



# Phenotypic plasticity in seedling defense strategies: compensatory growth and chemical induction

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Phenotypic plasticity in growth (leading to compensation) and secondary chemical production (leading to induction) in response to herbivory are key defense strategies in adult plants, but their role in seedling defense remains unclear. A pair of greenhouse studies was conducted to investigate compensation and induction in seedlings and juvenile plants, using *Plantago lanceolata* (Plantaginaceae) and the specialist buckeye caterpillar *Junonia coenia* (Nymphalidae) as a model system. Plants received 50% defoliation at two and four weeks of age, and groups of plants were harvested one week after herbivory and six to eight weeks after herbivory to investigate the duration of the responses. Plants damaged at two weeks showed no chemical induction and fully compensated for the lost leaf tissue by ten weeks of age. Plants damaged at four weeks showed a significant reduction in iridoid glycosides one week after herbivory and achieved full shoot compensation by ten weeks of age at the expense of root biomass. These results indicate that *P. lanceolata* seedlings use compensation, but not chemical induction, as a defense strategy. This research highlights the importance of considering ontogeny in studies of plant–herbivore interactions and suggests that seedling defense may differ markedly from adult plant defense.

Studies of plant demography have revealed that most plant mortality occurs during the seed and seedling stages (Harper 1977). In contrast to seeds, seedlings are more difficult to define, resulting in some inconsistencies among studies. However, in general, ecologists accept all young and very small plants to be seedlings, and there have been many attempts to identify sources of seedling mortality (Fenner 1987). A recent review of studies on natural communities reports that herbivores are the greatest source of mortality (above drought, fungal disease, physical damage and competition) and generally account for more than 38% of seedling mortality in natural populations (Moles and Westoby 2004). Under some circumstances, herbivores may even cause seedling mortality rates as high as 100% (Gardescu 2003). These observations are significant from an evolutionary perspective because they suggest that there is tremendous potential for strong selection pressure for seedling defense in most plant species. Yet, studies of plant–herbivore interactions have typically focused on adult plants, and relatively little is known about seedling defense against herbivores. Furthermore, research on seedling defense has focused primarily on a single defense trait – the constitutive production of defensive secondary chemicals. These studies have provided mixed results: in some species, seedling secondary chemical production is low (Cipollini and Redman 1999, Fritz et al. 2001), but in others, seedling secondary chemical production is high (Erwin et al. 2001, Schaffner et al. 2003). Few studies have investigated other defense strategies, even those that are

common among adult plants, such as plasticity in defense traits in response to herbivory (e.g. induction and compensation).

Phenotypic plasticity is a key aspect of the strategies that plants use to deal with herbivores. Plasticity can occur in traits that confer resistance to herbivores (e.g. secondary compounds, thorns, trichomes), leading to their induction, and in traits that confer tolerance to herbivory (e.g. compensatory growth, plant architecture). Although plants have often been assumed to be constrained by tradeoffs in the allocation of resources to resistance versus tolerance (Van der Meijden et al. 1988, Fineblum and Rausher 1995), it is becoming clear that these are not mutually exclusive plant defense strategies (Leimu and Koricheva 2006). Nonetheless, there is some evidence that tradeoffs can occur between traits underlying resistance and tolerance. For example, limited resources may lead to allocation costs expressed as tradeoffs between growth and the production of secondary compounds (Bergelson and Purrington 1996, Strauss et al. 2002). Such allocation costs may constrain seedlings from responding to herbivory with an increase both in secondary chemistry (leading to induction) and growth (leading to compensation).

Induced responses to herbivory in the expression of resistance traits are widespread among plant species (reviewed by Karban and Baldwin 1997). Induction of higher levels of resistance traits typically reduces the likelihood that plants will be attacked by herbivores in the future (‘induced resistance’), although it is also possible for induction to lead

to increases in herbivory ('induced susceptibility'). However, whether induction leads to greater resistance or susceptibility depends on the species of herbivores and their abilities to deal with plant resistance traits (Agrawal 2000, Van Zandt and Agrawal 2004). Theory predicts that induced resistance to herbivores is common in and advantageous for seedlings, and previous studies using artificial damage or the application of chemical signaling molecules have revealed the potential for seedlings to induce (Zangerl et al. 1997, Barto and Cipollini 2005). Although various factors may favor induced over constitutive resistance (Agrawal and Karban 1999), the most widely supported explanation for its prevalence is that it is a cost-saving strategy (Zangerl 2003). If the production of secondary compounds exacts allocation costs on plants by diverting limited resources away from growth and reproduction, plants may benefit from minimizing secondary chemical production when possible in order to optimize growth and reproduction. Induction of secondary compounds thus allows plants to reduce the allocation costs of defense until they are necessary, i.e. after experiencing herbivory. There is substantial evidence to support the cost-saving hypothesis of induction (Cipollini et al. 2003), although little of this evidence comes from seedling studies. Because allocation costs of defense are predicted to be higher in seedlings than in older plants due to the limitations of small size on resource acquisition (Bryant et al. 1992, Herms and Mattson 1992, Kelly and Hanley 2005), induction as a cost-saving strategy may benefit plants most at the seedling stage.

Herbivory can also lead to changes in plant traits related to growth, including rate of photosynthesis (Welter 1989), rate of nutrient uptake (Valentine et al. 2004), utilization of stored reserves (Van der Meijden et al. 1988), and plant architecture (Zangerl et al. 1997). Compensation occurs when plasticity in growth allows a plant to replace tissues lost to herbivores, thereby allowing damaged plants to 'catch-up' in size to undamaged plants. Compensation may be an important mechanism of tolerance to herbivory, allowing damaged plants to maintain fitness levels equivalent to that of undamaged plants (Tiffin 2000).

In general, seedlings are thought to be intolerant of herbivory, due in part to their inability to compensate for damaged tissues (Strauss and Agrawal 1999). For example, honey mesquite seedlings can survive multiple bouts of complete shoot removal (except for cotyledons), but they are not able to compensate for the lost tissues, resulting in significantly lower growth rates and smaller size than undamaged control plants (Weltzin et al. 1998). Weak compensation is a general pattern among tree seedlings: growth rates are reduced by herbivory more at the seedling stage than at the mature plant stage (Nykänen and Koricheva 2004), but few studies have investigated compensation for seedling herbivory in herbaceous plants.

The goal of this study was to investigate phenotypic plasticity in secondary chemistry (induction) and growth (compensation) as potential defense strategies in seedlings, using *Plantago lanceolata* (Plantaginaceae) as a model system. Seedlings were damaged by a natural herbivore, the buckeye caterpillar, *Junonia coenia* (Nymphalidae), in the greenhouse at two and four weeks of age. These age classes were selected because they include an obvious seedling stage at two weeks

(2–3 leaf stage) as well as an older juvenile plant stage (four weeks, 7–8 leaf stage), allowing early ontogenetic patterns in responses to herbivory to be examined. Furthermore, to characterize the timing and duration of responses to seedling herbivory, plants were harvested either shortly following herbivory (six days later) or much later, at the onset of flowering (six or eight weeks later).

*Plantago lanceolata* and *J. coenia* are a model system for investigating seedling induction and compensation for several reasons. First, they commonly interact in nature, providing a more realistic test of plant defense than studies using artificial damage or chemical elicitors. Second, *P. lanceolata* seedling mortality rates have been observed to be as high as 98% (Blom 1992) but may also be much lower (Barton unpubl.), revealing high variability among populations. At least part of this mortality is caused by herbivores (Hanley et al. 1996). These observations suggest that there is selection pressure for seedling defense, but because herbivore-mediated mortality is unpredictable, in contrast with competition which is very common and intense for *P. lanceolata* seedlings (Van der Toorn and Pons 1988), plastic defense traits may be advantageous over constitutive defense. A previous study documenting very low constitutive expression of iridoid glycosides in two- and four-week-old *P. lanceolata* plants supports this prediction (Barton 2007).

I predicted that *P. lanceolata* seedlings and juveniles would respond to herbivory by increasing iridoid glycoside production, leading to induced resistance. Because allocation costs are likely to constrain seedling/juvenile responses and due to limited storage and photosynthetic tissues, I predicted that compensatory growth would be weak, leading to significant herbivore-mediated reductions in adult plant size. Finally, as evidence of allocation costs, I expected to detect a tradeoff between induction and compensation, as evidenced by a negative correlation between chemical induction and compensatory growth.

## Methods

### Study system

*Plantago lanceolata* (narrowleaf plantain or ribwort plantain; Plantaginaceae) is a short-lived perennial herb native to Eurasia but now established as a weed all over the world (Van der Aart and Vulto 1992). It is gynodioecious and self-incompatible (Van Damme 1992), and so seedlings within maternal families are almost certainly half-sibs, although receiving multiple pollen from a single source could lead to the presence of full-sibs. *Plantago lanceolata* produces iridoid glycosides, terpenoid compounds that have been demonstrated to mediate interactions with both generalist and specialist herbivores (reviewed by Bowers 1991), as well as fungal pathogens (Marak et al. 2002). The two most abundant iridoid glycosides in *P. lanceolata* are aucubin and catalpol, which together occur in quantities as high as 10–12% dry weight (Bowers and Stamp 1993). In *P. lanceolata*, iridoid glycoside production is heritable (Marak et al. 2000) and genetically variable (Bowers and Stamp 1993, Adler et al. 1995). Levels of iridoid glycosides are low to undetectable in two- and four-week-old *P. lanceolata* seedlings (Barton 2007).

Previous studies investigating adult plant responses to herbivores have produced mixed results. Although some studies have shown no effect of herbivory on iridoid glycoside concentrations (Stamp and Bowers 1994, Adler et al. 1995), it is clear that under some circumstances, adult *P. lanceolata* can increase iridoid glycoside biosynthesis in response to herbivory (Bowers and Stamp 1993, Fuchs and Bowers 2004). In a study that included multiple herbivores, plants fed on by the specialist *J. coenia* induced higher levels of iridoid glycosides than plants fed on by the generalist *Spilosoma congrua* (Bowers and Stamp 1993). In contrast, a more recent study reported a reduction in iridoid glycosides in *P. lanceolata* plants fed on by the specialist *J. coenia* but an increase in iridoid glycosides in plants fed on by the generalist *Pyrrharctia isabella* (Stamp and Bowers 2000). Clearly, chemical induction in adult *P. lanceolata* depends on species of herbivore and other factors, such as genetics (i.e. variability among populations) or plant age (Fuchs and Bowers 2004). In general, adult plants are capable of compensating for leaf tissue lost to herbivores (Bowers and Stamp 1993, Fuchs and Bowers 2004). Seedling induction and compensation in response to herbivory have not been previously studied in this species.

Plants were damaged by a specialist nymphalid caterpillar, *Junonia coenia* (common buckeye), a species that uses iridoid glycosides as feeding and oviposition cues (Bowers 1984, Pereyra and Bowers 1988). *Plantago lanceolata* is one of their most common host plants (Bowers 1984, Scott 1986). A lab colony reared at the Univ. of Colorado and established from butterflies collected in Louisiana the previous summer provided caterpillars for the damage treatments. Fourth and fifth instar caterpillars were deprived of food for a 24-h period preceding the experiment to ensure that damage treatments could be completed within a single day. Damage levels were controlled at 50% defoliation before caterpillars were removed from the plants. Caterpillars dispersed their damage, and so induction was measured at the whole-plant level, rather than at the level of individual leaves.

## Sample summary

Seeds were collected from maternal plants in fall 2003 and stored separately. Genetic family thus refers to plants from a single maternal sibship. A total of 15 genetic families were used: six families from a 'Prairie' population within the State Natural Tallgrass Prairie Area at South Boulder Creek in Boulder County, Colorado, USA; five families from 'Ranch', a roadside weed population near residential ranchland in Boulder County, Colorado, USA; and four families from 'Median', a weed population growing in the median of a major intersection in Boulder County, Colorado, USA. Insufficient germination resulted in two families being represented in only the short-term experiment, and a single family represented in only the long-term experiment.

## Responses to seedling herbivory

### *Short-term responses to herbivory: experimental design*

The first experiment was designed to measure short-term responses to herbivory. Seeds were sown in flats filled with Fafard nursery mix on 14 May 2004, and germination

began approximately ten days later. Seedlings that germinated within a three-day period were transplanted immediately into 3.5-l pots (35 cm depth × 14 cm Ø) filled with a medium composed of equal parts sterilized sand, Metro Mix 350 (50–60% vermiculite, 25–40% peat moss, 9–19% bark ash), and Turface MVP. Pots were randomly placed on one of three greenhouse benches and were periodically re-randomized among benches to remove any effect of location within the greenhouse. Plants were watered daily. They were not fertilized during the experiment.

Seedlings were matched for size and randomly assigned to one of four treatment groups: 1) plants subjected to herbivory two weeks after germination, 2) plants subjected to herbivory four weeks after germination, 3) control plants not damaged by caterpillars, to be harvested with plants damaged at two weeks, and 4) control plants not damaged by caterpillars, to be harvested with plants damaged at four weeks. Each treatment group had two to six replicate plants per genetic family for a total sample size of 217 plants.

Two and four weeks after germination, plants in the herbivory treatment groups were subjected to herbivory by one *J. coenia* caterpillar (two weeks) or one to three *J. coenia* caterpillars (four weeks). A single caterpillar was placed on each plant and prevented from leaving by enclosing the entire pot within a customized sleeve made of Reemay and closed at the top with a clothespin. Additional caterpillars were added to four-week-old plants as needed to ensure 50% defoliation within the experimental time period of 12 h. To control for potential handling and shading effects, control plants were also enclosed within Reemay sleeves at the time of the herbivory treatments.

Six days after the herbivory treatments, damaged and undamaged control plants were harvested. This period of time was chosen because a previous study identified six days to be the optimal time for detecting induction in adult *P. lanceolata* (Fuchs and Bowers 2004). Leaves and roots were harvested separately, oven-dried at 50°C to a constant weight, and weighed to the nearest 0.01 g. Leaves were prepared for iridoid glycoside quantification by gas chromatography.

### *Long-term responses to herbivory: experimental design*

The protocol of this experiment was similar to that of the first in most regards, although there were a few key differences. This experiment was conducted later in the summer than the first experiment; seeds were sown on 10 July 2004. Seedlings were transplanted on 20 July and were randomly assigned to one of three treatment groups: 1) plants subjected to 50% leaf herbivory at two weeks, 2) plants subjected to 50% leaf herbivory at four weeks, and 3) control plants that were not damaged by caterpillars. For each treatment group, the number of replicates per family ranged from four to seven for a total sample size of 232 plants. Pots were placed randomly on two greenhouse benches, and placement was re-randomized within benches every two weeks; bench represents the block effect in this study. Plants in all treatment groups were harvested ten weeks after transplantation, just before the onset of flowering and at a time when naturalized plants are flowering in Boulder, CO (Barton unpubl.). At this time, plants were not showing any signs of being pot-bound, and upon

harvest, it was observed that some of the soil volume remained unoccupied by roots. Because all plants were harvested at the same time, the response time of the treatment groups differed by two weeks: plants damaged at two weeks had eight weeks to show compensation while plants damaged at four weeks had only six weeks. Although this design thus confounds age of damage and response time, it more realistically reflects what actually occurs in natural communities. In nature, plants damaged at various ages do differ in the amount of time they have to respond before the end of the growing season. To test whether seedling herbivory leads to long-term induction of iridoid glycosides, half of the replicates within each family were randomly selected for chemical analysis.

## Chemical analyses

Leaves were analyzed for iridoid glycosides. All leaves for each plant were ground into a fine powder, and 5–50 mg subsamples (entire aboveground tissue for many young seedlings) were processed for chemical analysis by gas chromatography using previously described methods (Gardner and Stermitz 1988, Bowers and Stamp 1993). Briefly, samples were extracted in methanol and then partitioned between water and ether to remove hydrophobic compounds and chlorophyll. An internal standard [phenyl- $\beta$ -D-glucose (PBG)] was added, and an aliquot was derivatized with Tri-Sil-Z. Derivatized samples were injected into a gas chromatograph, and chromatograms were integrated with HP 3365 Series II ChemStation A.03.34.

## Statistical analyses

Data were analyzed using SAS for Windows 9.1.3 mixed (proc mixed), regression (proc reg), and means (proc means) procedures. In all analyses, genetic family was nested within population. Data from the two experiments were analyzed separately. Aucubin and catalpol data were analyzed as proportions of dry mass (concentration) and were arcsine transformed to meet assumptions of normality. For the short-term experiment, shoot biomass, root biomass, and the concentrations of aucubin and catalpol were each analyzed with a mixed-model four-way factorial ANOVA. The full model included four main effects: herbivory (fixed), age (fixed), population (fixed), and genetic family (random), and all two-way interactions. For the long-term experiment, the same variables were each analyzed with a mixed-model three-way factorial ANOVA with the main effects herbivory (fixed), population (fixed), and genetic family (random) and a random block effect (bench). A significant effect of genetic family would signify genetic variation in the plant trait of interest (shoot mass, aucubin concentration, etc.), and a significant interaction between herbivory and genetic family would signify genetic variation in compensation and induction. No three-way interactions were found to be significant during preliminary analyses and were pooled into the error terms. The significance of the random effects (and interactions with random effects) were tested by running models with and without the random effect of interest and calculating the likelihood-ratio statistics, which can be compared to a

$\chi^2$ -distribution (Littell et al. 1996). Because block was not included in any interaction terms, block F-tests were computed with residual mean square error, similar to tests of fixed factors (Newman et al. 1997). Thus, results from the ANCOVA are reported as F-values for fixed factors and block and  $\chi^2$ -values for random factors.

### *Tradeoffs between induction and compensation*

To investigate whether plants experienced a tradeoff between induction and compensation, phenotypic correlations were analyzed. Because induction was detected only in the short-term experiment, these analyses were conducted on these data only. Phenotypic correlations demonstrate a relationship between plant size and chemistry at the individual plant level, which represents the sum of environmental and genetic factors (Koricheva 2002). Induction (I) was calculated within families as the difference in iridoid glycoside content between damaged plants and the mean of the control plants, standardized by mean control plant levels ( $I = (\text{damaged} - \text{control})/\text{control}$ ). Compensation (C) was similarly calculated within families as the mean difference in plant size between damaged plants and the mean of the undamaged control plants, standardized by mean control plant size ( $C = (\text{damaged} - \text{control})/\text{control}$ ). These measures reflect relative changes in chemistry (induction) and growth (compensation) of damaged plants.

## Results

### Short-term responses to herbivory

Herbivory had no short-term effect on the iridoid glycoside concentrations of two-week-old plants (Fig. 1A, Tukey-adjusted least square mean comparisons,  $p > 0.9$  for aucubin and catalpol). In contrast and counter to my prediction, plants damaged at four weeks of age showed a significant reduction in iridoid glycoside concentrations (Fig. 1A, Tukey-adjusted least square mean comparisons,  $p < 0.0001$  for both aucubin and catalpol). There was a significant interaction between age and herbivory (Table 1, Fig. 1A), which could have resulted from the difference in the mean effects of herbivory on two and four-week-old plants or from differences in variance.

Because iridoid glycoside concentrations increase dramatically during the first six weeks of growth in *P. lanceolata* (Barton 2007), it is possible that herbivory leads to lower levels of iridoid glycosides compared to undamaged control plants simply by slowing down development. Damaged plants may have lower levels of iridoid glycosides simply because they are 'younger' and smaller than undamaged plants. To investigate this, an additional ANOVA analysis was conducted that included total plant biomass as a covariate. Although biomass did significantly explain some of the variation in aucubin ( $F_{1,174} = 29.76$ ,  $p < 0.0001$ ) and catalpol ( $F_{1,174} = 23.76$ ,  $p < 0.0001$ ), herbivory still had no effect on iridoid glycoside concentrations at two weeks of age (Tukey-adjusted least square mean comparisons,  $p = 0.9940$  for aucubin and  $p = 0.9858$  for catalpol) and led to a significant reduction in iridoid glycoside concentrations at four weeks of age

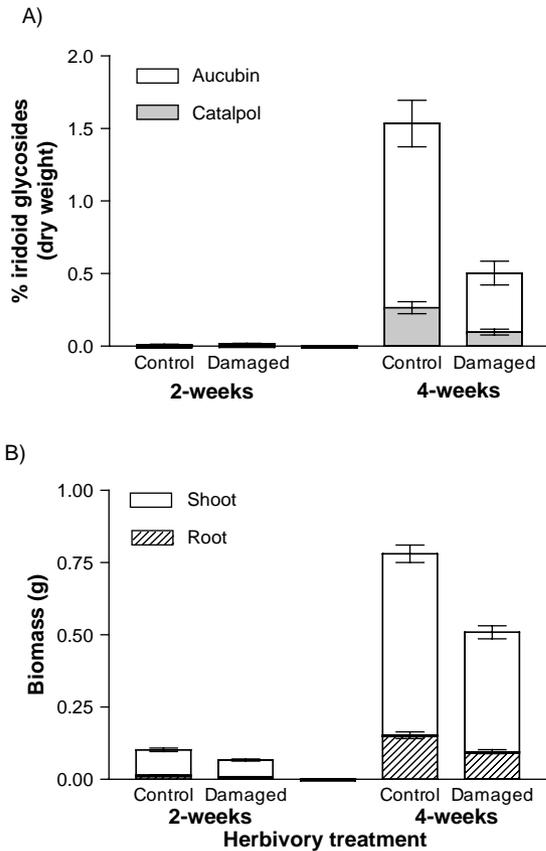


Fig. 1. Short-term effects of herbivory on (A) concentrations of iridoid glycosides (mean  $\pm$  1 SE), and (B) root and shoot biomass (mean  $\pm$  1 SE) in *P. lanceolata*. Damaged and undamaged control plants were harvested six days following the herbivory treatments at two and four weeks of age.

(Tukey-adjusted least square mean comparisons,  $p < 0.0001$  for aucubin and  $p = 0.0244$  for catalpol).

Concentrations of iridoid glycosides increased with age in all plants, resulting in a significant effect of age on both aucubin and catalpol (Table 1). Genetic variation was detected at the population but not at the family level for aucubin, although this effect depended on age (Table 1, age  $\times$  population interaction). Induction did not vary genetically at either the population or maternal family level (Table 1, herbivory  $\times$  population and herbivory  $\times$  family interactions not significant).

Seedlings and juveniles did not fully compensate for shoot herbivory within the brief duration of the first experiment. As a result, damaged plants had significantly less shoot biomass than undamaged control plants, and although the effect was proportionately similar in both age classes, the magnitude of reduction was greater in four-week-old plants (Table 1, herbivory  $\times$  age interaction; Fig. 1B). Despite experiencing 50% defoliation, plants were reduced in shoot biomass by approximately one-third (35% in both age classes) rather than one-half, indicating that damaged plants were beginning to compensate for the lost leaf tissue within only six days. Root biomass was also reduced in damaged plants, although this effect must have been indirect as caterpillars damaged only aboveground tissues. Like shoots, the reduction of root biomass by herbivory was also greater in four-week-old vs two-week-old plants (Table 1, herbivory  $\times$  age interaction; Fig. 1B). Plants grew during the experiment, resulting in a significant effect of age on both shoot and root biomass (Table 1, Fig. 1B). Genetic variation in compensation was not detected at either the maternal family or population level (Table 1, herbivory  $\times$  population and herbivory  $\times$  family interactions not significant).

### Long-term responses to herbivory

Seedling and juvenile herbivory did not lead to long-term induction of iridoid glycosides; concentrations of both aucubin and catalpol were similar for damaged and undamaged plants (Table 2, Fig. 2A). Similar to the short-term experiment, there was no detectable genetic variation in the chemical responses to herbivory, although mean levels of aucubin and catalpol did vary significantly among populations (Table 2).

Plants fully compensated for the 50% leaf tissue removal experienced at either two or four weeks of age, resulting in similar shoot biomass for all three treatment groups at ten weeks (Table 2, Fig. 2B). However, full shoot compensation was achieved at the expense of root biomass, at least in plants damaged at four weeks. Root biomass was significantly lower in plants damaged at four weeks of age than it was in undamaged control plants (Fig. 2B, Tukey-adjusted least square mean comparisons,  $p = 0.0011$ ). Genetic variation was not detected in either mean plant size or compensation at the population or maternal family level (Table 2).

Table 1. Summary of ANOVAs for plant biomass and iridoid glycoside concentrations. F-values are reported for herbivory, age, population, and their interactions.  $\chi^2$  values are reported for the genetic family main effect and interactions because genetic family is a random factor. Significance is displayed as  $p < 0.0001$  (\*\*\*),  $p < 0.01$  (\*\*),  $p < 0.05$  (\*).  $n = 217$ .

	Shoot mass	Root mass	Aucubin	Catalpol
Sources of variation (DF)				
Herbivory (1,10)	38.88***	25.49**	29.62**	15.60**
Age (1,10)	515.98***	313.68***	87.17***	26.75**
Population (2,10)	0.29	6.86*	7.10*	1.59
Genetic family	1.00	0.40	0.00	0.00
Herbivory $\times$ Age (1,175)	23.77**	15.52***	28.60***	16.09***
Herbivory $\times$ Population (2,10)	3.14	3.99	1.04	1.25
Herbivory $\times$ Family	0.00	0.00	0.00	0.00
Age $\times$ Population (2,10)	0.39	9.05**	6.78*	1.72
Age $\times$ Family	0.00	0.00	1.30	6.30

Table 2. Summary of ANOVAs for plant biomass and iridoid glycoside concentrations. F-values are reported for herbivory, age, population, and their interactions.  $\chi^2$  values are reported for the genetic family main effect and interactions because genetic family is a random factor. Significance is displayed as  $p < 0.0001$  (\*\*\*),  $p < 0.01$  (\*\*),  $p < 0.05$  (\*). For the biomass variables,  $n = 232$ ; for the chemistry variables,  $n = 117$ .

	Shoot mass	Root mass	Aucubin	Catalpol
Sources of variation (DF)				
Herbivory (2,20)	1.44	9.10***	1.71	0.51
Population (2,10)	0.43	0.06	19.21***	8.12**
Genetic family	20.70***	18.10***	0.00	1.50
Herbivory $\times$ Population (4,20)	1.19	0.65	0.56	0.51
Herbivory $\times$ Family	0.70	0.10	0.00	0.60
Bench (block) (1,192)	3.26	20.67***	3.37	2.04

Block significantly affected root biomass, but not shoot biomass or iridoid glycoside chemistry (Table 2). This likely reflects differences in shading, and associated water availability, among greenhouse benches.

### Tradeoffs between induction and compensation

Tradeoffs between induction and compensation were not detected for plants damaged at either two or four weeks of

age. In contrast, highly significant positive relationships were observed (Fig. 3).

### Discussion

This study revealed interesting patterns of phenotypic plasticity in *P. lanceolata* seedlings and juveniles in response to caterpillar herbivory. Most importantly, a significant reduction in iridoid glycoside concentration was detected following herbivory at four weeks of age, but plants damaged at two weeks showed no change in iridoid glycoside concentration. These responses are not consistent with the prediction that young plants would respond to herbivory with an increase in secondary chemical concentration in order to achieve induced resistance. Also unexpectedly, plants demonstrated complete compensatory growth at ten weeks of age for shoot tissue lost to herbivory at the seedling stage (at the expense of roots in plants damaged at four weeks). Considering that *P. lanceolata* plants showed full compensation and no fatalities from 50% seedling defoliation, this study suggests that *P. lanceolata* plants have a high tolerance of seedling herbivory.

### Induced susceptibility

Herbivory reduced iridoid glycoside concentration by over 65% in four-week-old *P. lanceolata* plants. The defensive properties of iridoid glycosides have been well documented (reviewed by Bowers 1991), suggesting that this dramatic decrease is likely to reduce juvenile plant resistance to generalist herbivores, thereby leading to induced susceptibility. Although induced susceptibility was originally thought to be uncommon, it is now widely recognized to occur under some circumstances (Nykänen and Koricheva 2004).

Because induced resistance and susceptibility are defined with respect to measures of herbivore performance on previously damaged plants, induction of higher levels of secondary compounds may lead to induced resistance to some herbivores and induced susceptibility to others (Karban and Baldwin 1997, Agrawal 2000). In contrast, this study reveals that juvenile *P. lanceolata* plants respond to herbivory with a decrease in secondary chemical concentration. Inspection of the total amounts of iridoid glycosides reveals that the decrease in concentration does not merely result from diluting effects due to changes in biomass. Plants damaged at four weeks had only 20% of

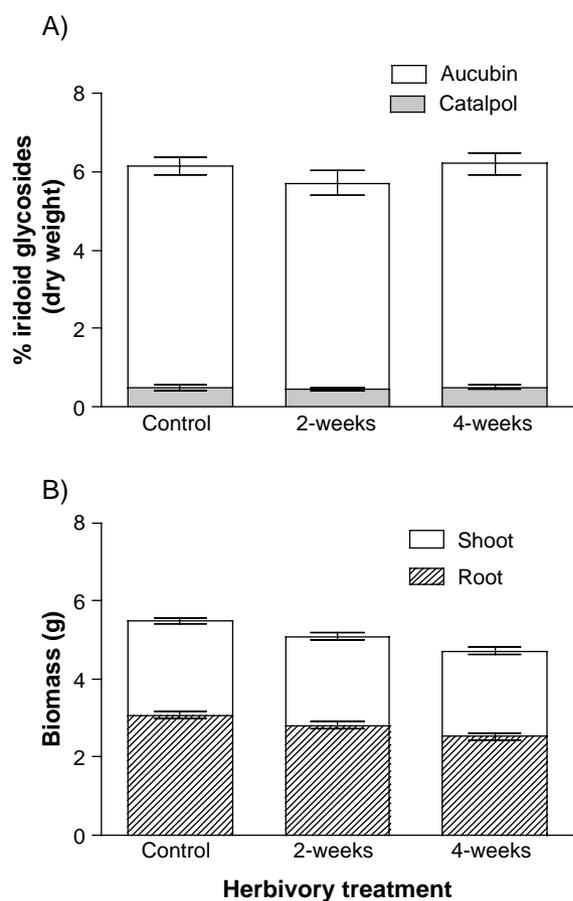


Fig. 2. Long-term effects of herbivory at two weeks and four weeks on ten-week-old *P. lanceolata* plants. Plants were damaged at two, four weeks of age, or not damaged at all (controls) and harvested at ten weeks of age to investigate herbivore-mediated changes in (A) iridoid glycoside concentrations (mean  $\pm$  1 SE), and (B) root and shoot biomass (mean  $\pm$  1 SE).

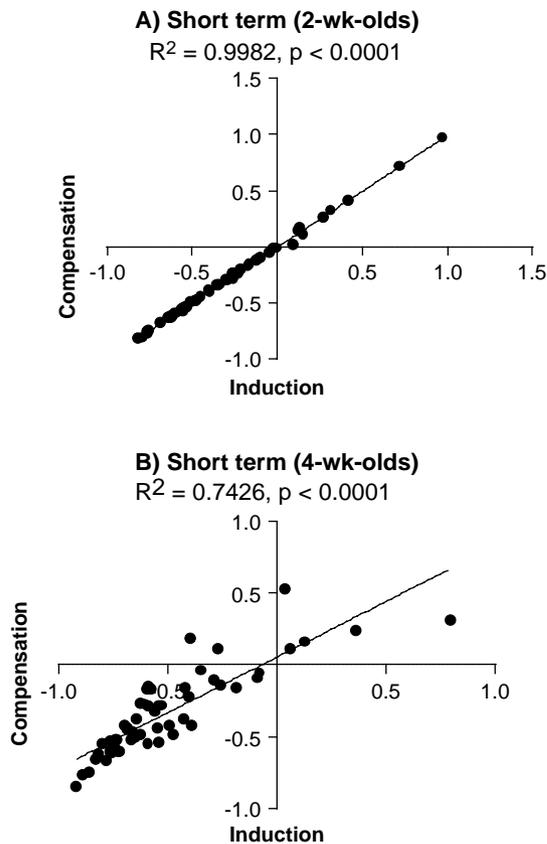


Fig. 3. Correlation analyses to investigate the relationship between plant size (compensation) and chemical defense (induction) responses following herbivory at two and four weeks of age in the short-term experiment. Induction (I) was calculated within families as the mean difference in iridoid glycoside content between damaged plants and the mean of undamaged control plants, standardized by mean control plant levels ( $I = (\text{damaged} - \text{control})/\text{control}$ ). Compensation (C) was similarly calculated within families as the mean difference in plant size between damaged and the mean of undamaged control plants, standardized by mean control plant size ( $C = (\text{damaged} - \text{control})/\text{control}$ ). Coefficient of determination, and p-value are reported. Sample sizes were as follows: two-week-olds ( $n = 53$ ); four-week-olds ( $n = 54$ ).

the iridoid glycoside content ( $2.16 \pm 0.402$  mg) of undamaged plants ( $10.72 \pm 1.60$  mg). Two mutually nonexclusive mechanisms could account for this dramatic difference between damaged and undamaged plants. Iridoid glycoside production may have decreased or ceased in damaged plants during the week between the herbivory treatment and harvest, while production continued in control plants, leading to significantly higher amounts in control plants. Alternatively, damaged plants may have metabolized iridoid glycosides following herbivory, perhaps in order to reallocate the components to other functions, such as compensatory growth. Detailed physiological studies could shed light on these potential mechanisms.

There are relatively few studies demonstrating a reduction in secondary chemical concentration or content following herbivory, and as a result, this response is not commonly considered a defense strategy of plants (Zangerl 2003). Most examples of herbivore-mediated decreases in

secondary chemical concentration involve terpenes in woody plants (Nykänen and Koricheva 2004), and these responses are thought to reflect source-sink dynamics within the plants following defoliation. Shifts in source-sink dynamics associated with herbivore induction may occur indirectly via reversions in ontogeny. For example, it has been suggested that induction in damaged birch and poplar trees (Bryant 1981, Bryant et al. 1983) results from a reversion to the juvenile stages (Chapin et al. 1985). *Plantago lanceolata* shows a significant ontogenetic increase in iridoid glycoside concentrations during the first six weeks of growth (Barton 2007). If herbivory slows ontogeny during this period such that damaged plants are 'younger' than undamaged plants, herbivory would be expected to decrease iridoid glycoside concentrations ('younger' damaged plants would have lower levels of iridoid glycosides than 'older' undamaged plants). Yet, the analysis including biomass as a covariate demonstrated that herbivory influences iridoid glycoside concentration above and beyond the associated effect on plant size.

Although a decrease in secondary compounds following herbivory is not typically considered an adaptive defensive response (Zangerl 2003), it is possible for it to be adaptive under some circumstances. In this study, seedlings were damaged by a specialist caterpillar, the buckeye *Junonia coenia* (Nymphalidae). Because buckeyes use iridoid glycosides as feeding cues (Bowers 1984), a reduction in the concentration of iridoid glycosides may be an adaptive response of seedlings to discourage continued feeding by buckeye caterpillars. Additional research with bioassays would shed light on this possibility. A reduction in secondary chemical production following damage may also be adaptive in the case that it allows limited resources to be allocated to other functions. In particular, plants that increase growth as a means of tolerating herbivory may forgo defense production in order to optimize compensation. However, compensation and induction were positively correlated in these experiments, indicating that *P. lanceolata* seedling responses to herbivory are not likely to be constrained by tradeoffs.

### Compensatory growth

In less than two months, *P. lanceolata* seedlings and juveniles were able to compensate entirely for shoot tissue consumed by caterpillars, although this was achieved at the expense of root biomass in plants damaged at four weeks of age. Even within six days following herbivory, damaged plants were showing remarkable regrowth of shoots and were reduced by only one-third, rather than one-half, compared to undamaged control plants. For both two-week and four-week-old plants, this compensation of shoots was being achieved through the reallocation of resources such that root growth was decreased. The long-term experiment revealed that the decrease in root growth rate was only temporary in plants damaged at two weeks, so that by ten weeks of age, they were indistinguishable from undamaged control plants in both shoot and root biomass. Although compensatory growth is high in plants of both age classes, these patterns suggest that seedlings are better able to replace tissues lost to herbivores than slightly older juvenile plants, a remarkable

result considering previous research showing seedlings to be poor tolerators of herbivory (Weltzin et al. 1998, Nykänen and Koricheva 2004).

Compensatory growth in adult plants is typically achieved through increases in photosynthetic rates, nutrient uptake, activation of dormant meristems and utilization of stored resources (Tiffin 2000). This study reveals that the same mechanisms that drive compensation in adult plants can also occur in seedlings. Following herbivory, resources stored in the roots are often reallocated to the shoots, leading to compensation of shoot tissues (Stowe et al. 2000), and often resulting in significantly less root biomass for damaged versus undamaged plants (Mabry and Wayne 1997). This is the pattern observed for all damaged plants in the short-term experiment and for plants damaged at four weeks of age in the long-term experiment, indicating that seedlings are plastic in the allocation of resources between shoots and roots. Had the experiment continued for longer, it is possible that the reduction in root biomass observed in plants damaged at four weeks would disappear, as it did for plants damaged at two weeks.

## Conclusion

Phenotypic plasticity is an important aspect of seedling defense strategies. In contrast to predictions based on previous studies, young *P. lanceolata* plants respond to caterpillar damage by decreasing chemical defense and increasing growth, ultimately leading to compensation by ten weeks of age. The long-term experiment demonstrates that this reduction in iridoid glycoside concentrations is only temporary; by ten weeks of age, damaged plants had similar levels of iridoid glycosides as undamaged control plants. Thus, seedlings experience only a temporary window of increased susceptibility to generalist herbivores. This study, along with a previous one demonstrating low constitutive levels of iridoid glycosides in seedlings (Barton 2007), reveals that in *P. lanceolata*, compensation is the primary defense strategy of seedlings. Furthermore, this study highlights the significance of ontogeny in patterns of plant–herbivore interactions by revealing that two-week-old seedlings and four-week-old juvenile plants differ in their responses to herbivory. By explicitly considering how ontogeny influences plant–herbivore interactions, we can gain insight into seasonal and yearly trends in food web dynamics and resolve seemingly conflicting patterns in defense traits measured among plants of different ages (Boege et al. 2007). Moreover, we can expand our understanding of the evolution of plant defense by focusing particularly on the seedling stage, when herbivory is likely to affect plant fitness the most.

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