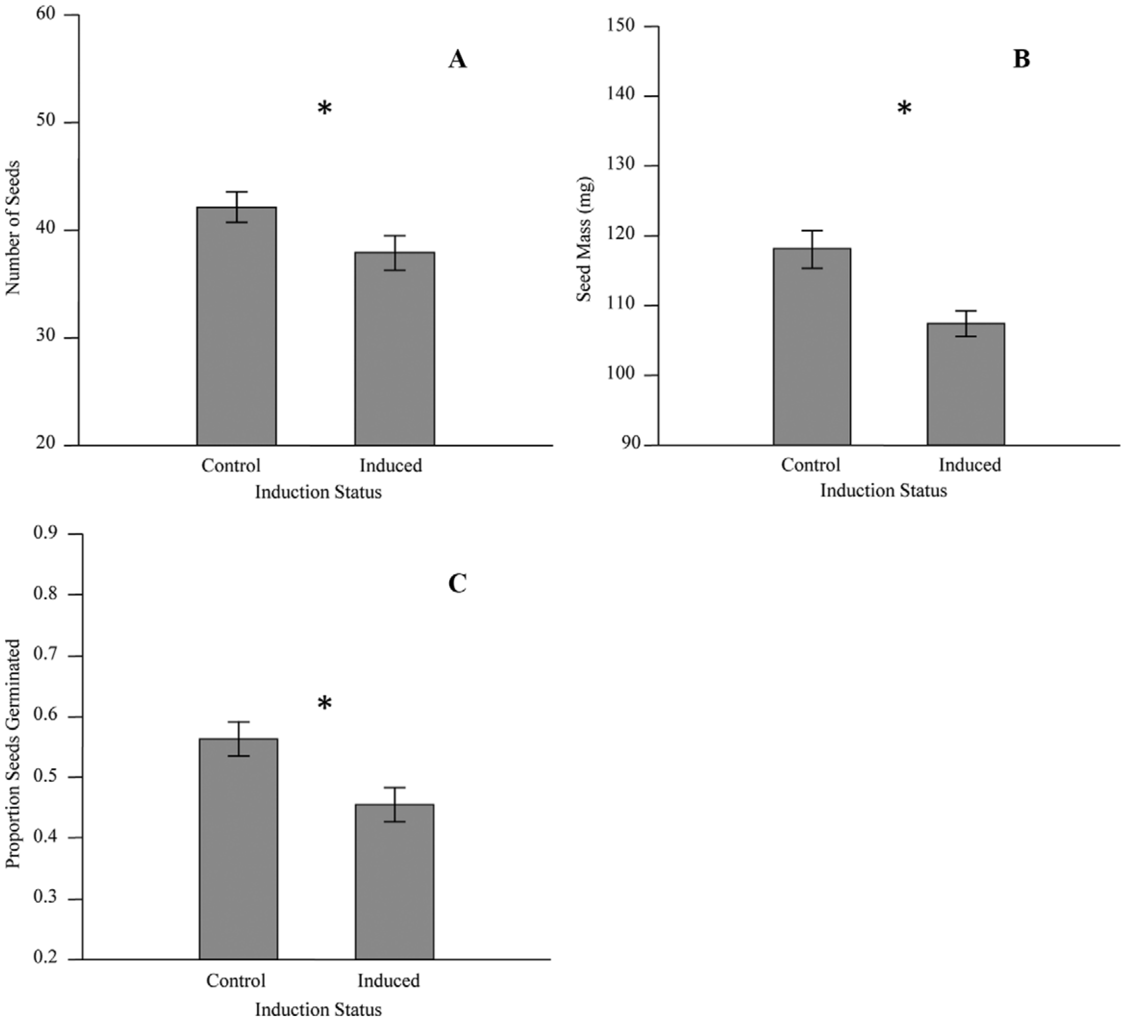

Fig. 1. The effect of induction status (control or induced) on number of seeds (A), seed mass (B), and proportion of seeds germinated (C) of soybean plants (±1 SE) in the absence of herbivores. Asterisks (*) denote a significant difference between means following a sequential Bonferroni correction of alpha.

duction status ($F_{1,47}$ = 0.002, P = 0.96) influenced soybean looper survivorship per plant but the interaction between the two had a marginally significant effect on survivorship ($F_{1,47}$ = 5.78, P = 0.02; Bonferroni-corrected alpha = 0.017; Fig. 2A). In the high herbivore density treatment soybean loopers exhibited 44.2% higher survivorship on control plants compared with previously induced plants, a difference that was marginally significant (t_{24} = 2.02, P = 0.055). At high density, soybean looper mortality occurred primarily in the larval stage (Fig. 3); an average of 25.9% and 14.4% of soybean loopers survived to eclosion on control and previously induced plants, respectively (Fig. 3). In the low density treatment, soybean looper survivorship did not differ significantly on previously induced plants compared with control plants (t_{23} = −1.50, P = 0.15). In the low density treatment, soybean looper mortality occurred entirely in the larval stage; an average of 9.62% and 20.83% of soybean loopers survived to eclosion on control and induced plants, respectively (Fig. 3).

Increased herbivore density resulted in significantly smaller soybean looper pupae ($F_{1,27}$ = 16.47, $P < 0.001$; Fig. 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 larval ($F_{1,27}$ = 1.88, P = 0.18; $F_{1,27}$ = 1.53, P = 0.23, $F_{1,27}$ = 3.35, P = 0.08, respectively, Fig. 2C) or pupal development time ($F_{1,27}$ = 1.89, P = 0.18; $F_{1,27}$ = 0.26, P = 0.61, $F_{1,27}$ = 0.04, P = 0.85, respectively, Fig. 2D).

Increased herbivore density resulted in greater defoliation and negative effects on components of soybean fitness, but induction status had no effect on

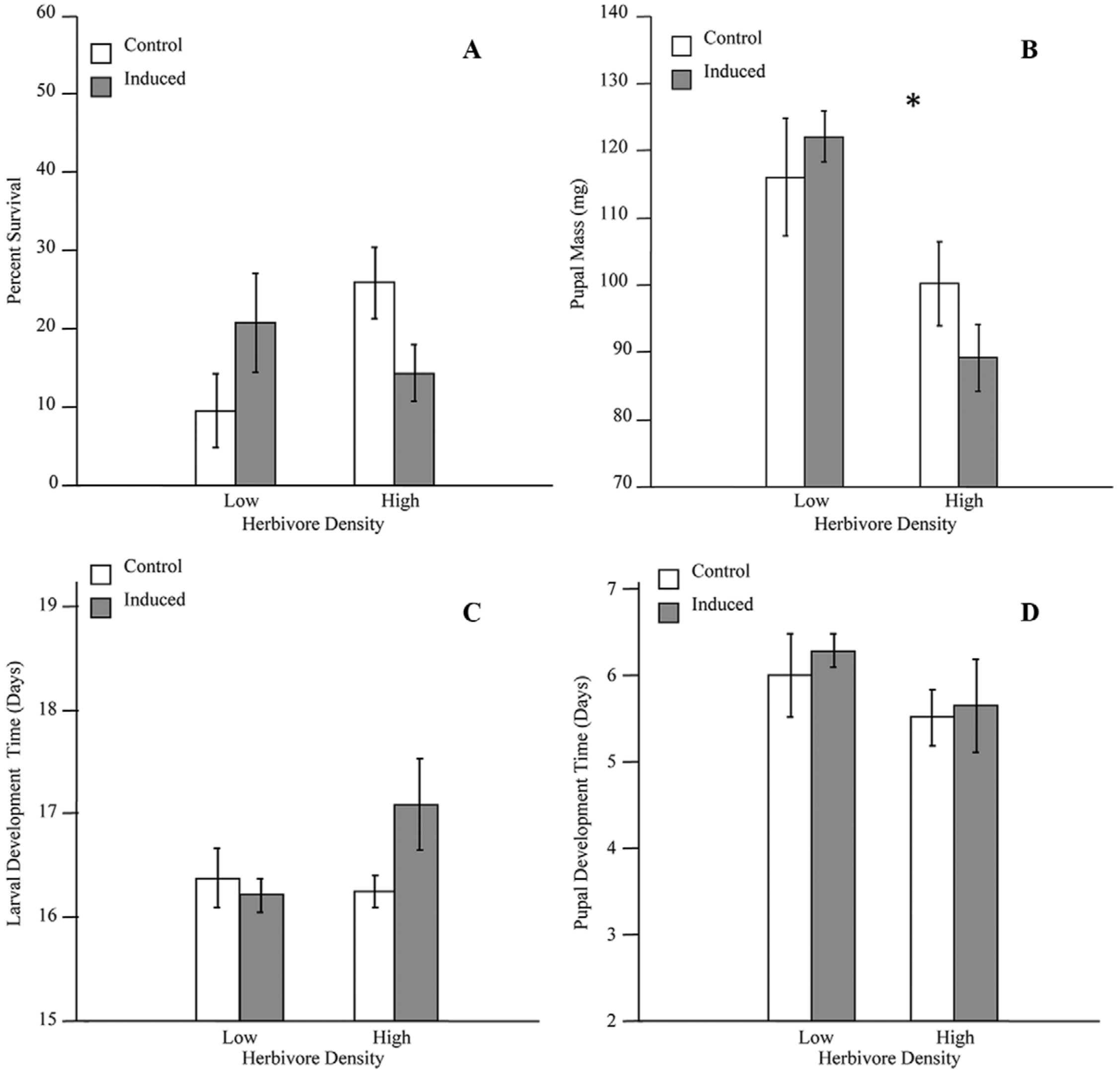

Fig. 2. The effect of prefeeding induction status and herbivore density on survivorship of soybean loopers (A), pupal mass (B), larval development time (C), and pupal development time (D). Error bars are ±1 SE. Asterisks (*) denote a significant difference between means following a sequential Bonferroni correction of alpha.

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 with an average of 11.6% ± 12.6% leaf area removed from plants in the low herbivore density treatment, differences that were statistically significant ($F_{1,47} = 34.26$, $P < 0.001$, Fig. 4A). 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 (Fig. 4B–D; Table 1). Neither induction status nor the interaction between induction status and herbivore density affected plant performance variables (Fig. 4A–D; Table 1).

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 1999). This study also demonstrates that induction benefits depend on environmental context, in this case, it varies with soybean looper density. Our finding of a significant fitness cost of induction is the first such demonstration for soybean, an economically important crop in the United States. In 2010, >77 million acres of soybean were planted with farm profits yielding over $38 billion (United States Department of Agriculture, Economic Research Service).

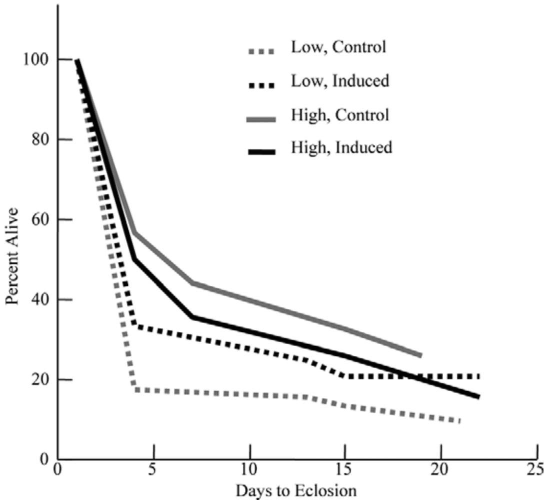

Fig. 3. Percent of soybean loopers surviving from the first instar (day 0) to adult eclosion. A separate curve is provided for each density (low, high) and prefeeding induction status (control, induced) treatment combination.

Induced soybean plants produced 10% fewer and 9% lighter seeds that had 19% lower germination rates compared with control plants, indicating that an induced defense strategy in soybean is costly in the absence of herbivores. Our study of the reproductive cost of soybean induction is consistent with an increasing number of studies in wild and cultivated plant species that have also found a cost of induction (e.g., Baldwin et al. 1990, Redman et al. 2001, Gómez et al. 2007). However, methodological differences among studies of plant defense often 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 often only yearly estimates of fitness are used in studies of perennial plants. Lastly, although we found a reproductive cost 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 (Sampedro et al. 2011); and competition for resources (Baldwin and Hamilton 2000) can affect the cost of an induced response. Different life history characteristics and the environmental context will likely be complicating, but important, factors in understanding how costs of induced defenses differ among plants species, and should be considered in future studies.

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). However, this negative effect of induction on soybean looper feeding did not translate into reduced damage in the no-choice experiment when soybean loopers were confined to previously induced plants. We discuss this seemingly contradictory finding below.

Prefeeding 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 previously induced plants compared with 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 prefeeding induction status.

It is unlikely that the reduced soybean looper survivorship found only at high conspecific densities resulted from competition among soybean loopers for food. At high densities, soybean loopers consumed on average only 31% of soybean tissue, suggesting that caterpillars may not have been entirely resource limited. A more likely explanation for the reduced soybean looper survivorship found only at high conspecific densities is that damage to previously induced plants (prefeeding induction treatment) caused even further induction, a pattern found in other species, including soybean (Karban and Baldwin 1997, Underwood 2000). Both control and jasmonic acid treated plants were exposed to low and high density herbivore loads. Herbivore feeding should have led to induction of control plants after three days. Likewise, herbivore feeding should have also caused further induction in previously induced plants. We can also assume that the greater damage (31% of leaf tissue lost) caused by higher densities of herbivores elicited greater responses in soybeans compared with soybeans exposed to low densities of herbivores (11% of leaf tissue lost). It was only under the combined conditions of prefeeding induction with jasmonic acid and subsequent feeding by high densities of herbivores that we documented reduced soybean looper survivorship.

We found no evidence for prefeeding induction-treatment effect on defoliation or plant fitness. 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 with control plants compensated for low host quality (because of an increase in phytoalexins and other phytochemicals) by increasing

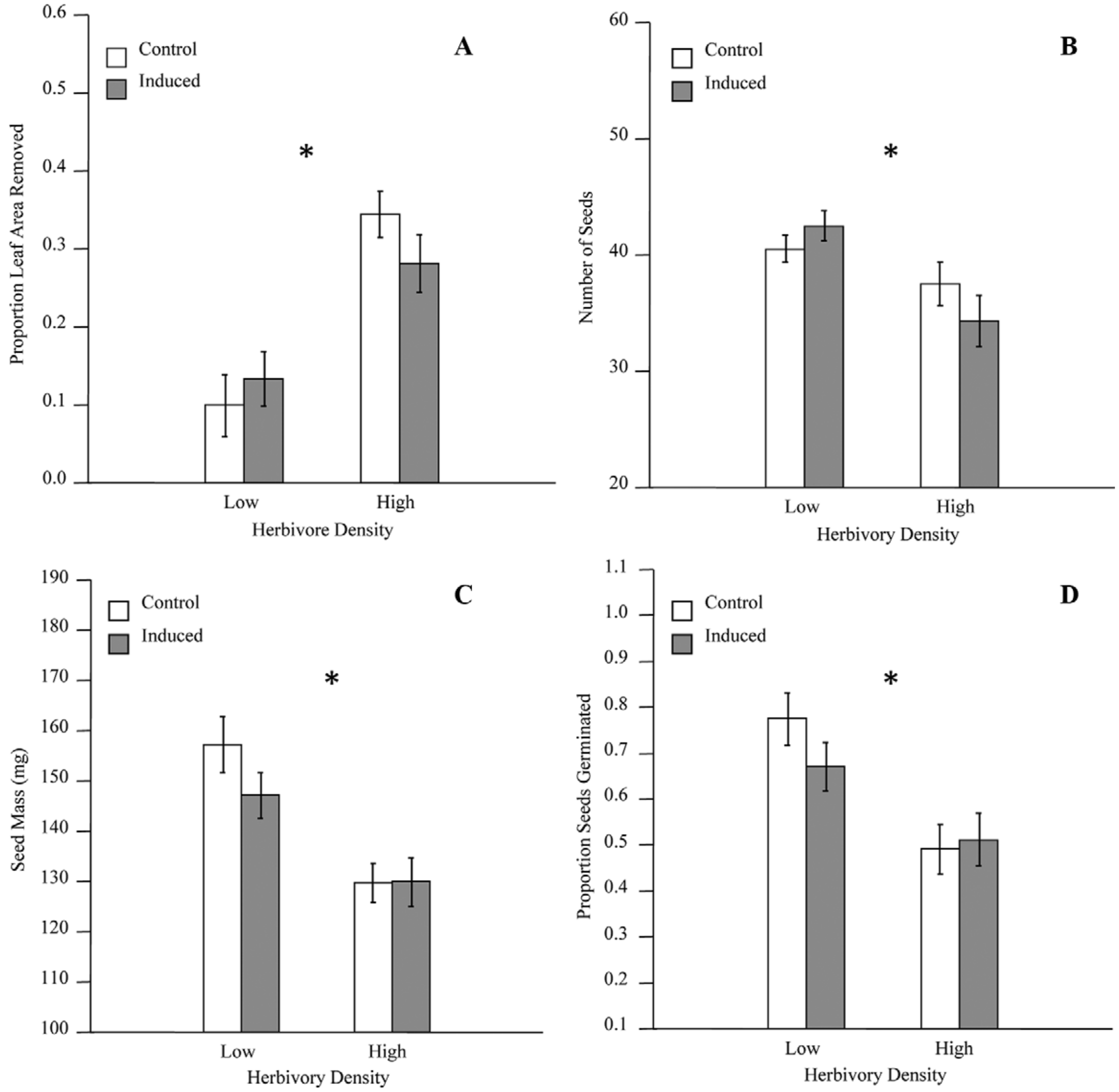

Fig. 4. The effect of prefeeding induction status and herbivore density on proportion of leaf area removed from soybean (A), number of seeds (B), seed mass (C), and proportion of seeds germinated (D). Error bars are ±1 SE. Asterisks (*) denote significance after a sequential Bonferroni correction to alpha.

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

Variable	Effect	$F_{1,47}$	P
Proportion leaf area removed	Herbivore density	34.26	<0.001
	Induction status	0.04	0.84
	Induction X density	2.57	0.12
Number of seeds	Herbivore density	11.53	<0.001
	Induction status	0.15	0.70
	Induction X density	2.48	0.12
Seed mass	Herbivore density	23.75	<0.001
	Induction status	1.16	0.29
	Induction X density	1.26	0.27
Proportion of seeds germinated	Herbivore density	17.49	<0.001
	Induction status	0.63	0.43
	Induction X density	1.37	0.25

the amount of leaf tissue they consumed (Slansky and Wheeler 1992, Lavoie and Oberhauser 2004). This type of behavior has been documented previously in soybean loopers feeding on soybean (Lin and Kogan 1990). 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 because of low statistical power of the test. However, an effect size (proportional difference in fitness between the induced and noninduced plants) of <1% in our high herbivore density treatments and our ability to find significant treatment effects on soybean looper performance, suggest that power was not an issue.

At high soybean looper densities, the reduced survivorship of caterpillars on previously induced plants did not translate into lower defoliation levels or higher fitness for soybeans. However, in the field, 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 because of 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.

A common dilemma in the development of cultivars with desirable agronomic traits (e.g., high yield, drought, and disease resistance) 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, Walters and Heil 2007, Gurr and Kvedaras 2010, Mészáros et al. 2011). 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 finding that defoliation of soybean was reduced only when soybean loopers were presented with a choice of induced and noninduced soybean tissue, induced resistance in soybean may be of limited use as a candidate for integration into soybean looper management programs. Current agricultural practices typically involve planting only a single soybean variety within a field. Thus, it will be important to evaluate whether greater heterogeneity in resistance traits (including inducible defense traits) within a field could be beneficial for farmers in terms of soybean pest management plan.

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