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Arbuscular mycorrhizal fungal species suppress inducible plant responses and alter defensive strategies following herbivory

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Abstract In a greenhouse experiment using *Plantago lanceolata*, plants grown with different arbuscular mycorrhizal (AM) fungal species differed in constitutive levels of chemical defense depending on the species of AM fungi with which they were associated. AM fungal inoculation also modified the induced chemical response following herbivory by the specialist lepidopteran herbivore *Junonia coenia*, and fungal species varied in how they affected induced responses. On average, inoculation with AM fungi substantially reduced the induced chemical response as compared with sterile controls, and inoculation with a mixture of AM fungi suppressed the induced response of *P. lanceolata* to herbivory. These results suggest that AM fungi can exert controlling influence over plant defensive phenotypes, and a portion of the substantial variation among experimental tests of induced chemical responses may be attributable to AM fungi.

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Introduction

The stationary status of plants leaves them vulnerable to repeated herbivory; however, plants limit herbivory using a variety of defense mechanisms. The production of secondary compounds that render plants unpalatable is likely the most effective and prevalent defense strategy. Concentrations of secondary compounds often increase following herbivory (Karban and Baldwin 1997 and references therein), and this induction of defense compounds can also affect the dynamics and stability of the plant–herbivore interaction (Underwood 1999; Underwood and Rausher 2002; Verschoor et al. 2004). Despite the large number of studies focused on inducible plant responses following herbivory, the presence and amount of induction often varies among studies on the same plant species. Variation in factors such as nutrients (Hamilton et al. 2001; Stamp 2003) and shade (Roberts and Paul 2006) have been shown to alter inducible chemical responses; however, these factors have explained only a portion of the variation in these systems, suggesting that other variables may be just as important.

Mutualisms, for example, may influence the inducibility of plant chemical responses. The majority of herbaceous plants maintain a mutualistic association with arbuscular mycorrhizal (AM) fungi (Smith and Read 1997), obligate plant mutualists that aid host plants in phosphorus, water, and trace-mineral uptake in return for carbon. Associations with these fungi often increase plant growth and reproduction (Smith and Read 1997), with AM fungal species

differing in their effects on plant growth (Bennett and Bever 2007; Pringle and Bever 2008). AM fungal species also vary in a number of ecological characteristics, including resource uptake rates (Bever 2002a; Cavagnaro et al. 2005; Smith et al. 2004), competitive abilities (Bennett and Bever 2009), and reproductive output (sporulation) (Bever et al. 1996), characteristics likely to influence host plants as well.

The presence or absence of AM fungi has been shown to affect plant defensive chemical composition (Gange and West 1994; Wurst et al. 2004) and herbivore growth rates (Gange et al. 2005; Goverde et al. 2000); for example, several studies have demonstrated that constitutive levels of plant defenses vary in the presence versus absence of AM fungi (Gange and West 1994; Gehring and Whitham 2002; Guerrieri et al. 2004; Rabin and Pacovsky 1985; Vicari et al. 2002; Wurst et al. 2004). Most of these studies have examined defenses in the presence or absence of a single fungal species (Gehring and Whitham 2002; Guerrieri et al. 2004; Rabin and Pacovsky 1985; Vicari et al. 2002; Wurst et al. 2004); although one study examined the presence or absence of an AM fungal field community (Gange and West 1994), specific AM fungal species were not identified. In addition, research by several groups has demonstrated that herbivore fitness and growth can be positively [for example, *Scopula ornate*, *Pyrausta aurata*, *Cryptomyzus ribis*, *Myzus persicae* (Gange et al. 2002b); *Epilachna varivestis* (Borowicz 1997); *Ozirhincus leucanthemi* (Gange et al. 2005)], or negatively [for example, *Heliothis zea* (Rabin and Pacovsky 1985); fall armyworm (*Spodoptera frugiperda*) larvae (Rabin and Pacovsky 1985); *Arctia caja* larvae (Gange and West 1994); noctuiid moth *Phlogophora meticulosa* larvae (Vicari et al. 2002); aphids (*Chaitophorus populicola*) (Gehring and Whitham 2002)] affected by host plant associations with AM fungi. Moreover, the presence of AM fungi can alter plant tolerance to herbivory (Bennett and Bever 2007; Borowicz 1997; Gange et al. 2002a; Kula et al. 2005). To date, however, no study has investigated how plant defensive chemical composition varies with species of AM fungi, or whether AM fungi alter plant inducible defenses.

Several studies focusing on AM fungal-aided defense against plant pathogens have provided evidence of cross-talk between salicylic acid and jasmonic acid pathways and priming of fungal defense systems in host plants (reviewed in Pozo and Azcon-Aguilar 2007). However, these studies did not demonstrate changes in, or priming of, aboveground herbivore defense pathways in AM fungal host plants. In addition, despite the large number of studies focused on mycorrhizal fungi and constitutive defenses and the suggestion that AM fungi might prime biochemical pathways involved in plant defenses, to our knowledge no study to date has examined how the presence of AM fungi interacts with aboveground herbivory to affect the induction of plant responses to herbivory. Here we show that the presence of AM fungi, as well as the species identity of AM fungi, alters constitutive and induced plant chemical responses in *Plantago lanceolata*.

Materials and methods

To address whether the presence of AM fungi, as well as the species identity of AM fungi, might alter constitutive and induced plant chemical defenses, we used specialist larvae of the butterfly *Junonia coenia* (Nymphalidae), its plant host narrow-leaved plantain, *Plantago lanceolata* (Plantaginaceae), and three AM fungal symbionts of *P. lanceolata*: *Glomus* white [*Glomus* d1 (Bennett and Bever 2007; Bever et al. 1996)] (Glomineae), *Archaeospora trappei* (Glomineae), and *Scutellospora calospora* (Gigasporineae). *Plantago lanceolata* associates with all three fungal species at the site where plants, larvae, fungi, and soil were collected (Bever et al. 1996). Only one of these three species, *S. calospora*, has been reported to associate with *Plantago lanceolata* in its European native range (Oehl et al. 2003). *P. lanceolata* contains carbon-based secondary compounds, iridoid glycosides (Bobbitt and Segebarth 1969; Duff et al. 1965), primarily aucubin and catalpol (Bowers and Stamp 1992), compounds shown to be toxic or deterrent to generalist, but not specialist, herbivores (Bowers 1991 and references therein), that are sequestered by *Junonia coenia* larvae (Bowers and Collinge 1992; Bowers and Puttick 1986) as defenses against enemies. Aucubin and catalpol vary in their degree of toxicity and cost of production. Aucubin is the less toxic compound and the biosynthetic precursor to catalpol (Bowers 1991; Jensen et al. 1975), thus relative concentrations of aucubin and catalpol provide an indication of resource allocation beyond the simple overall allocation to chemical defense. All three sets of organisms (butterflies, plants, and fungi) as well as soil, were collected from the same old field on the campus of Duke University, Durham, NC, USA. For the purposes of this study, we define constitutive chemical defense as the concentration of defensive compounds, iridoid glycosides, found within the leaf tissues of host plants that have not experienced herbivory. Induced chemical responses are defined as any change in the concentration of defensive compounds in leaf tissues within host plants that received herbivory.

We conducted a 5 × 2 factorial experiment with five different fungal treatments (plants grown with *Glomus* white, *Archaeospora trappei*, *Scutellospora calospora* individually, all three species in combination, and all three species combined and sterilized to create a sterile control treatment), and two herbivory levels (herbivory and no herbivory).

Soil was mixed 1:1 with sand to promote drainage in pots, and the mixture was then sterilized with steam. This soil combination was previously tested and found to contain 0.09% N and 0.03% P (Reynolds et al. 2006). Deepots (600 ml) (Stuewe & Sons, Corvallis, OR) were then filled with sterile soil and inoculated with 100 ml of one species or a mixture of species of the above fungi, or with a mixture of sterile inoculum (containing sterilized spores from all three fungal species). Inocula of *Glomus* white, *A. trappei*, and *S. calospora* (originally collected from the old field in North Carolina) were obtained from pure cultures grown with *Sorghum halepense* maintained in the greenhouses on the Indiana University campus. In order to control for genetic variation, seven genotypes of *P. lanceolata* were created by mating randomly chosen plants collected from the old field in North Carolina. These genotypes created a random background level of genetic variation that could be controlled for in the analyses. Four replicates (four pots with one plant per pot) of each of the seven genotypes of *P. lanceolata* were planted into each fungal treatment (for a total of 140 plants) and grown in the greenhouses on the Indiana University campus with supplemental lighting to increase day length to 14 h. After 5 weeks of growth, two replicates of each genotype in each soil treatment (14 plants per treatment) were subjected to a 20% defoliation event by *Junonia coenia* larvae using clip cages. Empty clip cages were placed on untreated plants to control for clip cage effects.

Three days following herbivory, aboveground plant tissue was harvested. Leaves of plants that experienced herbivory were separated by whether they received herbivory (damaged) or not (undamaged), and oven dried at 60°C. Plants not subjected to herbivory (control plants) were harvested at the same time. Leaves from control plants, and damaged and undamaged leaves of plants exposed to herbivores were subsampled and analyzed separately for aucubin and catalpol, using gas chromatography (Bowers and Stamp 1992; Gardner and Stermitz 1988). Remaining uneaten leaf tissue was analyzed for nitrogen content on a Perkin Elmer 2400 elemental analyzer.

We first analyzed the results using the mixed model analysis of variance (ANOVA) within the mixed procedure of SAS 2000. We tested for effects of mycorrhizal fungal treatment, herbivory, and their interaction on plant iridoid glycoside concentrations and nitrogen content with plant genotype as a random effect. We used orthogonal a priori contrasts to test three levels of hypotheses: first, does the presence of AM fungi affect levels of constitutive and induced plant defenses and plant nutrient content; second, does AM fungal species diversity affect constitutive and induced defenses and nutrient content; and finally, does AM fungal species identity affect these same defenses and host nutrient content? In addition, in order to confirm the robustness of our univariate analyses, we performed a multivariate analysis of variance (MANOVA) in the general linear models procedure of SAS 2000, testing the effect of mycorrhizal fungal treatment on the total amount of iridoids and the proportion of catalpol within damaged and undamaged leaves. We then conducted profile analyses to determine when values in damaged and undamaged leaves differed. In addition, we included the log-transformed effects of plant biomass and nitrogen content as covariates in analyses examining constitutive levels of defense, and we included nitrogen content as a covariate in the full mixed model to determine whether the inclusion of plant mass or nutrient content influenced the levels of chemical defense in *P. lanceolata* and mediated the effect of AM fungal inoculation. We tested for the effects of fungal inocula on aboveground plant biomass of uneaten plants using analysis of variance with block as a random factor within the mixed models procedure of SAS 2000.

Results

We found that constitutive iridoid glycoside levels in *P. lanceolata* that never received herbivory varied among plants grown with different AM fungal communities ($P < 0.0001$, Table 1). In particular, undamaged plants associated with the AM fungus *Scutellospora calospora* produced higher constitutive levels of iridoid glycosides than those associated with *Glomus* white, *Archaeospora trappei*, all three mycorrhizal fungal species in combination, or the sterilized control (Table 1; Fig. 1a). In addition, undamaged plants associated with the combination of all three fungal species produced a significantly greater proportion of catalpol than plants associated with any single fungal species treatment or plants associated with no fungi (Table 1; Fig. 1b). Genotypes of *P. lanceolata* did not differ in their total iridoids, and there were no significant interactions of genotype and herbivory or genotype and fungal inocula, indicating that genotypes respond similarly to these treatments (Table 1).

To examine how plants exposed to herbivores responded at the whole plant level, we calculated whole plant levels of iridoids by correcting for the biomass of damaged and undamaged leaves. This whole plant comparison revealed that when plants were exposed to herbivory by buckeye caterpillars there was a weak effect of herbivory on total iridoid glycosides ($P = 0.05$, Table 1; Fig. 1a), a significant overall effect of AM fungi on induction of chemical responses following herbivory (Fig. 1a, Table 1), and a significant among fungal species effect (Herbivory by Species of Inocula contrast $P = 0.03$, Table 1), indicating that plant response to herbivory depended upon the fungal

Table 1 ANOVA results for analyses of defensive chemistry of *Plantago lanceolata*

	Total iridoids			Proportion catalpol			Proportion N		
	df	*F*	*P*	*df*	*F*	*P*	*df*	*F*	*P*
Fungal Inocula[a]	4, 24	11.20	<0.0001	4, 24	5.91	0.0019	4, 24	14.73	<0.0001
Live Inocula versus Sterile Inocula	1, 24	0.47	0.4977	1, 24	0.75	0.3944	1, 24	42.34	<0.0001
Mixture versus Single Species Inocula	1, 24	20.18	0.0002	1, 24	13.28	0.0013	1, 24	2.22	0.1492
Among Species of Inocula	2, 24	13.46	0.0001	2, 24	5.06	0.0147	2, 24	6.52	0.0055
A. trappei versus *Glomus* white	1, 24	14.55	0.0016	1, 24	9.97	0.0084	1, 24	3.49	0.1480
A. trappei versus *S. calospora*	1, 24	0.69	0.8294	1, 24	1.75	0.3968	1, 24	2.51	0.2518
Glomus white versus *S. calospora*	1, 24	23.27	<0.0002	1, 24	3.26	0.1674	1, 24	12.98	0.0028
Herbivory[b]	1, 6	5.77	0.0531	1, 6	3.40	0.1149	1, 6	0.79	0.4096
Herbivory × Fungal Inocula	4, 73	4.83	0.0016	4, 80	0.84	0.5024	4, 68	2.33	0.0649
Herbivory versus Live Inocula	1, 73	6.30	0.0143	1, 80	0.62	0.4330	1, 68	0.06	0.8087
Herbivory versus Mixture	1, 73	5.06	0.0276	1, 80	0.83	0.3657	1, 68	5.02	0.0284
Herbivory versus Species of Inocula	2, 73	3.83	0.0262	2, 80	0.91	0.4075	2, 68	2.24	0.1146
A. trappei versus *Glomus* white	1, 73	7.66	0.0142	1, 80	0.44	1.0000	1, 68	1.96	0.3328
A. trappei versus *S. calospora*	1, 73	2.38	0.2544	1, 80	0.42	1.0000	1, 68	4.38	0.0802
Glomus white versus *S. calospora*	1, 73	1.37	0.4904	1, 80	1.81	0.3640	1, 68	0.69	0.8158

Results include arcsin-transformed percentage of iridoid glycosides in leaf tissue and proportion of iridoid glycosides that is catalpol in leaf tissue of *Plantago lanceolata* 3 days following an herbivory event as well as the log-transformed values of N content within uneaten leaves. Contrasts within the Fungal Inocula, and Herbivory by Fungal Inocula term are represented by indentations below their respective term. Nonorthogonal contrasts within the Among Species of Inocula contrasts are represented by additional indentation, and *P* values were adjusted using Scheffe's method. Genotype and block were considered as random effects, and thus terms were tested across the genotype interaction terms

[a] Fungal Species was tested across Genotype × Fungal Species Error

[b] Herbivory was tested across Genotype × Herbivory Error

treatment. Overall, induction of iridoid glycosides was greater in plants not associated with AM fungi than in plants associated with AM fungi (Herbivory by Fungal Inocula, Herbivory by Live Inocula contrast $P = 0.01$, Table 1; Fig. 1a). However, plants associated with *A. trappei* experienced an induced response (Single Species of Inocula contrast $P = 0.03$, Table 1), whereas *Glomus* white and *S. calospora* suppressed any increases in total iridoid glycosides both singly and in combination with *A. trappei* (Mixture versus Single Inocula contrast $P = 0.03$, Table 1). Induced responses by *P. lanceolata* plants associated with *A. trappei* or no fungi resulted in levels of iridoids three times greater in plants exposed to herbivores than in plants not exposed to herbivores (Fig. 1a). Although there was no increase in iridoid glycosides in response to herbivory in plants associated with *Glomus* white, those plants had a significantly greater proportion of catalpol, indicating an overall change in iridoid biosynthesis and allocation between aucubin and catalpol in plants associated with *Glomus* white (Table 1; Fig. 1b).

A comparison of damaged and undamaged leaves on plants exposed to herbivores revealed that tissue allocation of total iridoid glycosides varied among plants associated with the different fungal inocula ($P = 0.004$, Table 3), and plants associated with AM fungi differed from plants lacking AM fungi (sterilized spore treatment) (Tables 2, 3). Following herbivory, plants associated with no AM fungi showed a 2.5-fold increase in iridoid glycosides in undamaged leaves compared with damaged leaves, while plants associated with AM fungi showed no difference in iridoid glycoside concentrations between damaged and undamaged tissues (Live Inocula versus Sterile Inocula contrast $P = 0.03$, Table 3; Fig. 2a). In addition, there were significant differences in allocation between plants associated with multiple fungal partners and a single fungal partner (Mixture versus Single Species Inocula contrast $P = 0.01$, Table 3), and between plants associated with different fungal partners (Among Species of Inocula contrast $P = 0.05$, Table 3). Overall, however, there was no shift in the proportion of one iridoid over another (Figs. 1b, 2b).

Overall, plants associated with AM fungi increased the proportion of catalpol in damaged leaves where they may immediately impact herbivore feeding (Fig. 2b). This increase in damaged leaves was offset by reductions in allocations of catalpol in undamaged leaves in plants associated with all fungal species except *Glomus* white (Table 2; Fig. 2b).

The proportion of nitrogen in leaf tissues of *P. lanceolata* tended to follow the same pattern as the concentration of

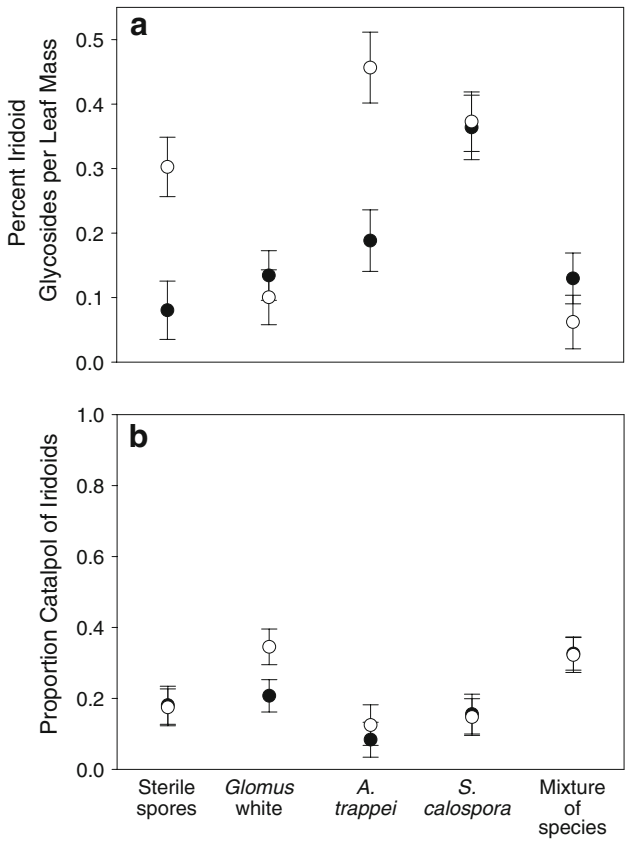

Fig. 1 (**a**) Percentage of total iridoid glycosides, and (**b**) proportion catalpol of total iridoid glycosides in leaves of *P. lanceolata* plants associated with five different mycorrhizal inocula. Plants that never received herbivory (representing constitutive levels of iridoid glycosides) are represented by *filled circles*; plants that received herbivory (representing induced response levels) are represented by *open circles*. *Error bars* represent one standard error

defenses within tissues (Table 1). Overall, plants associated with AM fungi had lower amounts of N within their tissues (Live Inocula versus Sterile Inocula contrast $P < 0.0001$, Table 1; Fig. 3), and plants associated with a mixture of species or *Glomus* white tended to have the lowest proportion of N in leaf tissues, followed by plants associated with *A. trappei* and *S. calospora*. Plants associated with no fungi had the highest amount of N in their tissues (Fig. 3). Overall, herbivory did not influence the amount of N in plant tissues ($P = 0.41$, Table 1) except in the case of plants associated with *A. trappei*, in which there was a decrease in N in plants that experienced herbivory (Fig. 3). There was no such shift for plants associated with no fungi, despite the increase in iridoid glycosides following herbivory.

Neither the inclusion of plant mass nor nitrogen content as covariates removed the significance of fungal inocula on the constitutive level of iridoids in plant tissues or the proportion of catalpol. In addition, the inclusion of nitrogen content as a covariate in the mixed model did not alter the significance of fungal inocula or the interaction between fungal inocula and soil, indicating that neither plant biomass nor nitrogen content mediated the effect of AM fungal inoculation on the plant defensive phenotype.

We found significant effects of fungal inocula on aboveground plant biomass at the time of harvest ($F_{4,62} = 5.06$, $P = 0.0014$; Fig. 4).

Discussion

While previous work demonstrated that the presence of AM fungi alters levels of defensive chemicals (Gange and

Table 2 ANOVA results for analyses of defensive chemistry within damaged and undamaged leaves of *Plantago lanceolata* plants that received herbivory

	df	Iridoids in damaged leaves		Iridoids in undamaged leaves		Proportion of catalpol in damaged leaves		Proportion of catalpol in undamaged leaves	
		F	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Fungal Inocula[a]	4, 24	5.06	0.0042	6.36	0.0012	2.75	0.0515	4.93	0.0048
Live Inocula versus Sterile Inocula	1, 24	0.02	0.8782	5.15	0.0326	3.24	0.0844	0.38	0.5419
Mixture versus Single Species Inocula	1, 24	9.21	0.0057	8.80	0.0067	1.90	0.1806	9.38	0.0053
Among Species of Inocula	2, 24	5.78	0.0089	5.75	0.0091	2.88	0.0758	5.10	0.0142
A. trappei versus *Glomus* white	1, 24	8.11	0.0178	10.54	0.0068	5.52	0.0546	10.18	0.0078
A. trappei versus *S. calospora*	1, 24	0.00	1.0000	0.67	0.8456	0.71	0.8146	2.22	0.2978
Glomus white versus *S. calospora*	1, 24	8.86	0.0132	5.83	0.0474	2.33	0.2794	2.79	0.2158

Results include log-transformed percentage of total iridoids in leaf tissue and proportion of catalpol in leaf tissue of *Plantago lanceolata* 3 days following an herbivory event. Contrasts within the Fungal Inocula term are represented by indentations. Nonorthogonal contrasts within the Among Species of Inocula contrast are represented by additional indentation, and *P* values were adjusted using Scheffe's method. Genotype was considered a random effect, and thus terms were tested across the genotype interaction terms

[a] Fungal Species was tested across Genotype × Fungal Species Error

Table 3 MANOVA results for analyses of defensive chemistry within damaged and undamaged leaves of *Plantago lanceolata* plants that received herbivory

	df	Wilk's lambda	*F*	*P*
Fungal Inocula	16, 64.794	0.2240	2.56	0.0040
Live Inocula versus Sterile Inocula	4, 21	0.6237	3.17	0.0348
Mixture versus Single Species Inocula	4, 21	0.5580	4.16	0.0123
Among Species of Inocula	8, 42	0.4933	2.22	0.0446
A. trappei versus *Glomus* white	4, 21	0.5494	4.31	0.0214
A. trappei versus *S. calospora*	4, 21	0.9057	0.55	1.0000
Glomus white versus *S. calospora*	4, 21	0.6543	2.77	0.1078

Results include log-transformed percentage of total iridoids in leaf tissue and proportion of catalpol in leaf tissue of *Plantago lanceolata* 3 days following an herbivory event. Contrasts within the Fungal Inocula term are represented by indentations below their respective term. Nonorthogonal contrasts within the Among Species of Inocula contrast are represented by additional indentation, and *P* values were adjusted using Scheffe's method. Terms were tested across the Fungal Inocula by Genotype interaction error

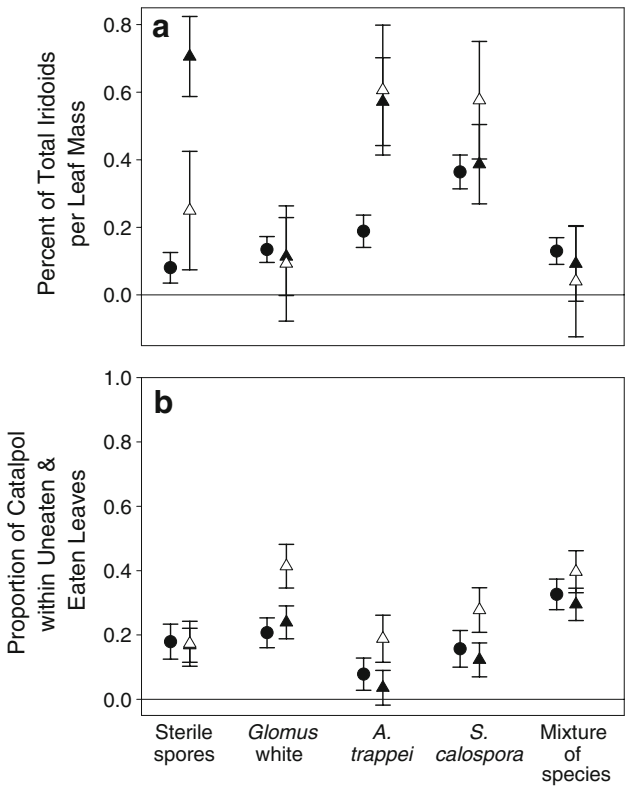

Fig. 2 (**a**) Percentage of total iridoids and (**b**) proportion of catalpol in damaged and undamaged leaves of *P. lanceolata* plants associated with five different mycorrhizal inocula that received herbivory. Proportion of defensive compounds in damaged leaves are represented by *open triangles*, and proportion of defensive compounds in undamaged leaves are represented by *filled triangles*. The (**a**) percentage of total iridoids and (**b**) proportion of catalpol present in plants that never received herbivory are represented by the *filled circles* to the left of each pair of points. *Error bars* represent one standard error

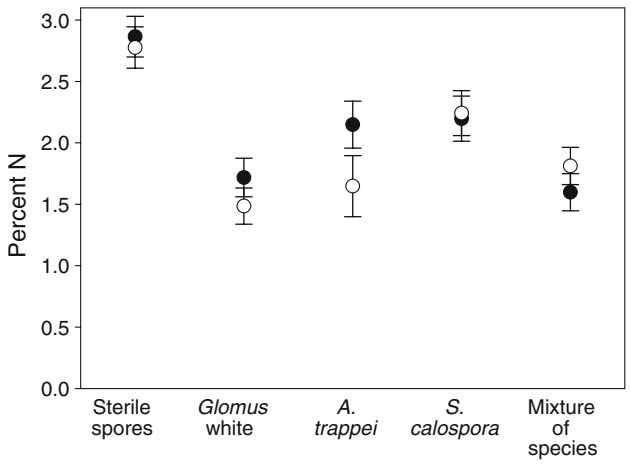

Fig. 3 The percentage of nitrogen in plant tissues associated with five different mycorrhizal inocula, and subject to two different herbivory treatments. Plants that never received herbivory are represented by *filled circles*; plants that received herbivory are represented by *open circles*. *Error bars* represent one standard error

West 1994; Wurst et al. 2004), our results provide the first demonstration that AM fungal species identity and composition can strongly affect plant defensive phenotype, as both constitutive and inducible levels of iridoid glycosides in *P. lanceolata* varied with association with different AM fungal species. Moreover, the alteration of plant defensive chemistry by individual AM fungal species was not a simple consequence of improved plant nutrition, because there was no effect of plant biomass, or N content of plant tissues, on iridoid glycoside content of plants within different AM fungal treatments.

Constitutive levels of iridoid glycosides within plant tissues were highest in plants associated with *S. calospora*, intermediate in plants associated with *A. trappei*, and lowest for plants associated with no AM fungi, *Glomus* white, and a mixture of all three species (Fig. 1a). While there was no difference in growth among plants associated with any fungal community (except the growth-promoting mixture of species) (Fig. 4), we believe these are early growth results and not indicative of the overall growth

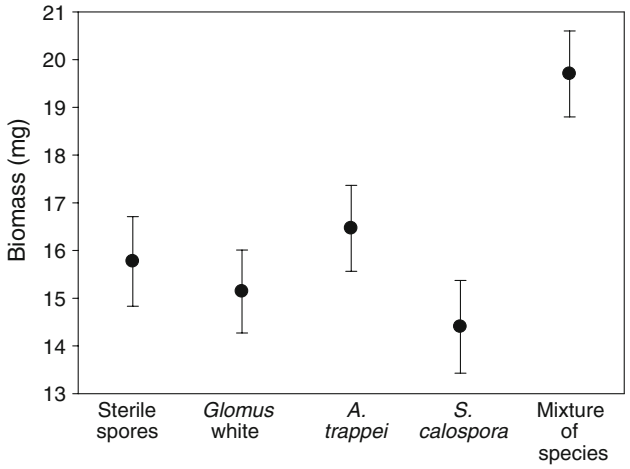

Fig. 4 Biomass (mg) of plants that never experienced herbivory associated with the five different inocula in the experiment

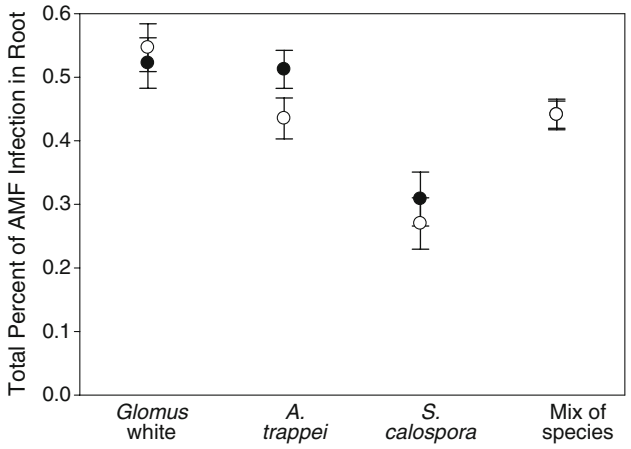

Fig. 5 Percentage of *P. lanceolata* root length colonized by four different AM fungal inocula types. *Filled circles* represent root length colonized in the absence of herbivory, and *open circles* represent root length colonized in the presence of herbivory. *Error bars* represent one standard error (Bennett and Bever, Oecologia, 2009)

pattern with the fungal species. In a concurrent, longer-term experiment, *P. lanceolata* plants associated with *Glomus* white and a combination of all three fungal species achieved the greatest biomass, while plants associated with *S. calospora* and the sterile control grew slowest (Bennett and Bever 2007). Fungal infection measured in a concurrent experiment (conducted with the same genotypes with the same treatments in the same greenhouse at the same time) revealed that all inocula infected roots, while infection by sterile inocula did not differ from zero (Bennett 2005; Bennett and Bever 2009; Fig. 5). In addition, there were no correlations between defensive chemistry and plant biomass when looking across treatments, nor between defensive chemistry and patterns of AMF infection (Bennett, unpublished data).

While plants not associated with AM fungi increased the total amount of iridoid glycosides and also allocated a greater proportion of compounds toward undamaged leaves following herbivory, plants associated with AM fungi did not increase concentrations of iridoid glycosides in damaged or undamaged leaves. Although the total iridoid concentration did not change, the relative abundance of the component iridoids in damaged and undamaged leaves shifted. In particular, the proportion of catalpol, the more costly and effective defense compound (Bowers 1991; Jensen et al. 1975), increased in damaged leaves and decreased in undamaged leaves (Fig. 2b), allowing plants associated with AM fungi to defend immediately threatened tissues following herbivory.

Plants associated with different AM fungi exhibited different patterns of allocation of iridoid glycosides. Plants associated with *Glomus* white increased overall proportions of catalpol and reduced overall proportions of aucubin (Fig. 1b), a shift likely due to both the movement of compounds to damaged tissues and the conversion of aucubin into catalpol (Duff et al. 1965). Increasing the proportion of catalpol, but not the total concentration of iridoid glycosides, may be a strategy employable only by plants with greater resources (Bennett and Bever 2007). By contrast, plants associated with *S. calospora* had higher constitutive levels of iridoid glycosides (Fig. 1a) and N content than *A. trappei* with *Glomus* white intermediate (Fig. 3). High constitutive levels of iridoid glycosides in plants associated with *S. calospora* may be a result of early induction by the infection of a fungus that does not benefit growth in the long term, resulting in a stress response to limited resources (Bennett and Bever 2007; Bever 2002a), a strategy to counter herbivory in a nonoptimal environment, or a response to increased nitrogen in plant tissues. Although increased nutrients result in decreased concentrations of iridoid glycosides in *P. lanceolata* tissues (Jarzomski et al. 2000), nutrient concentrations are not likely responsible for the increased iridoid glycosides in plants associated with *S. calospora* because they had an intermediate amount of nitrogen in tissues (Fig. 3). Plants associated with *A. trappei* increased overall levels of iridoid glycosides following herbivory (Fig. 1a), but this response was suppressed in combination with *Glomus* white and *S. calospora*. Finally, plants associated with all three fungi exhibited a potential combination of strategies including higher constitutive levels of iridoids (as in plants associated with *S. calospora*), particularly catalpol (Fig. 1b) (as in *Glomus* white). Shifting allocation to stronger resistance compounds such as catalpol might be an alternative defense strategy employed by some plants associated with mutualistic AM fungi.

Previous work demonstrated that the presence of AM fungi from the native range of *Plantago* could modify the constitutive levels of iridoid glycosides (Gange and West

1994; Wurst et al. 2004). In a greenhouse study there was no variation in catalpol concentration between *P. lanceolata* plants associated with or without *G. intraradices* (Wurst et al. 2004) (although aucubin was not measured). In a field experiment the concentration of iridoid glycosides in *P. lanceolata* tissues was greater in control plants associated with a mixture of fungi than plants (although species of fungi were not identified) subjected to fungicide application (Gange and West 1994). Given that the present study was done with plants and fungi from the introduced range of the *P. lanceolata*, we can suggest that AM fungal modification of plant defensive phenotype is general across continents. Moreover, the possibility exists that modification of *Plantago* defensive phenotype by novel AM fungal species may have contributed to the plant invasion in North America. Recent work on *Hypericum* suggests that modification of the plant's relationship with mycorrhizal fungi contributed to plant invasion of North America (Seifert et al. 2009).

Although studies of inducible responses in *P. lanceolata* have not controlled for AM fungi, there is variation between tested environments. In previous studies of *P. lanceolata* inducible responses (Adler et al. 1995; Bowers and Stamp 1993; Darrow and Bowers 1999; Fuchs and Bowers 2004; Jarzomski et al. 2000; Stamp and Bowers 1994, 1996, 2000) there was often greater induction of iridoid glycosides in plants likely lacking AM fungi (e.g., in greenhouses) versus environments where AM fungi are likely present (e.g., field settings). While many factors may be responsible for the differences in induction observed between greenhouse and field studies, this result is consistent with our observation that the presence of multiple AM fungal species reduces the induced response in *P. lanceolata*, and may suggest that in environments where AM fungi are common, defense strategies other than induced chemical responses are likely to be important.

Here we show that the presence and identity of arbuscular mycorrhizal fungi play an important role in the production of constitutive *P. lanceolata* chemical defense, as well as the expression of inducible chemical responses. This observation of symbiont control shares similarities with the control of plant defensive chemistry exercised by foliar fungal endophytes (Clay 1988), which has been found to have cascading effects through terrestrial ecosystems (Clay and Holah 1999; Finkes et al. 2006). Unlike the case of fungal endophytes, in which endophytic fungi produce their own compounds, AM fungi manipulate the defensive chemical concentrations already produced by *P. lanceolata*. Nevertheless, given the ubiquity of AM fungal infection in terrestrial communities, the influence of AM fungi on plant defense will likely also have important cascading effects through terrestrial ecosystems, especially given the lack of a trade-off between growth and constitutive defense in *P. lanceolata* (Barton 2007) and that induction of iridoid glycosides has been shown to have broad-spectrum effects against other plant antagonists (Biere et al. 2004). While previous models demonstrate that mycorrhizal modification of plant defense can have profound effects on the dynamics of such plant–herbivore interactions (Bennett et al. 2006), these models have not yet considered the implications of mycorrhizal modification of the induction of plant responses and do not scale up these potential effects through terrestrial food webs. The present study suggests this is an important area of future exploration.

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