Functional Ecology

British Ecological Society

Functional Ecology 2010, **24**, 293–300 doi: 10.1111/j.1365-2435.2009.01647.x

Support from the underground: Induced plant resistance depends on arbuscular mycorrhizal fungi

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Summary

1. Mycorrhizal symbiosis is thought to affect interactions between plants and herbivores by its influence on plant growth, nutrition and the plants defence system. Moreover, arbuscular mycorrhizal fungi (AMF) may enhance the inducibility of resistance responses. Until now, induction of plant resistance has not been considered to be a mechanism affecting the outcome of mycorrhization for plant-herbivore interactions.

2. Here, we test the hypothesis that the resistance of plants against herbivores depends on the induction of plant resistance by previous herbivory and mycorrhization. With a full factorial experiment in a greenhouse, we examined responses in growth of seven herbaceous plant species to AMF and the induction of resistance. To evaluate whether induced resistance is higher in plants with AMF we analyzed the combined effects of AMF and induction on herbivory, using bioassay caterpillars (*Spodoptera littoralis*).

3. Across all species, mycorrhization increased growth of plants and performance of the bioassay herbivore feeding on them. If, however, we induced resistance by allowing a caterpillar to feed for a short period on the plants, mycorrhization did not increase plant growth and performance of a subsequent herbivore that fed on the plant. This suggests that the increased plant resistance after induction was dependent on the symbioses with AMF.

4. Our results indicate that induction interacts with the allocation of the additional resources provided by mycorrhization towards plant growth and plant resistance. Therefore, mycorrhiza may play an important but hitherto overlooked role in the induction of plant resistance against herbivores.

Key-words: aboveground-belowground interactions, arbuscular mycorrhiza, induced resistance, insect herbivory, plant defence, plant-herbivore interactions

Introduction

The symbiosis with arbuscular mycorrhizal fungi (AMF) increases the performance of plants by improving nutrient acquisition, resulting in positive effects on growth and reproduction (Smith & Read 1997). Furthermore, AMF may indirectly affect hosts by mediating interactions with natural enemies, e.g. pathogens and herbivores. These effects include the increase of plant nutritive value, plant quality and plant tolerance as well as changes in the plant defence system (Bennett, Alers-Garcia & Bever 2006). These influences interactively affect plant resistance, e.g. against herbivores. In experiments analyzing the effects of AMF on herbivorous insects the performance of generalists feeding on mycorrhized plants did not increase as one would expect due to the nutritional value but rather decreased (e.g. Rabin & Pacovsky 1985; Gange & West 1994; Gange & Nice 1997; Gange, Bower & Brown 1999; Vicari, Hatcher & Ayres 2002). This suggests that AMF increases plant resistance against herbivores (Gange & West 1994), a mechanism that may also apply to other root symbionts (Kempel, Brandl & Schädler 2009). In contrast, specialist herbivores, which can cope with the defence mechanisms of their host plants, may even benefit from fungal associations (Gehring & Whitham 2002).

Bennett, Alers-Garcia & Bever (2006) suggested that the additional resources provided by mycorrhiza relax the trade-offs between growth, tolerance and defence. However, the effects of mycorrhization on resistance against plant enemies cannot be exclusively explained by an increase in available resources (see Liu *et al.* 2003; Fritz *et al.* 2006). Therefore, a modulation of the plant's ability to defend may occur during

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symbiosis. Recent advances revealed that AMF might genetically precondition the plant for a quicker and more effective activation of defence responses upon attack, a process called priming (Pozo & Azcon-Aguilar 2007). This suggests that herbivore feeding (induction) and mycorrhization on plants interact causing an effect on future resistance. This leads to the prediction that induction should result in particularly negative effects on herbivore performance on mycorrhized plants. Moreover, induction may lead to the allocation of resources to resistance thereby constraining plant growth (Herms & Mattson 1992). Consequently, induction of resistance may counteract the positive effect of mycorrhization on plant growth.

However, we are not aware of experiments, which evaluated the interactive effects of herbivore induction and AMF on plant growth and future herbivory, although such interactions have the potential to explain the observed variability of the effect of AMF on plant-herbivore interactions (reviewed in Gehring & Whitham 2002). Therefore, we designed an experiment to investigate the combined effects of induction and mycorrhization by the fungus *Glomus intraradices* on the growth of a range of herbaceous plants. We further investigated the effects of induction and AMF on the performance of the polyphagous caterpillar (*Spodoptera littoralis*) to analyze whether AMF amplifies induced resistance to herbivory. Only a few studies have used grasses to investigate mycorrhiza-insect interactions (Hartley & Gange 2009). Thus, we used four grasses and three dicotyledonous species to assess these effects across a wider range of possible defence mechanisms. Specifically, we addressed the following questions:

1. Are growth responses of plants to AMF negatively affected by the induction of resistance?
2. Is induced resistance to herbivory higher in mycorrhized plants than in non-mycorrhized plants?

Materials and methods

STUDY SPECIES

We used seeds of the four grass species *Poa pratensis* L., *Festuca rubra* L., *Agrostis capillaris* L. and *Deschampsia flexuosa* (L.) Trin. and the three dicots *Senecio jacobaea* L., *Plantago lanceolata* L. and *Artemisia vulgaris* L. (obtained from Appels Wilde Samen GmbH, Darmstadt/Germany). All species are typical grassland plants and known to be associated with AMF (Data base of 'The Ecological Flora of the British Isles at the University of York'; http://www.york.ac.uk/res/ecoflora). Dicots used in the experiment are known to show induced resistance to herbivory (Fuchs & Bowers 2004; Hol *et al.* 2004; Held & Baldwin 2005), whereas for grasses investigations on the role of chemical defence and its induction focused almost exclusively on crop species (Frey *et al.* 1997; Degenhardt 2009).

We used caterpillars of the herbivore Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) – a polyphagous insect species whose caterpillars are known to feed on plants of at least 40 plant families (Brown & Dewhurst 1975). The extreme polyphagy of these caterpillars allows us to compare leaf quality among plant species from different families (e.g. Hendriks, de Boer & van Groenendael 1999; Schädler *et al.* 2005, 2007; Hendriks, Luijten & van Groenendael 2009). Thereby feeding and performance of caterpillars is used as an integrative and functionally relevant measure of plant resistance (Van Zandt 2007). Furthermore, *S. littoralis* is known to induce plant resistance (Anderson, Jonsson & Morte 2001) and behaves similarly to other generalist herbivores with respect to its susceptibility to plant constitutive and induced resistance (see references in Van Zandt 2007). Caterpillars originated from a lab stock bred on a bean based artificial diet to avoid adaptation of the insects to specific plants.

EXPERIMENTAL DESIGN

The experiment was conducted in a greenhouse with the temperature maintained at 15 to 25 °C, a constant day length of 14 h, and additional light supplied by high-pressure sodium lamps (Philips Son-T Agro, 400 W). At the beginning of February 2007, we surface sterilized seeds of all seven plant species with 1% H_2O_2 and sowed them into seed trays filled with steam-sterilized (100 °C for 4 h) potting soil.

The experimental pots (diameter 9 cm, height 7 cm) were filled with steam-sterilized soil and sand (ratio 1 : 1, v/v). We used soil from an old fallow grassland site on sandstones (Lahnberge near Marburg; Hesse, Germany). To leach nutrients from the soil, which became available during steaming, we irrigated the pots each day with 40 mL of deionised water for 3 days prior to the start of the experiment. This resulted in an initial nitrogen availability of 2·9 mg NH_4^+ kg^{-1} soil and 1·2 mg NO_3^- kg^{-1} soil.

The experiment was set up in a full factorial-design using AMF, induction and herbivory as treatments. All eight possible combinations were replicated five times for each plant species leading to 40 pots per plant species. We randomly assigned pots to five blocks in the greenhouse and randomized pots within the blocks several times during the experiment. We irrigated all pots every 2 days with 50-100 mL of water.

MYCORRHIZA TREATMENT

After 2 weeks, we transplanted the seedlings into the experimental pots. Mycorrhizal inoculum was provided by spreading 2 g of clay granules, containing a mixture of root fragments, spores and hyphae of the generalist fungus *Glomus intraradices* (AMykor, Greppin, Germany) in a layer 4 cm below the soil surface in half of the pots (M+ in Figs 2 and 3). The control plants (M− in Figs 2 and 3) received sterilized clay granules.

We are aware that the natural soil community contains many organisms, which may modulate the effects of mycorrhization with independent effects on plant growth (Barea 1997; Frey-Klett, Garbaye & Tarkka 2007). We decided not to include a mycorrhiza-free soil inoculum to all treatments in our experiment to exclude confounding effects, which may arise through interactions with other soil organisms and may complicate the interpretation of results.

INDUCTION AND BIOASSAY OF RESISTANCE

Gange & West (1994) demonstrated that the fungus needs 8 to 10 weeks for the colonization of roots to have effects on plant growth and herbivores. Therefore, we started the other two treatments after 8 weeks. To induce resistance in plants, in half of the plants of each AMF treatment we allowed one 3rd instar larva of *S. littoralis* (mean

weight 0·034 g) to feed on each plant for 1 day. We enclosed all pots with nylon gauze (200 μm mesh) to prevent escape of the caterpillars.

Direct measurements of the production of plant defence compounds across all experimental plant species and the effects of these compounds on herbivores was beyond the scope of our experiment. The plant species used in our experiments produce a plethora of compounds, which may have very different effects on herbivores, which are furthermore difficult to compare. Moreover, induced plant resistance is defined as a reduced preference or performance of herbivores in response to stress or injury of the plant (Karban & Myers 1989). Therefore, the direct response of herbivores is a more appropriate indicator of plant defence than the measurement of concentrations of secondary compounds (Hamilton *et al.* 2001) and we used caterpillars to assess the effects of mycorrhization and induction on herbivore performance. We call these caterpillars bioassay caterpillars to distinguish them from the induction caterpillars. Immediately after the removal of the induction caterpillar, we added one 3rd instar larva of *S. littoralis* as bioassay caterpillars to half of the experimental pots and allowed them to feed for 5 days. Caterpillars consumed only a small amount of the foliage and therefore available plant biomass was not limiting for the herbivores (see also Results). For unknown reasons only three caterpillars survived on *Poa pratensis*. We therefore excluded this species from further analysis. The other half of the plants were left without herbivory to investigate growth responses of plants to the combined effects of induction and mycorrhization. Those plants grew for a further 4 weeks.

MEASUREMENTS

To quantify the mycorrhizal status of plants, we took root samples from each plant, washed them to remove soil and fixed them in FAA (6% formaldehyde, 2·3% glacial acetic acid, 45·8% ethanol, 45·9% H_2O). We cleared root samples in 10% KOH and stained them with Trypan blue (Phillips & Haymann 1970). We determined mycorrhizal colonization (arbuscules, vesicles, hyphae) with a Leica DMRB microscope using the line intersect method with 300 segments per root sample (Ambler & Young 1977; modified by Schmitz *et al.* 1991). Arbuscules are the major site of nutrient exchange (Cox *et al.* 1975). Therefore, we use arbuscule colonization as a measure of the intensity of the symbiosis.

To estimate the increase in weight of the bioassay caterpillars, we recorded fresh mass of caterpillars before and after feeding. For the herbivore-free treatment, aboveground plant parts were harvested, dried at 70 °C and weighed.

STATISTICAL ANALYSES

We visually checked all data for normality of residuals. Prior to statistical analyses, we arcsine square root transformed percentage data (mycorrhizal colonization). We analyzed the effect of block, induction and species on mycorrhizal colonization using a three-way ANOVA. Where a significant induction × species interaction occurred, we then compared the mean colonization within species using least-square mean contrasts.

We analyzed the effects of block, species, induction and mycorrhiza on aboveground plant biomass using a four-way ANOVA (STATISTICA 6; Statsoft, Tulsa, Oklahoma, USA). The effects of block, species, induction and mycorrhiza on final caterpillar mass were analyzed using an ANCOVA (STATISTICA 6) with initial caterpillar mass as the covariate. We used type I sums of squares to remove the confounding effects of initial caterpillar mass (Raubenheimer & Simpson 1992; Horton & Redak 1993). The effects of block, induction and mycorrhiza on the survival of the bioassay caterpillars we tested using a binary logistic model computed in R 2.5.1 (R Development Core Team, 2007).

To test whether general trends between the functional groups dicots and grasses do exist, we applied a nested ANOVA, with the factor plant species nested in the factor functional group. For this, mean squares of the factor functional group and its interactions were tested against the mean squares of the factor species and its interactions, respectively. In all analyses, we pooled interactions with the factor block in the error term (Newman, Bergelson & Grafen 1997).

Results

MYCORRHIZATION

We found on all plants of the AMF treatment mycorrhizal structures. We found no such structures on roots of the mycorrhizal-free treatment. Arbuscule colonization was strongly related to the total colonization by all mycorrhizal structures ($r = 0{\cdot}92$, $P < 0{\cdot}001$) and the treatment effects on arbuscule colonization as well as on total colonization of roots were consistent. Degree of arbuscule colonization and total colonization differed significantly between plant species ($F_{6,52} = 38{\cdot}9$, $P < 0{\cdot}0001$ respectively $F_{6,52} = 76{\cdot}48$, $P < 0{\cdot}001$). We found a positive effect of induction on the degree of arbuscule colonization ($F_{1,52} = 6{\cdot}88$, $P = 0{\cdot}011$) but not on total colonization ($F_{1,52} = 2{\cdot}03$, $P = 0{\cdot}16$). However, an inspection of the means showed that only in one plant species, Deschampsia flexuosa, did induction have a positive effect on arbuscule colonization: in D. flexuosa induced plants showed higher arbuscule colonization than non-induced plants (Fig. 1, interaction species × induction: $F_{6,52} = 4{\cdot}25$, $P = 0{\cdot}002$). Similarly, total colonization was higher in induced plants of D. flexuosa than in non-induced plants (interaction species × induction: $F_{6,52} = 2{\cdot}41$, $P = 0{\cdot}039$).

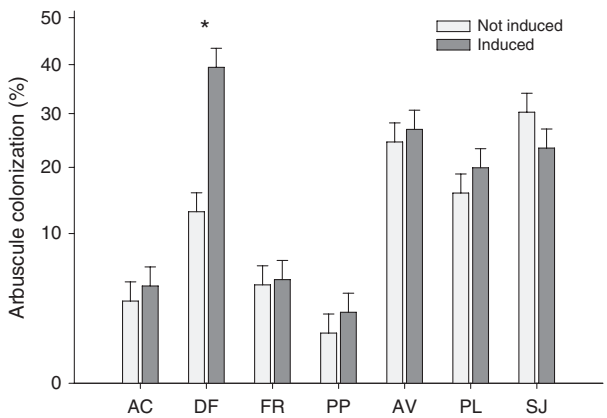

Fig. 1. Percentage arbuscule colonization on non-induced and induced plants of the species *Agrostis capillaris* (AC), *Deschampsia flexuosa* (DF), *Festuca rubra* (FR), *Poa pratensis* (PP), *Artemisia vulgaris* (AV), *Plantago lanceolata* (PL) and *Senecio jacobaea* (SJ). Note that the *y*-axis is arcsine square-root transformed. The asterisk indicates a significant difference after contrasting the means within species (least-square means contrasts, $P < 0{\cdot}05$).

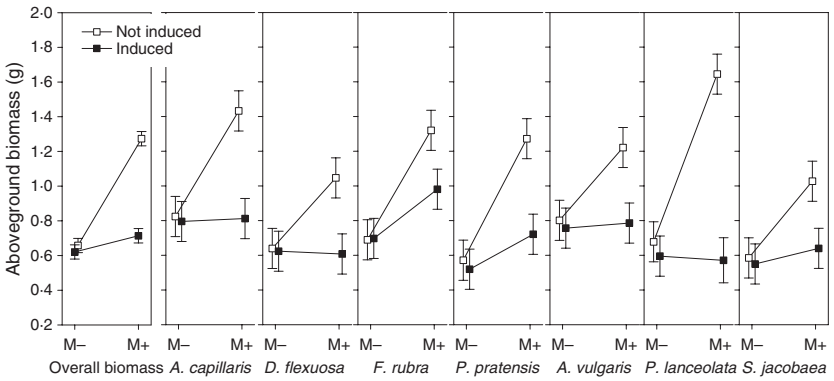

Fig. 2. The interacting effects of mycorrhiza and induction on aboveground dry biomass of the investigated plant species and across all species (overall). Values are means with standard error.

ABOVEGROUND PLANT BIOMASS

Without herbivores, AMF increased aboveground plant biomass by 87% (Table 1, Fig. 2). However, this increase in biomass disappeared for plants in the induction treatment (Fig. 2, highly significant mycorrhiza × induction interaction, Table 1). We did not observe such an effect for plants without AMF suggesting that induction of plants with AMF leads to a shift of resources from aboveground growth to resistance. We found no significant species × induction interaction (Table 1). Therefore patterns were consistent across plant species and did not differ between functional types of plants (dicots and grasses; nested ANOVA, plant functional type (T): $F_{1,5} = 0{\cdot}25$, $P = 0{\cdot}64$; T × mycorrhiza: $F_{1,5} = 0{\cdot}44$, $P = 0{\cdot}54$; T × induction: $F_{1,5} = 0{\cdot}65$, $P = 0{\cdot}47$; T × mycorrhiza × induction: $F_{1,5} = 0{\cdot}71$, $P = 0{\cdot}43$).

PERFORMANCE OF BIOASSAY CATERPILLAR

The biomass of the caterpillars was far smaller than the available plant biomass (see Fig. 2, 3). Therefore, feeding was never limited by the availability of plant tissue (note that plant biomass is given as dry mass whereas caterpillar mass represents fresh mass). Caterpillar mortality was not affected by induction and mycorrhization (logistic regression, all effects $P > 0{\cdot}1$). Overall, if plants were not induced by previous herbivory, the association with mycorrhiza significantly increased caterpillars' mass (corrected for initial caterpillar mass; Table 1). However, this positive response to AMF disappeared on induced plants (Fig. 3, significant mycorrhiza × induction interaction, Table 1). Therefore, induction reduced only the growth of caterpillars feeding on plants with AMF, whereas there was no effect of induction on the growth of caterpillars feeding on plants without AMF (Fig. 3).This pattern was consistent across all plant species (Fig. 3), as well as across and within the functional groups (nested ANOVA, all $P > 0{\cdot}1$).

Discussion

We demonstrate that the effect of mycorrhization on aboveground growth of plants and herbivore performance depends on induction. Our results suggest that the symbiosis of plants with AMF is an important trigger of induced resistance by shifting resource allocation from aboveground growth towards resistance upon herbivore attack.

POSITIVE EFFECT OF MYCORRHIZA ON PLANT BIOMASS AND HERBIVORE PERFORMANCE ON NON-INDUCED PLANTS

The presence of AMF resulted in an almost twofold increase in aboveground biomass of plants. This supports other studies demonstrating positive effects of AMF on plant growth when nutrient availability is scarce (e.g. Smith & Read 1997; van der Heijden 2002). Further, mycorrhization resulted in a significant increase in caterpillar growth of the polyphagous

Table 1. Results of the ANOVA (aboveground dry plant biomass) and the ANCOVA (caterpillar growth) for the effects of block, mycorrhiza, induction and plant species on aboveground dry plant biomass and final caterpillar fresh mass. Significance levels of the effects are denoted with $*P < 0{\cdot}05$, $**P < 0{\cdot}01$ and $***P < 0{\cdot}001$

	Aboveground plant biomass			Caterpillar growth		
Source of variation	d.f.	Mean squares	*F*-values	d.f.	Mean squares	*F*-values
Initial caterpillar mass	-	-	-	1	0·011	3·64*
Block	4	0·18	2·66*	4	0·019	6·18***
Mycorrhiza (M)	1	4·01	59·97***	1	0·019	6·15*
Induction (I)	1	2·97	44·45***	1	0·031	9·84**
Plant species (S)	6	0·21	3·12**	5	0·023	7·27***
M × I	1	2·29	34·23***	1	0·012	3·98*
M × S	6	0·07	1·03	5	0·003	0·87
I × S	6	0·09	1·34	5	0·003	1·16
M × I × S	6	0·06	0·93	5	< 0·001	0·19
Residuals	107	0·07		73	0·003	

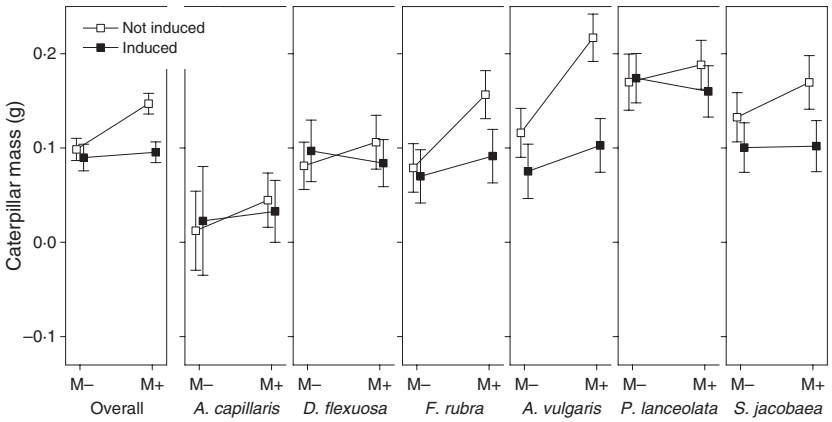

Fig. 3. The interacting effects of mycorrhiza (M−/M+) and induction on final caterpillar mass (corrected for initial caterpillar mass) of the caterpillar *S. littoralis* feeding on the investigated plant species and across all species (overall). Values for *Poa pratensis* were not included since the herbivore failed to establish on this species. Values are means with standard error.

caterpillar *S. littoralis* on plants not treated by an induction caterpillar. We suppose that the efficient uptake of nutrients by plants with AMF improved food-quality for the herbivore. A number of studies have already shown that an association with mycorrhizal fungi affects interactions between plants and their enemies in a variety of ways (reviewed in Borowicz 2001; Gange, Bower & Brown 2002; Gehring & Whitham 2002; Bennett, Alers-Garcia & Bever 2006; Gange 2007). Moreover, genotypic variation of both plants and AMF may affect the influence on herbivores and plant growth (reviewed in Hartley & Gange 2009). Nevertheless, the available information suggests that the effect of AMF on herbivores depends on the susceptibility of the herbivore to secondary plant compounds. Specialists that can cope with the defensive compounds of their host, and sap-feeding herbivores seem to benefit from the fungal association. In contrast, studies analyzing interactions between AMF and leaf-chewing, generalistic herbivores often revealed negative effects of the fungi on the herbivore (Gange & West 1994; Rabin & Pacovsky 1985; reviewed in Gehring & Whitham 2002). In those studies, the negative response was attributed to changes in the carbon/nitrogen ratio, which was increased on mycorrhized plants due to higher rates of photosynthesis, thereby favouring the production of carbon-based defensive compounds (Bryant, Chapin & Klein 1983). In contrast, our experiments showed in general positive effect of AMF for the herbivore, which was also found by Hoffmann *et al.* (2009). Therefore, we conclude that the direction and magnitude of the effect of AMF on herbivores depends on the net influence of increased nutrient uptake by the plant as well as changes in the production of defence compounds. For instance, on nutrient poor soil (as in our study) the positive effects of mycorrhiza on plant nutritive quality may be the prevailing influence on herbivore performance at least for non-induced plants. Further experiments are needed to highlight the interacting effects of nutrient supply and effects of AMF on herbivores.

NO POSITIVE EFFECT OF MYCORRHIZA ON PLANT BIOMASS AND HERBIVORE PERFORMANCE ON INDUCED PLANTS

The increase in aboveground plant biomass due to mycorrhizal colonization disappeared with induction. This pattern was consistent across all plant species and did not differ between dicots and grasses. Since aboveground biomass of plants without AMF was not reduced by the induction treatment, the decrease of biomass in the induced plants with AMF is not due to removal of biomass during the short period of feeding by the induction caterpillar. Therefore, feeding by the induction caterpillar seemed to control the allocation of the additional resources provided by the AMF. Growth of plants with and without AMF at the time when we added the induction caterpillar was very similar. This explains why aboveground biomass of induced plants is not higher with AMF than without, suggesting that AMF did not substantially affect plant growth during the 8 weeks prior to the induction treatment. This corresponds well with the data of Gange & West (1994), who demonstrated that a period of 2 months is necessary for mycorrhizal colonization and effects on plant growth and herbivore feeding.

Similarly, previous feeding by the induction caterpillar for only 24 h was sufficient to impede positive effects of mycorrhization on the performance of a subsequently feeding herbivore. This supports findings of Viswanathan, Lifchits & Thaler (2007), where responses induced by the initial herbivore made the plants less responsive to subsequent attack. When we placed bioassay caterpillars on the plants they encountered either 'good' or 'bad' (induced) plant tissue. These different initial conditions may be crucial for larval development (Zalucki *et al.* 2001).

Investment in anti-herbivore defences constrains the use of resources for growth and reproduction (reviewed in Bergelson & Purrington 1996; Koricheva 2002; Strauss *et al.* 2002). Although we did not measure the production of defence compounds, our study demonstrated a decrease in plant biomass as well as a decrease in herbivore performance in plants with AMF plants after induction. In contrast, induction had no effect on the growth of plants or the bioassay herbivores feeding on them in plants without AMF. The effects of induction are therefore not due to removal of meristematic or nutritious tissues of the plant by the herbivore. Rather, our result points to an increased allocation of resources to resistance in plants with AMF.

If we consider only non-induced plants, the increase of herbivore performance on plants with AMF may also be interpreted as a suppressed investment of resources to

resistance with AMF. However, performance of herbivores on induced plants with AMF was similar when compared to induced plants without AMF. This suggests that either the lower investment to resistance in plants with AMF is compensated by induction or that induced resistance in plants with AMF is compensated by the additional supply of nutrients by fungi. The existing literature, however, does not point to a suppression of plant resistance to herbivores with mycorrhization (Hartley & Gange 2009). We therefore regard the induction of resistance in plants with AMF as the more likely explanation for the patterns found during our experiment.

Our experiments do not provide information for other components of plant investment, e.g. reproduction and root growth. Reproduction did not occur during our experiment. Furthermore, roots were used for the assessment of mycorrhization and therefore were not weighed. Further experiments are needed to study the interactions of mycorrhization and induction on trade-offs involving reproduction as well as root/shoot ratios.

Herbivory may impair mycorrhizal colonization as a result of the consumption of photosynthetic tissue leading to a limited carbon transfer of the plant to the fungi (reviewed in Gehring & Whitham 1991; Gange 2007). However, we could not observe a general effect of induction on the degree of mycorrhizal colonization (except in *D. flexuosa* where mycorrhization even increased with induction). We suspect that the short time of induction was not sufficient to create an important sink for carbon. Moreover, herbivore-induced exudation of carbon-based compounds of plants in the rhizosphere may explain the positive response of mycorrhizal fungi to induction in *Deschampsia flexuosa* (reviewed in Gange 2007). However, the decrease in plant and herbivore performance in our study was independent from the degree of mycorrhizal colonization.

INDUCED RESISTANCE– DEPENDENT ON MYCORRHIZA, NUTRIENTS OR BOTH?

Remarkably, the increase of plant resistance to herbivory caused by induction was only found in plants with AMF in our experiment. Although at present our understanding of the mechanisms behind this mycorrhiza-constrained shift in resource allocation is limited, we nevertheless suggest two possible explanations. Firstly, low availability of nutrients in the soil used in our experiments may have constrained the ability of plants without AMF to direct resources to resistance. AMF increase resource availability for the plant, thereby enabling a shift in resource allocation towards anti-herbivore defences. However, studies including nutrient-supplemented controls showed that an enhanced resistance of plants with AMF at least against pathogens cannot be explained exclusively by an improved nutritional status of the plant, suggesting similar effects for insect herbivores (Liu *et al*. 2003; Fritz *et al*. 2006). Therefore, the importance of AMF in resistance against pathogens and herbivores may sometimes exceed their importance in plant nutrition (see e.g. Newsham, Fitter & Watkinson 1995). Secondly, the maintenance of the mutualistic association between AMF and plants may activate defence mechanisms against natural enemies (Garcia-Garrido & Ocampo 2002). Pozo & Azcon-Aguilar (2007) reviewed evidence that the effect of mycorrhization on induction of plant resistance is triggered by the activation of the plant's defence system and the expression of defence-related genes.

Our results indicate that the effects of mycorrhizal fungi on plant-herbivore interactions interferes with trade-offs in the plant's ability to allocate resources to plant quality, growth and defence (Bennett, Alers-Garcia & Bever 2006). Based on current advances in the understanding of the role of mycorrhiza as a trigger of induced defences (summarized in Pozo & Azcon-Aguilar 2007) our findings also point to the importance of AMF for the induction of plant resistance against herbivores. Future experiments should disentangle the relative effects of AMF as a nutrient provider or as an activator of defence related genes.

INDUCED RESISTANCE EXISTS IN GRASSES AND DICOTS

One important result of our experiment is that grasses did not differ from dicots in their response to mycorrhization and induction. Compared to dicots the role of chemical defences in grasses is supposed to be low (but see Frey *et al*. 1997). Grasses react to herbivory by regrowth and physical defences such as silica (Vicari & Bazely 1993; see Massey, Ennos & Hartley 2007a). Nevertheless, repeated damage by herbivores increases the silica content of grasses and seems to induce other chemical responses (McNaughton *et al*. 1985; Massey, Ennos & Hartley 2007a; Keeping & Kvedaras 2008). This may have substantial costs for the growth of grasses (Ma *et al*. 2006; Massey, Ennos & Hartley 2007b), and may lead to a reduction in the performance of herbivores (Massey & Hartley 2009). However, we are not aware of any study demonstrating increased silica levels after induction within a few days. In addition, just like dicots grasses may also exhibit considerable levels of chemical defence, e.g. phenolics (Massey, Ennos & Hartley 2007b). Maier *et al*. (1995) showed that AMF increased the levels of terpenoid glycosides in roots of cereal grasses. Furthermore, induction of indirect chemical defence responses (volatiles) is well documented for grasses (Degenhardt 2009).

Endophytic fungi often infect grasses and these endophytes may contribute to resistance against herbivores (Hartley & Gange 2009 for review). In the only available study on the effects of mycorrhization on an endophytic fungus, Mack & Rudgers (2008) found no effects of AMF on infestation by the endophyte in a grass. Of course, we are not able to rule out certain effects of AMF on the production of secondary compounds by endophytes in our experiments, but such a production is not able to explain the interacting effects of AMF and induction. Therefore, our findings that AMF enhances induced plant resistance to herbivory seem to be fundamental rather than group specific.

Conclusions

Whereas mycorrhization *per se* may positively affect plant growth and the performance of a generalist herbivore, this effect was shown to be cancelled out by the induction of plant resistance by previous feeding, whereas induction on plants without AMF had no effect on plant biomass or herbivore performance. Mycorrhization increases the effectiveness of inducible resistance at the cost of plant growth. Therefore, our results point towards an important role of AMF during induction of plant resistance against herbivores. We showed that these patterns exist across a range of dicots and grasses and seem to be a fundamental component in the defence system of plants. The mechanisms behind these effects may include trade-offs in resource allocation patterns as well as in signalling pathways and their priming by mycorrhiza. Further investigations are required, especially regarding simultaneous effects on plant growth and plant quality. Since mycorrhizal symbiosis dates back to the colonization of land ecosystems, mycorrhizal fungi may have been an important but overlooked factor during the evolution of plant defences.

Acknowledgements

We kindly thank Angelika Spindler (AMykor, Greppin, Germany) for providing the mycorrhizal inoculum and useful comments. We further thank Oliver Bossdorf, Markus Fischer and two anonymous referees for commenting on earlier versions of the manuscript.

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Received 3 March 2009; *accepted 11 August 2009*
Handling Editor: John Klironomos