

# Neighbor species differentially alter resistance phenotypes in *Plantago*

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**Abstract** In this study, we investigated how neighbors (i.e., competitors) altered resistance phenotypes, namely plant size and levels of secondary compounds (iridoid glycosides), of individual plants and specifically tested whether neighbor identity mattered. We conducted a greenhouse experiment with *Plantago lanceolata* and *Plantago major* (Plantaginaceae) in which each species served as focal plants as well as neighbors in a factorial design. In addition, we harvested plants six and nine weeks after transplantation to test whether effects changed as plants grew. In both species, competition reduced plant size, and this effect increased over time. *Plantago lanceolata* neighbors suppressed growth of both focal plant species more than *P. major* neighbors. Effects of competition on levels of secondary compounds were more complex. Concentrations of iridoid glycosides were increased by competition in both species at harvest one. By the second harvest, an effect of competition on iridoid glycosides was found only in *P. major*. Neighbor identity influenced levels of iridoid glycosides in *P. lanceolata* at harvest one; concentrations were higher in plants grown with *P. lanceolata* neighbors than in plants grown with *P. major* neighbors. We also tested whether there was a trade-off between growth (biomass) and defense (levels of iridoid glycosides). Biomass and iridoid glycoside content were significantly correlated only

in plants grown with competition and harvested at nine weeks, and this relationship was positive in both species, indicating that there was no trade-off between growth and defense. This study suggests that neighbor identity could play an important role in interspecific interactions, including the interactions of plants with other trophic levels.

**Keywords** Allocation costs · Competition · Iridoid glycosides · Phenotypic plasticity · Trait-mediated indirect effects

## Introduction

It is well-documented that neighbors can influence levels of resistance and herbivory of plants (e.g., Cipollini and Bergelson 2001; Agrawal 2004). However, whether neighbor species differ in their effects on plant resistance and herbivory is of particular interest and is poorly understood. The mechanisms driving these neighbor effects can be broadly categorized into two classes: (1) plant trait-mediated indirect effects, and (2) associational effects. Plant trait-mediated indirect effects occur when neighbors interact directly with focal plants and alter phenotypic traits involved in plant resistance, thereby indirectly affecting herbivory (Peacor and Werner 2001; Werner and Peacor 2003; Agrawal 2004). Alternatively, neighbors can influence herbivory without directly interacting with focal plants via associational effects. Plants benefit by associational resistance when neighbors reduce levels of herbivory by repelling herbivores or attracting natural enemies (Tahvanainen and Root 1972; Hay 1986; Letourneau 1986; Karban 1993). In contrast, focal plants might

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experience associational susceptibility if neighbors attract herbivores (Rand 2003; Agrawal 2004).

For both trait-mediated indirect effects and associational effects, the identity of the neighbor is likely to be important. Most studies on associational effects explicitly consider neighbor identity (e.g., Hay 1986; Agrawal 2004). However, the role of neighbor identity has been relatively overlooked in studies of trait-mediated indirect effects, especially in those studies looking at how competition influences plant resistance. This is perhaps because the effects of competition on resistance have been viewed generically in terms of resource availability, and although it is known that species vary in how they alter resource availability (Harper 1977; Tilman 1997), few competition studies have specifically tested whether neighbor species differ in their effects on focal plant resistance.

In this study, we investigated the role of neighbor identity in trait-mediated indirect effects. The most common plant resistance traits altered by neighbors are plant size and secondary chemistry. Although allelopathy (Ridenour and Callaway 2001) and volatile chemical signaling (Karban et al. 2000) can cause changes in plant size and secondary chemistry, competition is likely to be the most common way in which neighbors alter focal plant resistance. A substantial body of research has investigated how competition and resource availability contribute to plant resistance phenotypes, instigated by early theoretical models, including the carbon–nutrient balance hypothesis (Bryant et al. 1983), the resource availability hypothesis (Coley et al. 1985), and the growth–differentiation balance hypothesis (Herms and Mattson 1992). Although the generality of specific models is debated (Hamilton et al. 2001; Lerdaun and Coley 2002; Stamp 2003, 2004), there is considerable evidence that competition and resource availability account for some of the variation in plant resistance (e.g., Fajer et al. 1992; Höft et al. 1996; Richardson et al. 1999; Glynn et al. 2003).

To examine competition-mediated effects of neighbors on plant resistance, we conducted a greenhouse experiment with *Plantago lanceolata* and *P. major* (Plantaginaceae) in which each species served as focal plants as well as neighbors in a factorial design. *Plantago lanceolata* and *P. major* are common weeds that often co-occur in disturbed habitats (Haeck 1992), making them a meaningful species comparison for this experiment. Both species produce iridoid glycosides; carbon-based terpenoid compounds that impact generalist and specialist herbivores (reviewed in Bowers 1991; Harvey et al. 2005). By using two neighbor species, we could test whether neighbor identity influenced competition-mediated effects on focal plant

resistance. Furthermore, because the same plant species were used both as focal plants and neighbors, we could contrast intra- and interspecific competition, which often differ in their effects on plant traits (e.g., Linhart 1988; Miller 1995). To investigate whether the neighbor effects changed as plants grew, we harvested plants at two time intervals.

One mechanism by which competitors might alter focal resistance traits is by causing changes in plant allocation to defense. To investigate this, we characterized the relationships between plant biomass and levels of iridoid glycosides in order to determine whether competition altered allocation costs of defense in these species. Trade-offs can occur when limited resources are allocated to defense at the expense of growth or reproduction (Bazzaz et al. 1987). Early hypotheses predicted that, in general, as resources became more limiting, trade-offs would increase (Rhoades 1979; Coley et al. 1985). However, experimental research has revealed that the magnitude of allocation costs is influenced not only by general resource availability, but also by the kind of resources that are limiting, the defense traits involved, and by the methods used to detect these costs (Koricheva 2002). Although very low resource availability may increase the trade-off between growth and defense, as predicted above, moderate nutrient or water limitation may actually alleviate the trade-off (Herms and Mattson 1992). Specifically, for carbon-based secondary compounds, such as the iridoid glycosides examined in this study, moderate resource availability can constrain growth more than photosynthesis, allowing surplus carbon to be allocated to defense without exacting allocation costs (Herms and Mattson 1992), which may explain why trade-offs are less common as resource availability decreases (Koricheva 2002).

In this study, we addressed four specific questions:

- Does competition alter resistance traits of focal plants?
- Do neighbor species differ in their effects on focal plant resistance traits?
- Does the effect of competition change with time?
- Is there a trade-off between resistance and growth?

## Materials and methods

### Study system

*Plantago lanceolata* and *P. major* are short-lived perennial herbs native to Eurasia. Both species now have naturalized weed populations all over the world (van der

Aart and Vulto 1992) and are common in Colorado. *Plantago lanceolata* and *P. major* were selected as competing species because they often grow together in sites where they experience mixed densities similar to those used in this experiment (Haeck 1992; K. E. Barton, personal observation; M. D. Bowers, personal observation). These species are common in disturbed habitats, and *P. lanceolata* is also found in native prairies and grasslands, both within its native range (Haeck 1992) and in Colorado (K. E. Barton, unpublished data).

*Plantago lanceolata* and *P. major* produce iridoid glycosides, carbon-based secondary compounds that impact both generalist and specialist herbivores (reviewed in 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 occur in quantities as high as 10–12% dry weight (Bowers and Stamp 1993). *Plantago major* does not produce catalpol (Rønsted et al. 2000) and produces aucubin in relatively low amounts (Barton KE, submitted to Am J Bot, 2006). In *P. lanceolata*, iridoid glycoside production is heritable (Marak et al. 2000), and significant genetic variation occurs among adult plants (Bowers et al. 1992; Bowers and Stamp 1992, 1993; Adler et al. 1995). Concentrations of iridoid glycosides in *P. lanceolata* change during ontogeny (Bowers et al. 1992; Stamp and Bowers 1994; Jarzomski et al. 2000; Barton KE, submitted to Am J Bot, 2006) and demonstrate high phenotypic plasticity, varying with respect to nutrient availability (Fajer et al. 1992; Darrow and Bowers 1999; Marak et al. 2003) and herbivory (Bowers and Stamp 1993; Darrow and Bowers 1999; Fuchs and Bowers 2004).

### Experimental design

To examine the effects of focal species (two levels), neighbor species (three levels), and harvest time (two levels) on focal plants, we conducted a greenhouse experiment with a  $2 \times 3 \times 2$  factorial design. Each replicate consisted of a single plant of either *P. lanceolata* or *P. major* (“focal species”) planted in the center of the pot and surrounded by no neighbors (control), or by four individuals of either *P. lanceolata* or *P. major* (“neighbor species”). Fifteen replicates of each of the focal species  $\times$  neighbor species groups were harvested six and nine weeks (“harvest time”) after transplantation. Due to a few plant fatalities during the experiment, the final sample size was 174 focal plants (89 *P. lanceolata*, 85 *P. major*).

Plants were grown from seed collected from three *P. lanceolata* and one *P. major* naturalized populations in Boulder County, CO. Seeds from many plants (>20 per

population) were collected and mixed together so that individuals in this experiment represented a random sampling of offspring from many genetic families. On 3 February 2002, seeds were sown in flats with Fafard nursery-mix and placed in a growth chamber for germination (14:10 h day/night cycle; 25 °C day and 10 °C night temperatures). Seeds began to germinate five days later and continued to germinate until 15 February, when the flats were transported to the greenhouse and covered with perforated plastic to provide shading and maintain moisture for early seedling growth.

To prevent root-binding effects, extra-deep seven-liter pots (70 cm depth  $\times$  14 cm diameter) were constructed by cutting the bottom off one 3.5-l pot (35 cm depth  $\times$  14 cm diameter) and attaching it to the top of a second pot with duct tape. These pots were filled with equal-parts medium of sterilized sand, Metro Mix 350 (50–60% vermiculite, 25–40% peat moss, 9–19% bark ash, Scotts-Sierra Horticultural Products Company, Marysville, OH, USA), and Turface. Seedlings were transplanted at the two true-leaf stages on 3 March.

Pots were placed on three benches within the same room of the greenhouse. Pots were routinely moved within a bench, rerandomizing the placement of replicates. Bench represents the block effect for this experiment. Plants were watered daily. Fertilizer was not added to the plants at any time during the experiment.

*Plantago lanceolata* plants did not flower during this experiment, but most *P. major* plants did (68% at six weeks and 90% at nine weeks). After being harvested, the aboveground biomass of the focal and neighbor plants was placed in paper bags, and inflorescences, when present, were stored and dried separately. Because it was not possible to untangle the focal and neighbor roots, the roots were not harvested. Plants were oven-dried at 50 °C to a constant weight and weighed to the nearest 0.01 g.

### Chemical analyses

Leaves of focal plants were analyzed for iridoid glycosides. Leaves were ground to a fine powder, and 25–50 mg subsamples were processed for chemical analysis by gas chromatography using previously described methods (Gardner and Stermitz 1988; Bowers and Stamp 1993).

### Statistical analyses

Data were analyzed using SAS for Windows V8 regression, means, and general linear model (GLM) procedures (SAS 2002). Aucubin and catalpol data were analyzed as proportions of dry leaf mass and were

arcsine-transformed to meet assumptions of normality (Quinn and Keough 2002). In all models, the main factors were considered fixed, and block was considered random. However, because interactions between block and main effects were not significant in preliminary analyses (and were not of interest), *F*-tests were computed with residual mean square for all factors, including block (Newman et al. 1997). Because of a few missing samples, the data were unbalanced, and type III sums of squares are reported. To avoid heteroscedasticity in the data, *P. lanceolata* and *P. major* were analyzed separately.

A multifactorial ANOVA was used to determine to what degree neighbor species, harvest time, bench, and harvest by neighbor interaction accounted for the variation in aboveground biomass. Aboveground biomass included leaves only for *P. lanceolata*, but leaves and inflorescences, when appropriate, for *P. major*. To test a priori hypotheses about the neighbor treatment, two planned contrasts were included in the model (Judd and McClelland 1989). The first (N1) tested for mean differences between control plants and plants grown with neighbors. The second (N2) tested whether plants grown with *P. lanceolata* neighbors differed from those grown with *P. major* neighbors. We also constructed interaction contrasts ( $H \times N1$  and  $H \times N2$ ) to separately test the interactions between harvest and each of the neighbor contrasts.

Because concentrations of aucubin and catalpol were correlated in *P. lanceolata* ( $R^2=0.2699$ ,  $P<0.0001$ ), they were initially analyzed with a MANOVA to test the effects of neighbor treatment, harvest time, and bench. Each was subsequently analyzed separately with a multifactorial ANOVA (Quinn and Keough 2002) with neighbor species (contrasts), harvest time, bench, and neighbor by harvest interaction (contrasts) as effects. *Plantago major* plants had only aucubin, the concentrations of which were analyzed with the same multifactorial ANOVA and contrasts.

To investigate whether there was a trade-off between iridoid glycosides and growth, we performed a series of regressions between iridoid glycoside levels and aboveground biomass. To address concerns about the appropriate data to use to detect costs (Koricheva 1999), we tested for relationships between leaf biomass and iridoid glycoside content, and between leaf biomass and iridoid glycoside concentration (concentration = content/biomass). To assess whether detection of a cost was influenced by harvest time or competition, separate regression analyses were done at harvests 1 and 2, both for control plants and plants grown with neighbors (preliminary analyses demonstrated that species of neighbor was not important: in *P. lanceolata*,  $P=0.3313$ , in *P. major*,  $P=0.6608$ ). A significant negative relationship

between iridoid glycosides and leaf biomass would suggest that a cost existed. Because the data were analyzed in two different ways (concentration and content), we applied a Bonferroni correction to give an adjusted  $\alpha$  level of 0.025 ( $\alpha=0.05/2$ ; Quinn and Keough 2002).

## Results

### Plant size: biomass

The responses of focal plant biomass to the experimental factors were similar in both *P. lanceolata* and *P. major*. Plants nearly tripled in size during the three weeks between harvest times, which resulted in a highly significant effect of harvest time on biomass (Table 1, Fig. 1). Neighbor presence and identity also significantly influenced aboveground biomass. Focal plants grown with neighbors were significantly smaller than plants grown alone (Table 1, Fig. 1). Although competition reduced plant size at both harvest times, the magnitude of this reduction was much higher at the second harvest time (Fig. 1), contributing to a significant interaction between harvest time and the presence/absence neighbor effect (Table 1:  $H \times N1$  term). Focal plants grown with *P. lanceolata* neighbors were significantly smaller than plants grown with *P. major* neighbors (Table 1, Fig. 1). This pattern was found in both species, and the magnitude of the biomass difference increased between harvest times (Fig. 1), and likely explains the significant harvest by neighbor identity interaction (Table 1:  $H \times N2$  term).

To determine whether neighbor effects on aboveground biomass were due to neighbor size rather than neighbor identity per se, we repeated the original analyses but with the addition of mean neighbor biomass as a covariate. In this analysis, for *P. lanceolata*, neighbor biomass did significantly affect focal plant biomass ( $F_{(1,80)}=6.66$ ,  $P=0.01117$ ), and neighbor identity was no longer significant ( $F_{(1,80)}=2.40$ ,  $P=0.1249$ ), suggesting that for this species, neighbor plant size, and not identity per se, drove effects on focal plant biomass. All other factors were unchanged in significance from the original analysis. In contrast, for *P. major*, neighbor biomass did not explain variation in focal plant biomass ( $F_{(1,76)}=2.21$ ,  $P=0.1413$ ), indicating that it was the identity of the neighbor, not its size, that was important.

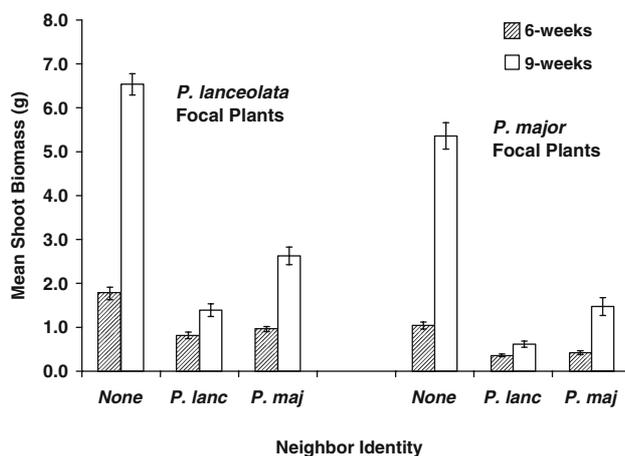
### Secondary chemistry: iridoid glycosides

The multivariate analysis of variance on plant chemistry revealed that in *P. lanceolata* leaves, neighbor treatment (Wilks'  $\lambda$   $F=2.97$ ,  $P<0.0212$ ) and harvest time

**Table 1** ANOVA results on focal shoot biomass of *P. lanceolata* and *P. major* grown alone or with four neighbors (*P. lanceolata* or *P. major*) and harvested at two times

Source	<i>Plantago lanceolata</i>		<i>Plantago major</i>	
	<i>F</i> ( <i>df</i> )	<i>P</i> value	<i>F</i> ( <i>df</i> )	<i>P</i> value
Neighbor				
N1: controls versus plants with neighbors	396.35 (1)	<b>&lt;0.0001</b>	448.98 (1)	<b>&lt;0.0001</b>
N2: <i>P. lanceolata</i> versus <i>P. major</i> neighbors	20.46 (1)	<b>&lt;0.0001</b>	10.63 (1)	<b>0.0017</b>
Harvest time (H)	320.76 (1)	<b>&lt;0.0001</b>	276.42 (1)	<b>&lt;0.0001</b>
Interactions				
H × N1	171.35 (1)	<b>&lt;0.0001</b>	253.36 (1)	<b>&lt;0.0001</b>
H × N2	14.15 (1)	<b>0.0003</b>	8.26 (1)	<b>0.0052</b>
Bench	2.18 (2)	0.1191	8.46 (2)	<b>0.0005</b>
Error	(81)		(77)	

The neighbor treatment effect was separated into two contrasts: N1 and N2. The interaction term refers to interactions between each of the neighbor treatment contrasts and harvest time. Bench is a random block effect and represents three greenhouse benches used in the experiment. All significant *P* values are highlighted in bold



**Fig. 1** Mean aboveground biomass ( $\pm 1$  standard error) for *P. lanceolata* and *P. major* focal plants at both harvest dates. Biomass includes leaves only for *P. lanceolata*, but leaves and reproductive tissues for *P. major* (the patterns are the same if only leaves are analyzed)

(Wilks'  $\lambda$   $F=124.80$ ,  $P<0.0001$ ) significantly contributed to variance in aucubin and catalpol concentrations in the leaves. Bench did not influence levels of aucubin and catalpol (Wilks'  $\lambda$   $F=0.92$ ,  $P<0.4520$ ), nor was there an interaction between neighbor treatment and harvest time (Wilks'  $\lambda$   $F=1.58$ ,  $P<0.1808$ ).

Separate univariate ANOVAs revealed that concentrations of aucubin and catalpol in *P. lanceolata* were influenced by the experimental treatments. The most significant source of variation was time; concentrations of both aucubin and catalpol increased two- to twentyfold during the three weeks between harvests (Table 2; Fig. 2). Neighbor presence and identity influenced concentrations of aucubin but not catalpol in *P. lanceolata* focal plants (Table 2). On average, *P. lanceolata* focal plants with neighbors had higher levels of aucubin than control

plants, although this effect was only evident at six weeks (Fig. 2), and this could explain the nearly significant interaction between harvest time and neighbor presence (Table 2: H × N1 term). *Plantago lanceolata* focal plants growing with conspecific neighbors had significantly higher levels of aucubin than plants growing with *P. major* neighbors (Table 2; Fig. 2). Although this pattern was consistent between harvest times (Table 2: H × N2 interaction not significant), the difference was especially marked at six weeks when plants with *P. lanceolata* neighbors had concentrations of aucubin three times as high as plants grown with *P. major* neighbors (Fig. 2).

Aucubin concentrations also increased significantly over time in *P. major* focal plants (Table 2, Fig. 2). Neighbor treatment further contributed to variation in aucubin. The presence of neighboring plants increased aucubin concentrations (Table 2: N1 term significant), although the species of neighboring plants did not matter (Table 2: N2 term not significant). These effects of neighbor treatment were consistent between harvest times (Table 2: interactions not significant).

Similar to the analyses on biomass, we repeated the original models but with the addition of mean neighbor biomass as a covariate in order to determine whether neighbor effects on aucubin concentrations were due to neighbor size rather than neighbor identity. Neighbor biomass did not significantly affect levels of aucubin in either *P. lanceolata* ( $F_{(1,80)}=0.04$ ,  $P=0.8431$ ) or *P. major* ( $F_{(1,75)}=0.24$ ,  $P=0.6248$ ), indicating that neighbor identity, and not size, is the key factor.

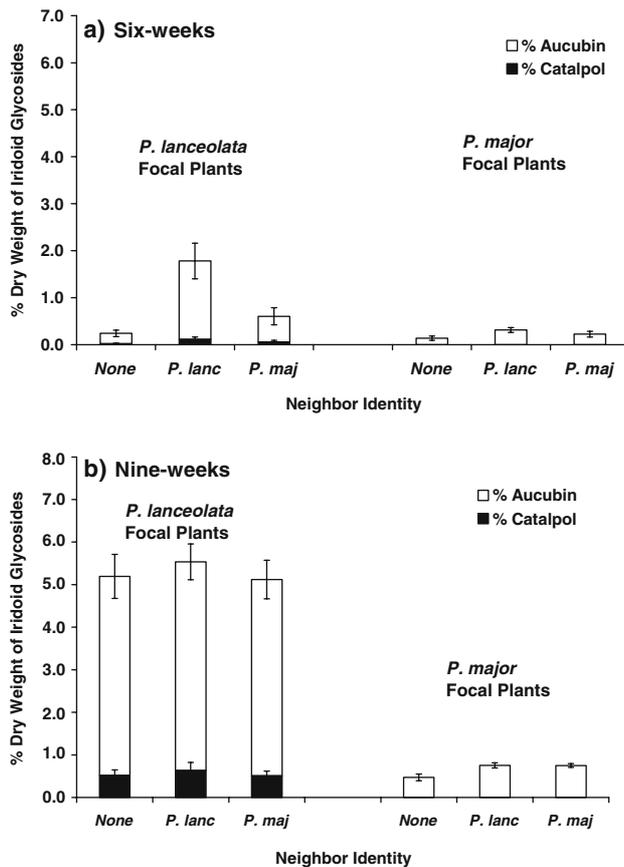
#### Trade-offs

Different results were obtained depending on whether iridoid glycoside content or iridoid glycoside

**Table 2** ANOVA results on iridoid glycoside concentrations in *P. lanceolata* and *P. major* focal plants grown alone or with four neighbors (*P. lanceolata* or *P. major*) and harvested at two times (six and nine weeks)

Source	<i>Plantago lanceolata</i>				<i>Plantago major</i>	
	Aucubin		Catalpol		Aucubin	
	<i>F</i> ( <i>df</i> )	<i>P</i> value	<i>F</i> ( <i>df</i> )	<i>P</i> value	<i>F</i> ( <i>df</i> )	<i>P</i> value
Neighbor						
N1: controls versus plants with neighbors	4.91 (1)	<b>0.0295</b>	0.63 (1)	0.4287	15.57 (1)	<b>0.0002</b>
N2: <i>P. lanceolata</i> versus <i>P. major</i> neighbors	6.50 (1)	<b>0.0127</b>	0.84 (1)	0.3612	0.51 (1)	0.4752
Harvest time (H)	223.10 (1)	<b>&lt;0.0001</b>	39.62 (1)	<b>&lt;0.0001</b>	88.85 (1)	<b>&lt;0.0001</b>
Interaction						
H × N1	3.83 (1)	0.0537	0.12 (1)	0.7258	1.91 (1)	0.1709
H × N2	2.45 (1)	0.1216	0.06 (1)	0.8108	0.57 (1)	0.4535
Bench	1.74 (2)	0.1822	0.10 (2)	0.9065	2.62 (2)	0.0793
Error	(81)		(81)		(77)	

The neighbor treatment effect was separated into two contrasts: *N1* and *N2*. The interaction term refers to interactions between each of the neighbor treatment contrasts and harvest time. Bench is a random block effect and represents three greenhouse benches used in the experiment. All significant *P* values are highlighted in bold



**Fig. 2a–b** Mean concentrations (% ±1 standard error) of iridoid glycosides in *P. lanceolata* and *P. major* focal plants at six (a) and nine (b) weeks. Open portions of the bars represent aucubin, and solid portions represent catalpol

concentration was used to test for defense costs (Table 3). For 13 of the 16 analyses, there was no relationship between biomass and iridoid glycosides. The only

**Table 3** Cost analyses

Samples	Content	Concentration
<i>Plantago lanceolata</i>		
Harv 1, controls ( <i>n</i> = 15)	$-R^2 = 0.06,$ $P = 0.375$	<b><math>-R^2 = 0.34,</math></b> <b><math>P = 0.024</math></b>
Harv 1, with neighbors ( <i>n</i> = 29)	$+R^2 = 0.01,$ $P = 0.656$	$-R^2 = 0.06,$ $P = 0.192$
Harv 2, controls ( <i>n</i> = 15)	$+R^2 = 0.25,$ $P = 0.060$	$+R^2 = 0.04,$ $P = 0.490$
Harv 2, with neighbors ( <i>n</i> = 30)	<b><math>+R^2 = 0.65,</math></b> <b><math>P &lt; 0.0001</math></b>	$+R^2 = 0.001,$ $P = 0.887$
<i>P. major</i>		
Harv 1, controls ( <i>n</i> = 15)	$+R^2 = 0.19,$ $P = 0.100$	$+R^2 = 0.02,$ $P = 0.618$
Harv 1, with neighbors ( <i>n</i> = 29)	$+R^2 = 0.01,$ $P = 0.583$	$-R^2 = 0.05,$ $P = 0.234$
Harv 2, controls ( <i>n</i> = 13)	$+R^2 = 0.10,$ $P = 0.292$	$-R^2 = 0.00,$ $P = 0.983$
Harv 2, with neighbors ( <i>n</i> = 28)	<b><math>+R^2 = 0.91,</math></b> <b><math>P &lt; 0.0001</math></b>	$+R^2 = 0.01,$ $P = 0.710$

Separate regression analyses to test relationship between iridoid glycoside content and concentration and leaf biomass. Direction of trend (+, -), coefficient of determination, and *p* value are reported. Harv 1 and 2 refer to the first (six weeks) and second (nine weeks) harvest times, respectively. A Bonferroni correction was applied to give an adjusted  $\alpha$  level of 0.025; significant tests are highlighted in bold

negative relationship between biomass and iridoid glycosides (concentration) was found in *P. lanceolata* control plants from the early harvest. In both species, there was a highly significant positive relationship between biomass and iridoid glycoside content for plants grown with competition and harvested late (nine weeks).

## Discussion

Few studies have investigated how neighbor species might differentially alter resistance phenotypes of focal plants via competition (trait-mediated indirect effects). Here, we have shown that *P. lanceolata* and *P. major* differ in their effects on two resistance traits of *Plantago* focal plants, namely plant size and secondary chemistry.

### Plant size: biomass

As expected, neighbors reduced biomass of both *P. lanceolata* and *P. major* focal plants. With time, the effects of neighbors on biomass increased, likely reflecting increasing limitation of resources. In both species, plants growing with *P. lanceolata* neighbors were smaller than plants growing with *P. major* neighbors. This indicates that beyond the importance of intra- versus interspecific competition, neighbor identity matters, and *P. lanceolata* is a better competitor than *P. major*. Plants likely competed for both soil nutrients and light in this experiment. Nutrient availability was probably relatively low considering that the soil medium we used had a low organic matter content, and plants were not fertilized. Furthermore, because both *P. lanceolata* and *P. major* have rosette growth forms that fail to achieve much height, and the surfaces of competition pots (with five plants) were very crowded, it is also likely that plants competed for light. At six weeks, leaves from neighboring plants were in contact, but by nine weeks, leaves were entirely overlapping, and it was difficult to identify individual plants. Thus, although resource availability, especially nutrients, may have been low for all plants in this experiment, control plants can be considered to have experienced relatively high resource availability (nutrients and light) compared to plants grown with neighbors. In addition to competition for resources, it is possible that neighbors reduced plant size via allelopathy. However, although iridoid glycosides have been demonstrated to inhibit seed germination and early root elongation in the lab (Pardo et al. 1998), there is no evidence that these effects can occur when plants grow in soil with microbial communities that can degrade leached iridoid glycosides.

Reduction in shoot size by competition could affect interactions with herbivores either positively or negatively. For example, competition might reduce herbivory of plants by reducing plant size, thereby constraining the amount of space available to sedentary herbivores (Linhart et al. 2005). Or, small plants might escape herbivores simply by being less apparent

(sensu Feeny 1976; Juenger et al. 2005). Alternatively, small plants can be totally consumed more easily. Whether competition-mediated reductions in plant size translate into positive or negative effects on herbivory probably depends on the behavior and diet breadth of the herbivores.

### Secondary chemistry: iridoid glycosides

Competitors might alter herbivory of plants by causing changes in secondary chemicals important for defense. For both *Plantago* species, concentrations of iridoid glycosides were higher in plants with competition than in control plants. Furthermore, *P. lanceolata* focal plants had higher concentrations of iridoid glycosides when competing with *P. lanceolata* than *P. major*, although this effect disappeared by nine weeks when iridoid glycoside levels were similar across the three treatment groups. There are several possible mechanisms that may drive these patterns in levels of iridoid glycosides.

First, as predicted by the carbon–nutrient balance hypothesis, plants with limited nutrients (i.e., with competition) could be constrained in growth and so allocate more photosynthate to carbon-based defenses than control plants that are not so constrained (Bryant et al. 1983). If the carbon–nutrient balance of plants was driving levels of iridoid glycosides, we would have expected neighbor size to significantly influence chemistry, as it did biomass. However, neighbor size did not significantly affect plant chemistry in either species, suggesting that nutrient depletion by neighbors and associated shifts in carbon–nutrient balance of focal plants may not account for differences in levels of iridoid glycosides.

Second, plants growing under competition and in resource-limited conditions are likely to be constrained in the degree to which they can compensate for tissues lost to herbivores (Coley et al. 1985). Therefore, the adaptive plasticity hypothesis (Sultan 1995; Agrawal 2001) predicts that plants should allocate more to resistance when they experience competition or limited resources. Several previous studies have shown levels of iridoid glycosides in *P. lanceolata* to be very plastic in response to variation in resource availability and in the direction predicted by the adaptive plasticity hypothesis (Fajer et al. 1992; Darrow and Bowers 1999; Jarzomski et al. 2000). No previous studies have looked at plasticity in iridoid glycosides of *P. major*, and although we did detect plasticity, it is unlikely to be adaptive merely because of the very small differences between control and competing treatment groups (<0.02%). These small differences are unlikely to be biologically relevant.

Third, it may be that the differences in iridoid glycoside concentrations for *Plantago* focal plants at the first harvest (six weeks) are merely dilution effects due to differences in shoot biomass. While average biomass does vary among treatment groups, these differences alone do not account for all differences in concentration. Total amounts of iridoid glycosides are more than three times higher, on average, in *P. lanceolata* focal plants grown with *P. lanceolata* neighbors (mean 0.0145 mg) as in control plants (mean 0.0043 mg). Amounts of iridoid glycosides are only slightly higher in plants grown with *P. major* neighbors (mean 0.0058 mg) than in control plants (mean 0.0043 mg). Apparently, *P. lanceolata* focal plants produce more iridoid glycosides when growing with competitors than they do when growing alone, and focal plants growing with *P. lanceolata* neighbors produce more iridoid glycosides than focal plants growing with *P. major* neighbors. In contrast, differences in concentrations of iridoid glycosides in *P. major* focal plants do seem to result from dilution. Average total amounts of aucubin are actually higher in control plants than in either competition group, despite having lower concentrations (0.0013 mg control plants vs. 0.0011 mg *P. lanceolata* neighbors vs. 0.00089 mg *P. major* neighbors).

Assessing which of these three mechanisms drives the patterns observed in this experiment is difficult, in part because we were unable to harvest roots. Within competition pots, it was impossible to untangle the roots of the individual plants, and because root–shoot ratios differ considerably between the two species (mean  $\pm 1$  standard error, *P. lanceolata*:  $0.611 \pm 0.019$ ,  $n=96$ ; *P. major*:  $0.449 \pm 0.015$ ,  $n=70$ ; K. E. Barton, unpublished data), it was not prudent to estimate mean per plant root mass from the entire-pot root mass. Because roots are likely to be involved in both the carbon–nutrient balance of plants and in possible dilution effects, it is difficult to definitively refute these two potential mechanisms without root biomass data. If changes in root–shoot ratio lead to shifts in carbon–nutrient balance that constrain growth more than photosynthesis, the production of carbon-based secondary compounds like iridoid glycosides may increase. Evidence from a similar greenhouse study in which it was possible to harvest roots suggests that root–shoot ratio in *P. lanceolata* and *P. major* is highly plastic in response to competition (K. E. Barton, in preparation). However, this response changed over time, presumably as resource availability changed. Competition led to an early decrease in root–shoot ratio (consistent with competition for light), followed by an increase in root–shoot ratio (consistent with competition for soil nutrients) only a few weeks later (K. E. Barton, in preparation).

Future work investigating the relationship between competition and resource availability (light and soil nutrients) is needed to explain this plant response and this would allow us to rigorously test the carbon nutrient balance and dilution hypotheses.

#### Trade-offs

A fundamental assumption of most plant defense theories is that limited resources lead to allocation constraints, often depicted as growth–defense trade-offs (reviewed in Stamp 2003). However, growth–defense trade-offs have been notoriously difficult to document (Bergelson and Purrington 1996; Koricheva 2002), perhaps in part because of the methods used to test for trade-offs. A negative correlation between biomass (growth) and secondary chemical concentration (defense) is interpreted to reflect a trade-off and is the most common measure of defense costs. However, this calculation can lead to spurious results because biomass is in both correlated factors (concentration = content/biomass) (Koricheva 1999). To avoid this problem, a correlation between biomass and secondary chemical content is more appropriate; a negative correlation would indicate a trade-off (Koricheva 1999). Our analyses indicate that iridoid glycoside content and concentration differ in their relationships with biomass, and because of the possibility for spurious results with concentration, we consider the analyses with content to be more robust and include the analyses with concentration only for illustrative purposes.

We observed no trade-offs between growth and defense (measured as content) for any treatment group in either species. In fact, the only significant correlations between growth and defense were for plants growing with competition and harvested late, and these were positive correlations, indicating that bigger plants had, on average, more iridoid glycosides. This probably reflects variation among plants in their resource acquisition abilities (Valverde et al. 2003), such that plants that obtained more resources had more biomass and more defensive chemicals. Plants performing poorly in resource acquisition were constrained in both growth and resistance. Evidence from previous experiments on the effects of competition on resistance costs is equivocal; approximately the same numbers of studies have found that competition increases, decreases or has no effect on allocation costs (Jones and Coleman 1991; Agrawal 2000; Cipollini 2002; Siemens et al. 2003, and references therein). Because trade-offs depend not only on resource limitation, but also on the kind of resources and secondary compounds involved (Koricheva 2002), it is not surprising that studies report

different effects of competition on allocation costs of defense. Determination of the mechanism driving the positive relationships between plant size and secondary chemistry (and the lack of trade-offs) in this study requires specific information about how competition altered light and nutrient availabilities.

It is important to emphasize that although we did not detect trade-offs between growth and defense in this study, this does not mean that there are not other costs associated with the production of iridoid glycosides. We specifically tested for an allocation cost of iridoid glycosides, but we did not look for ecological costs (Strauss et al. 2002) or biochemical production costs, which have been estimated to be relatively high for terpenoids such as iridoid glycosides (Gershenson 1994).

## Conclusion

To revisit our initial questions, we found that: (a) competition did alter resistance traits of both *P. lanceolata* and *P. major* focal plants. Competition led to decreases in plant size and increases in levels of iridoid glycosides. (b) Neighbor species did matter. *Plantago lanceolata* was a better competitor than *P. major*, and competition with *P. lanceolata* led to greater reductions in plant size and increases in levels of iridoid glycosides. (c) Effects of competition did change over time. Competition further suppressed growth, but no longer increased levels of iridoid glycosides above levels of control plants. (d) We did not detect a trade-off between growth and resistance. In contrast, plants of both species growing under competition and harvested at nine weeks showed a positive relationship between growth and defense.

Although we did not measure the effects of competition on herbivory, our results allow us to make predictions about how competition between *P. lanceolata* and *P. major* might influence herbivory in the field. For example, this study indicates that *Plantago* plants growing with neighbors are smaller but better chemically defended than control plants at six weeks. Thus, although small plants are more vulnerable, the higher level of chemical defense might prevent total consumption by generalist herbivores. Plants growing near *P. lanceolata* are expected to be even more resistant to herbivores than plants growing near *P. major*. By nine weeks, levels of iridoid glycosides are similar for plants growing with and without competition, but plants with neighbors, especially *P. lanceolata* neighbors, are severely reduced in size. This means that any chemical advantage that small plants might have had at six weeks has disappeared three weeks later.

This study highlights the importance of neighbor identity when considering trait-mediated indirect effects of neighbors on plant–herbivore interactions. Although it has been previously recognized that competition can alter resistance phenotypes of plants, the role of competitor identity has not been appreciated. Including this component increases the complexity of the interaction, which better reflects what occurs in natural communities. Future work to test for effects in the field would verify whether these responses can occur under natural conditions.

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