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PLANT-ANIMAL INTERACTIONS - ORIGINAL PAPER

Specific bottom–up effects of arbuscular mycorrhizal fungi across a plant–herbivore–parasitoid system

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Abstract The majority of plants are involved in symbioses with arbuscular mycorrhizal fungi (AMF), and these associations are known to have a strong influence on the performance of both plants and insect herbivores. Little is known about the impact of AMF on complex trophic chains, although such effects are conceivable. In a greenhouse study we examined the effects of two AMF species, *Glomus intraradices* and *G. mosseae* on trophic interactions between the grass *Phleum pratense*, the aphid *Rhopalosiphum padi*, and the parasitic wasp *Aphidius rhopalosiphi*. Inoculation with AMF in our study system generally enhanced plant biomass (+5.2%) and decreased aphid population growth (−47%), but there were no fungal species-specific effects. When plants were infested with *G. intraradices*, the rate of parasitism in aphids increased by 140% relative to the *G. mosseae* and control treatment. When plants were associated with AMF, the developmental time of the parasitoids decreased by 4.3% and weight at eclosion increased by 23.8%. There were no clear effects of AMF on the concentration of nitrogen and phosphorus in plant foliage. Our study demonstrates that the effects of AMF go beyond a simple amelioration of the plants' nutritional status and involve rather more complex species-specific cascading effects of AMF in the food chain that have a strong impact not only on the performance of plants but also on higher trophic levels, such as herbivores and parasitoids.

Keywords *Aphidius rhopalosiphi* · Insect herbivory · Multitrophic interactions · Parasitoid performance · *Rhopalosiphum padi*

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Introduction

Multitrophic interactions between above- and belowground organisms are powerful forces shaping the structure and diversity of natural communities (van der Putten et al. 2001). For example, belowground herbivores can influence aboveground herbivores via a shared host plant and vice versa (van Dam et al. 2003; Wurst and van der Putten 2007). One interaction that has been found to affect the performance of both above- and belowground organisms is the symbiosis between plants and arbuscular mycorrhizal fungi (AMF; Bennett et al. 2006; Bezemer and van Dam 2005; Gehring et al. 2002). The infection of plants by AMF affects the interactions of the former with root pathogenic fungi (Newsham et al. 1995), Collembola (Gange 2000), saprotrophic fungi (Tiunov and Scheu 2005), above- and belowground herbivores (Gange 2001; Goverde et al. 2000), and parasitic plants (Stein et al. 2009).

Aphids, as one guild of herbivores directly feeding on plant phloem, can be influenced by AMF colonizing the roots of their host plants (e.g., Gange et al. 1999; Guerrieri et al. 2004; Wurst et al. 2004), but the direction of the effects have varied between different experiments. While Gange and West (1994) and Gange et al. (1999) found a positive influence of AMF on weight and fecundity of two *Myzus* species reared on *Plantago lanceolata*, negative AMF effects were reported with *Chaitophorus populicola* reared on *Populus angustifolia* × *P. fremontii* (Gehring and Whitham 2002) and *Macrosiphum euphorbiae* reared on *Lycopersicon esculentum* (Guerrieri et al. 2004). One possible explanation for this inconsistency in results may be the variability of arbuscular mycorrhizal symbiosis itself, which ranges from mutualism to parasitism depending on various biotic and abiotic factors (Johnson 1993; Klironomos 2003). In addition, infection by different AMF species can have different effects on several plant traits, such as biomass or nutrient capture (van der Heijden et al. 1998). There are also indications that AMF infection of plants can have cascading effects in the food chain up to higher trophic levels (Gange et al. 2003). For example, there is evidence that AMF symbioses with plants can affect both the rate of aphid parasitism by parasitoid wasps (Gange et al. 2003) and parasitoid preference, where aphid infested non-mycorrhizal plants are as attractive to parasitoid wasps as uninfested mycorrhizal plants (Guerrieri et al. 2004). However, both of these studies did not directly assess parasitoid performance, although it is likely that the strong effects of AMF reported on primary producers (plants) and primary consumers (herbivores) cascade upwards in the food chain and thus also affect several traits in predator or parasitoid performance, such as food consumption or reproductive output (Bezemer et al. 2005).

The objective of this study was to test AMF species effects on the tri-trophic interaction of a typical grassland plant species (*Phleum pratense*), its insect herbivore *Rhopalosiphum padi* L., and the parasitoid *Aphidius rhopalosiphi*. In a greenhouse experiment, the grass was inoculated with either one of the two AMF species, *Glomus intraradices* or *G. mosseae*, and the results compared to a non-mycorrhizal control. These three treatments were combined with three insect treatments: (1) plants only (no insects), (2) plant + aphid, and (3) plant + aphid + parasitoid.

We hypothesized that:

1) the association with AMF improves plant biomass and nutrient capture;
2) there is an increase in food quality which benefits aphid reproduction and supports larger aphid populations on mycorrhizal plants;
3) larger aphid populations allow female parasitoids to choose more suitable aphids for parasitization, which leads to an increase in parasitoid weight and a decrease in parasitoid development time; and that
4) the two AMF species have different effects on the tri-trophic interaction.

Materials and methods

Plant, aphid, and parasitoid material

Plant seeds and soil were collected from a hay meadow in the Franconian Forest in Central Germany (11°26′44″E/50°23′04″N). We collected seeds from *Phleum pratense* L. (timothy grass) in the summer and autumn of 2006. *Phleum pratense* is common in European grasslands and an important grass for hay production. The substrate used in the experiment consisted of 50% sieved soil (1 cm) collected from the top 10 cm of the field site and 50% washed silica sand. The substrate was heated for 48 h at 200°C to kill soil organisms, including AMF. Pre-experimental soil analyses showed soil nutrient contents of 0.48% organic carbon (C), 0.1% nitrogen (N), and 36.85 μg g^{-1} plant available phosphorus (P) at a pH (H_2O) of 6.6.

Inoculum of two AMF species, *Glomus intraradices* N.C. Schenck & G.S. Sm. isolate BEG140 and *G. mosseae* (T.H. Nicolson & Gerd.) Gerd. & Trappe isolate BEG25, were purchased as two separate mixtures of spores and mycorrhizal roots from a commercial supplier (SYMbio-M, Lanškroun, Czech Republic). Both isolates have been widely used in greenhouse experiments, and both species are commonly found in grasslands (Hempel et al. 2007; Rosendahl and Stukenbrock 2004).

Aphids (*R. padi* L., cherry oat aphid) were purchased from Katz Biotech AG (Bayreuth, Germany) and propagated

on wheat (*Triticum aestivum* L.). *Rhopalosiphum padi* has been shown to be compatible with *Phleum pratense* (Orlob 1961) and is widely used in greenhouse experiments (Ponder et al. 2000; Vestergård et al. 2004). We chose the parasitoid wasp species *Aphidius rhopalosiphi* (DeStefani-Perez), which is a natural enemy of *R. padi* (e.g. Gonzáles et al. 1999) commonly occurring throughout Northern Europe (Muratori et al. 2004). Wasps were bought as mummies (Katz Biotech AG) and allowed to hatch and mate. After 2 days, the wasps were anaesthetized with CO_2 and sorted according to sex. Single female wasps between 2 and 4 days old were then introduced into the parasitoid treatments.

Experimental set-up

The experiment was set up in ten blocks in the greenhouse in a full randomized block design. Three mycorrhizal treatments (non-mycorrhizal control, inoculation with *G. intraradices* or with *G. mosseae*) were combined with the three insect treatments (no insects added, aphids added, or aphids and female parasitoid added). These nine treatments were replicated 20 times, resulting in 180 pots in total. Two plants from each of the nine treatments were randomly assigned to each block.

Each 1-l pot (height 13.5 cm, diameter approx. 10 cm) was filled with 2 cm expanded slate and 1 cm washed sand for drainage. Pots were then filled with sterile substrate, with the mycorrhizal inoculum placed 1 cm below the surface. Each third of the pots received 15 g inoculum of either *G. intraradices*, *G. mosseae* or an autoclaved mixture of both (non-mycorrhizal control). To establish a natural microbial community, we irrigated all pots with 10 ml soil suspensions from the field soil filtered through a Whatman filter paper No. 4 with pore sizes of 20–25 µm (Whatman International, Kent, UK) to exclude AM propagules from the suspension (Schroeder and Janos 2004). A bulk seed collection of *Phleum pratense* was germinated in sterile substrate. After 2 weeks, one seedling was planted into each pot and its height recorded as initial plant size. Temperatures in the greenhouse ranged from 18°C (14-h day) to 13°C (10-h night) with additional light provided by 400 W lamps. Plants were watered three times a week with tap water.

The plants were cut 2 cm above the soil surface 15 and 21 weeks after planting to mimic the mowing regime of the grassland the plants originated from. This time period also provided the plants with enough time to establish mycorrhizal symbiosis. One week after the second cut, five *R. padi* instars (3–5 days old) were added to the respective treatments (120 pots) using a fine brush. All pots were encaged in air-permeable cellophane bags (width 185 mm, length 390 mm). Twenty-five days after the aphids had been introduced, single females of *Aphidius rhopalosiphi* were introduced into the parasitoid treatments (60 pots) and allowed to parasitize aphids for 12 h during daytime, after which they were removed from the cellophane bags. Plants were harvested 2 weeks after introduction of the parasitoids (i.e., 39 days after aphid introduction), when visible mummies had developed. The shoots were cut at the soil surface, and aphids and mummies were carefully separated from plant material.

Plant measurements

Plant roots were washed free of soil, and a 2-g root aliquot from each pot was stored in formaldehyde–acetic acid (FAA: aqueous solution of 6.0% formaldehyde, 2.3% glacial acetic acid, 45.8% ethanol, all v/v). Root subsamples stored in FAA from five pots of each mycorrhizal and control treatment were stained in lactophenol blue solution according to Phillips and Hayman (1970), with modifications of Schmitz et al. (1991). We studied 300 stained root segments under a light microscope at 200× magnification using the line intersect method (Brundrett et al. 1996) and detected mean mycorrhizal colonization rates of 42 and 21% in the *G. intraradices* and *G. mosseae* treatments, respectively. The AMF structures were absent in the control treatment.

Above- and belowground plant material was dried at 60°C for 48 h and then weighed. Phosphorus concentrations and total N and C content from five plants in the mycorrhizal and control treatments were also determined using plant material ground in a ball mill. The P concentrations were analyzed with a CIROS ICP spectrometer (SPECTRO Analytical Instruments, Kleve, Germany) following combustion of the subsamples at 550°C and dissolution of the ash with 4 *N* nitric acid. Total N and C contents were measured with an Elementar Vario EL element analyzer (Elementar Analysengeräte, Hanau, Germany).

Aphid and parasitoid measurements

The numbers of aphids per plant were determined 11 days after the aphids had been added to the plant system in order to monitor the establishment of their populations. No counts were carried out thereafter during the experiment to avoid aphid disturbance (Godfray 1994). Aphids, winged aphids and mummies were counted at the end of the experiment (day 39). Mummies were placed singly into gelatine capsules and put in a growth chamber (16/8-h light/dark photoperiod, with 22:12°C day/night temperatures, 50% relative humidity) until emergence. Capsules were checked three times per day. Freshly hatched wasps were immediately frozen and their developmental time recorded until all wasps had emerged 1 week after the end of the experiment (day 46). Wasps were sexed, dried at 60°C for 24 h, and weighed. For each mummy, we determined whether the

aphids were adult at mummification using the shape of the cauda as a criterion (Minks and Harrewijn 1987).

Data analysis

Calculations were carried out with JMP ver. 7 (SAS Institute, Cary, USA), with a few exceptions (given below). One plant inoculated with *G. mosseae* and one control plant died during the experiment and were excluded from the analysis. As mortality caused our data to be unbalanced, we used type III sum of squares (Shaw and Mitchell-Olds 1993). Data on initial plant size, plant dry weights, and numbers of aphids were log transformed, and proportion data (sex ratio and proportion of adults among mummies) were arcsine-square root transformed to achieve normal distribution. The combined effects of mycorrhizal treatments and aphids on shoot and root biomass were analyzed in separate analyses of co-variance (ANCOVA). Initial plant size was used as a covariate; block, aphid presence, AMF, and the interaction of the latter two were used as main effects in both analyses. Additionally, using orthogonal contrasts, we tested the following two initial hypotheses: (1) plants perform better with AMF than without—"control versus AMF" and (2) plants are differently affected by the two AMF isolates—"*G. intraradices* versus *G. mosseae*". These contrasts were also calculated on the level of aphids and parasitoids (see below). In addition, we compared root and shoot biomass of aphid-infested and uninfested plants within each mycorrhizal treatment using orthogonal contrasts. Plant C, N, and P content data were compared in a one-way analysis of variance (ANOVA) between the fungal and control treatments.

To test for possible effects of mycorrhizal treatments on aphid population establishment (i.e., the number of aphids detected 11 days after adding), we used an ANCOVA with initial plant size as the covariate and block and AMF treatment as main effects. According to this analysis, initial aphid population establishment was independent of mycorrhizal treatments ($F_{2,107} = 2.33$; $P = 0.10$). It is conceivable that some of the released aphids were not able to localize adequate feeding sites on the plants in time and thus died due to starvation. Therefore, we used the result of our counting exercise 11 days after the addition of aphids to the plant system as a starting point and excluded all pots in the aphid treatments showing no aphids at this time point from further analysis. Aphid population growth rates per day were calculated between day 11 and 39 (harvest) for each pot. The impact of AMF treatments on aphid population growth rates was analyzed using an ANCOVA. We included initial plant size as the covariate, and block, parasitoid presence, and AMF were used as main effects. As the population growth rates were negative in one third of all pots, we analyzed the AMF treatment effect on the proportion of pots with this negative growth pattern. We used an analysis of deviance with quasi *F*-statistics, binomial error distribution, and logit link function with the same covariate and main effects as for aphid population growth rates. This model fits our data reasonably well, as indicated by the goodness-of-fit statistics (model deviance = 85.0, $df = 75$, $P = 0.2$).

We calculated the average parasitoid dry weight and the average development time for each pot. The impact of AMF inoculation on parasitoid dry mass and development time was then assessed using ANCOVA. To account for the highly variable number of mummies in each pot, we used this number as a weighting factor in the ANCOVA. Blocks were poorly replicated due to the extinction of aphid populations on some plants and therefore excluded from the analyses. We used the number of aphids present on the respective plant at harvest as the covariate and parasitoid sex ratio together with AMF as the main factors in the analysis. Aphid numbers and parasitoid sex ratio are very likely to have an influence on the dry weight and developmental time of parasitoids; as in larger aphid populations, ovipositions can be made in more suitable aphid stages, and male parasitoids are usually smaller and develop faster (Sequeira and Mackauer 1992). We also used ANCOVAs to separately analyze the impact of the different mycorrhizal inoculations on sex ratio and the number of adults among parasitized aphids, using the number of mummies as a weighing factor, the number of aphids as the covariate, and the AMF treatment as the main effect.

To test for the impact of AMF inoculation on rates of parasitism, we used major axis (MA) regression (SMATR ver. 2.0, Falster et al. 2006). Major axis regression is an appropriate method for evaluating the association between variables that have been measured with error, and where error variances are unknown, but expected to be within similar dimensions (Sokal and Rohlf 2003). With the algorithms given in SMATR we also compared intercept and slope between the MA regression of each mycorrhiza treatment to test for changes in the rates of parasitism and aphid density-dependent reactions of parasitoids, respectively.

Results

Plant responses

Shoot biomass at harvest increased due to AMF inoculation (Fig. 1a), with orthogonal contrasts showing significant differences compared to control plants; however, there were no differences between the two AMF species (Table 1). A similar pattern was found for root biomass (Fig. 1b), which showed an even stronger mycorrhizal effect (Table 1).

Aphids had a significant negative impact on shoot biomass, with a reduction of 14.2, 10.3, and 5.2% in the

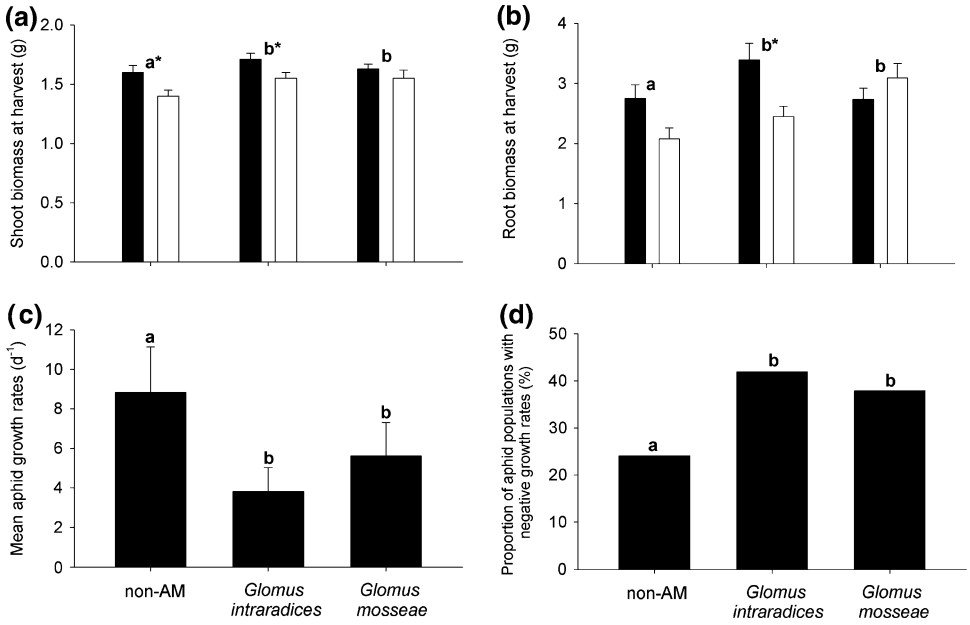

Fig. 1 Mean shoot biomass (**a**) and mean root biomass (**b**) at harvest. *Closed bars* without aphids, *open bars* with aphids. **c** Aphid population growth rates per day, **d** proportion of aphid populations with negative growth rates. *Different letters above bars* indicate a significant difference between arbuscular mycorrhizal fungal (*AMF*) treatments, *asterisks* indicate a significant difference between aphid-infested and -uninfested plants within the AMF treatments ($P < 0.05$) according to orthogonal contrasts, *whiskers* standard error

Table 1 Results of ANCOVA on shoot and root biomass of *Phleum pratense* at harvest

Source of variation	*df*	Shoot biomass			Root biomass		
		MS	*F* value	*P* value	MS	*F* value	*P* value
Initial plant size	1	0.09	3.21	0.08	0.63	4.13	0.04*
Block	9	0.10	3.34	<0.001*	0.92	6.06	<0.001*
Aphid presence	1	0.43	14.57	<0.001*	0.61	3.99	0.05*
AMF	2	0.12	3.91	0.02*	1.04	6.84	0.001*
AMF vs. control	1	0.21	6.94	0.009*	2.07	13.60	<0.001*
Intra vs. moss	1	0.03	0.86	0.36	0.01	0.10	0.76
Aphid presence × AMF	2	0.01	0.47	0.62	0.53	3.48	0.03*
Residuals	162	0.03			0.15		

* Significant at $P < 0.05$

AMF, Arbuscular mycorrhizal fungi; ANCOVA, analysis of covariance

Orthogonal contrasts are calculated for AMF treatments (see Materials and methods for details)

control, *G. intraradices*, and *G. mosseae* treatments, respectively (Fig. 1a). The ANCOVA showed a significant effect of the interaction between AMF and aphids on root biomass. This reflects the increase in root biomass (+12.8%) in the *G. mosseae* treatment under aphid presence (Fig. 1b), whereas root biomass was strongly reduced under aphid presence in the control (−32.3%) and *G. intraradices* (−38.3%) treatments, respectively.

Plant C, N, and P contents are given in Table 2. The values varied slightly between the fungal treatments, but ANOVA showed no significant differences.

Aphid population development

Aphid population growth rates were significantly smaller in the AMF-inoculated plants (growth rate per day ± SE: 2.5 ± 0.8 and 3.5 ± 1.2 for *G. intraradices* and *G. mosseae*, respectively) compared to control plants (6.2 ± 1.6, Fig. 1c; Table 3). No difference between the effect of the two AMF species was found in aphid population growth rates. According to the analysis of deviance, aphid populations with negative growth rates were also more frequent on mycorrhizal plants (42 and 38% for *G. intraradices* and *G. mosseae*, respectively) than on non-mycorrhizal plants (24%; Fig. 1d; Table 3). Parasitoid presence had no effect on aphid numbers at harvest.

We detected winged aphids on two pots in the non-mycorrhizal control only, with four and one winged aphid, respectively.

Parasitoid responses

Emerging parasitoid wasps were significantly heavier when they developed on plants of the two AMF treatments (mean

Table 2 Mean foliar nutrient concentrations and element ratios of five randomly chosen plants

Foliar nutrients	Control	*Glomus intraradices*	*Glomus mosseae*	*F* value	*P* value
C (mg g^{-1})	417 ± 2.6	410 ± 1.0	414 ± 1.0	3.78	0.06
N (mg g^{-1})	23.5 ± 1.9	23.4 ± 1.6	21.4 ± 1.1	0.53	0.60
P (mg g^{-1})	4.15 ± 0.27	4.25 ± 0.07	3.73 ± 0.15	1.79	0.21
C/N ratio	18.3 ± 1.49	17.8 ± 1.13	19.6 ± 1.05	0.87	0.44
N/P ratio	5.6 ± 0.2	5.5 ± 0.3	5.7 ± 0.2	0.25	0.78

Values are given as the mean ± standard error (SE)

There were no significant differences due to AMF inoculation

Table 3 Results of ANCOVA on aphid population growth rates and of the analysis of deviance on the proportion of aphid populations with negative growth rates

Source of variation	*df*	Aphid population growth rates			Populations with negative growth rates	
		MS	*F* value	*P* value	Log likely-hood ratio	*P* value
Initial plant size	1	23.00	0.27	0.61	3.12	0.07
Block	9	154.11	1.78	0.09	23.46	0.005*
Parasitoid presence	1	71.35	0.82	0.37	0.16	0.69
AMF	2	280.84	3.24	0.04*	6.01	0.05*
AMF vs. control	1	491.36	5.67	0.02*	45.47	0.01*
Intra vs. moss	1	60.53	0.70	0.41	42.50	0.90
Residuals	75	86.59				

* Significant at $P < 0.05$

Orthogonal contrasts are calculated for AMF treatments (see Materials and methods for details)

dry weight 53.7 ± 3.9 and 54.0 ± 2.6 µg for *G. intraradices* and *G. mosseae*, respectively) than on non-mycorrhizal plants (43.5 ± 4.1 µg, Fig. 2; Table 4). Additionally, the development of parasitoids was significantly faster in the AMF treatments (mean developmental time 17.0 ± 0.2 and 16.8 ± 0.2 days for *G. intraradices* and *G. mosseae*, respectively) than in the control treatment (17.7 ± 0.2 days; Table 4). There was no significant difference in parasitoid dry weight and developmental time between the two AMF species. Sex ratio had a significant effect on dry weight, with females being heavier than males, but there was no difference between the sexes in terms of development time. The opposite was true for the number of aphids, which had a significant impact on the developmental time of emerging wasps, but not on parasitoid dry weight (Table 4).

A comparison of sex-specific mean values for dry weight at eclosion and development time in the control and in the two AMF treatments showed rather uniform responses of males and females in the control and *G. intraradices* treatments, with a better performance in the latter (Fig. 2). In contrast, *A. rhopalosiphi* expressed a clear sex-specific reaction in the *G. mosseae* treatment, with males developing faster and females getting heavier. Nevertheless, there was no significant difference in parasitoid sex ratio (mean 49 ± 15, 37 ± 10, and 51 ± 13% females in control, *G. intraradices*, and *G. mosseae* treatments, respectively, $F_{2,22} = 0.47$, $P = 0.63$). The proportion of adult aphids among mummies was also not significantly different

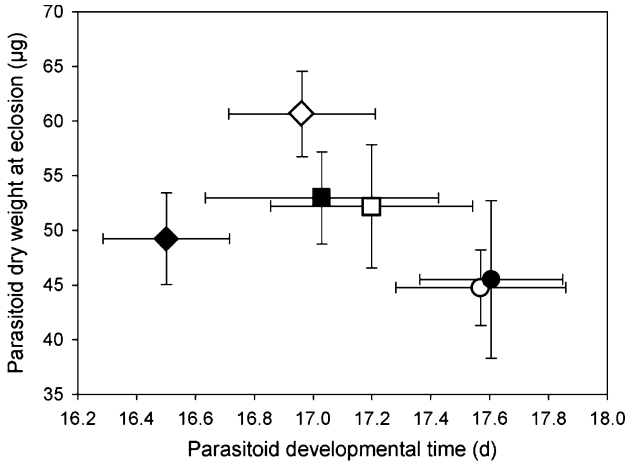

Fig. 2 Mean parasitoid dry weight (+SE) plotted against mean parasitoid developmental time (+SE) for both sexes separately. *Circles* Non-mycorrhizal control, *squares* plants inoculated with *Glomus intraradices*, *diamonds* plants inoculated with *G. mosseae*. *Open and closed symbols* represent data for female and male parasitoids, respectively

between the AMF treatments (mean 27 ± 8, 51 ± 8, and 50 ± 12% in control, *G. intraradices*, and *G. mosseae* treatments, respectively, $F_{2,21} = 0.23$, $P = 0.80$).

The rate of parasitism (expressed as intercept in Fig. 3) was significantly different between the *G. intraradices* (15.9 ± 3.7%) and the two other treatments (6.6 ± 1.6 and 6.3 ± 1.0% in control and *G. mosseae* treatments, respectively, $P = 0.007$). However, MA regression showed no

Table 4 Results of ANCOVA on mean parasitoid dry mass and development time as means per pot

Source of variation	*df*	Dry weight			Development time		
		MS	*F* value	*P* value	MS	*F* value	*P* value
Number of aphids	1	357.3	0.44	0.52	28.84	9.14	0.007*
Sex ratio	1	4316.9	5.27	0.03*	1.99	0.63	0.44
AMF	2	2726.6	3.33	0.05*	19.64	6.22	0.008*
AMF vs. control	1	5401.8	6.59	0.02*	33.78	10.70	0.004*
Intra vs. moss	1	28.4	0.03	0.85	4.84	1.53	0.23
Residuals	21	819.8			3.16		

* Significant at $P < 0.05$

Orthogonal contrasts are calculated for AMF treatments (see Materials and methods for details)

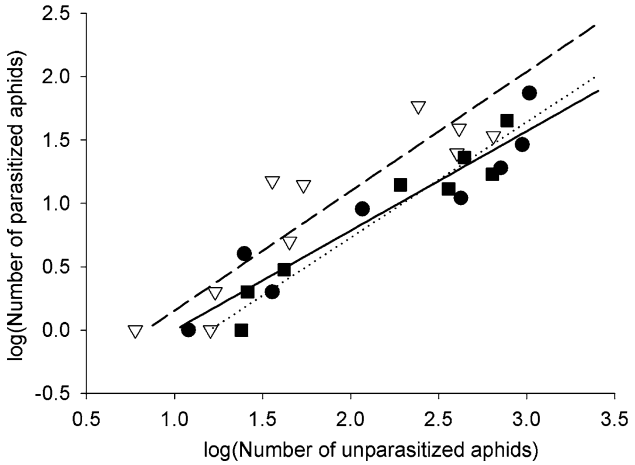

Fig. 3 Scatterplot of the number of unparasitized against the number of parasitized aphids per pot for control (*circles*), *G. intraradices* (*squares*), and *G. mosseae* (*diamonds*) treatments. Major axis regression: control (*solid line*, $y = 0.785 \times -0.787$, $R^2 = 0.89$), *G. intraradices* (*dashed line*, $y = 0.942 \times -0.789$, $R^2 = 0.82$), *G. mosseae* (*dotted line*, $y = 0.913 \times -1.097$, $R^2 = 0.93$)

significant difference in aphid density-dependent reactions of parasitoids between the fungal treatments (expressed as slope, $P = 0.64$). The rates of parasitism decreased with increasing number of aphids (slope < 1).

Discussion

In contrast to our initial hypotheses, positive effects of AMF inoculation on performance could only be observed at two trophic levels. One is the level of primary producers (*Phleum pratense*), which benefitted from the association in terms of an increase in biomass, and the other level is that of the parasitoids (*Aphidius rhopalosiphi*), which showed increased weight at eclosion and shorter developmental time on mycorrhizal plants (Figs. 1, 2). Population growth rates of aphids (*R. padi*) as primary consumers decreased on plants inoculated with AMF. Although we did not detect significant differences between the two inoculated AMF species concerning aphid population growth rates, such differences were clearly present in terms of plant biomass under aphid attack and in the rates of parasitism. These differences may reflect direct physiological effects of the two AMF species, but they may also result from the observed differences in mean colonization rate between *G. intraradices* and *G. mosseae* (42 and 21%, respectively). The different mycorrhization rates, in turn, may be an artefact of our experiment, but we suggest that they instead reflect innate differences between AMF species, as shown by Hart and Reader (2002), because we used the same amount of inoculum for both AMF species. Nevertheless, the results of our experiment do not allow the indirect effects of different colonization rates to be disentangled from the direct physiological effects of AMF species, and we also have to consider with caution any conclusion regarding species-specific effects on higher trophic levels.

It has to be considered that the AMF inoculum (a commercial cultivar) and plant seeds used in this study share no common ecological background and conceivably are not adapted to each other (Fitter et al. 2005). Klironomos (2003) showed that the combination of non-adapted AMF and plants can narrow the range of host plant responses. Yet, our study demonstrates *potential* effects of different AMF species on plants and higher trophic levels, rather than revealing the *actual* outcome of these interactions under natural conditions. However, a positive impact of the two AMF isolates on plant biomass was present. Highly adapted AMF can be expected to provide even more benefits to their host plants (Helgason et al. 2007); as such, comparable or even stronger effects on higher trophic levels may be expected under more natural conditions.

Arbuscular mycorrhizal fungi and aphid effects on plants

The positive effect of AMF inoculation on plant biomass was also present at the two interim clippings (data not shown). In the case of *G. mosseae*, this positive effect was clearly not reflected in foliar N and P contents (Table 2), as these values tended to be lower than in the control. Such species-specific effects of AMF on several plant variables are also in accordance with previous studies (see Jansa et al. 2008; Maherali and Klironomos 2007; van der Heijden et al. 1998), which showed that biomass and nutrient capture of a

plant community varied independently with the identity of the inoculated AMF species.

Although aphid presence had a consistently negative impact on shoot biomass, this reduction was only significant in the control and *G. intraradices*-inoculated plants, indicating a higher tolerance to aphid feeding in the *G. mosseae*-inoculated plants (Fig. 1a). In contrast, an inconsistent pattern was observed in root biomass. Plants inoculated with *G. mosseae* increased their root biomass under aphid presence, whereas lowered biomass was detected in control and *G. intraradices*-inoculated plants under aphid herbivory (Fig. 1b). Such interactive effects of AMF species and aphids have been reported previously (Gange and West 1994) and may reflect differences in nutrient allocation within plants under aphid attack (Vestergård et al. 2004).

AMF effects on aphids

The negative effect of AMF inoculation on aphid population growth rates found in our experiment (Fig. 1c, d) contradicts some results of other studies on AMF-aphid interactions (e.g., Gange et al. 1999; Gange and West 1994). However, negative interactions have also been reported by other authors (Gehring and Whitham 2002; Guerrieri et al. 2004; Wurst et al. 2004). In most of the respective publications, AMF effects on aphids were measured using the reproductive fitness of individual females (e.g. Gange et al. 1999; Wurst et al. 2004). In our study, however, we measured aphid response to AMF in terms of population growth rather than in terms of individual fitness. Although individual fitness and population growth rate may be correlated (Ponder et al. 2000), we would like to emphasize that we can draw conclusions only for AMF effects on the population growth of aphids.

Three potential mechanisms limiting aphid growth in our experiment must be considered: aphid crowding, nutrient limitation, and plant defense compounds. Winged morphs, a good indicator of aphid crowding (Hodgson 2001), were rarely detected (in two pots only). Nutrient limitation is also unlikely, as aphid population growth rates (Fig. 1c) and plant nutrient contents at harvest (Table 2) show no correlation. Inoculation with *G. intraradices* induced the highest decrease in aphid population growth rates, but the respective plants contained as much N as control plants and tended to contain even more P than plants of the two other fungal treatments. This lack of correlation between N contents and aphid performance is in accordance with a field study by Gange and West (1994), who hypothesized that changes in aphid numbers were more related to a changed leaf morphology (phloem location and size) in mycorrhizal plants than to differences in N content. In contrast, previous studies (Bezemer et al. 2005; Ponder et al. 2000) reported decreased aphid population sizes in parallel with decreased foliar N concentrations using the same aphid species (*R. padi*) as was used in our study. Another explanation for the high proportion of aphid populations with negative growth rates on mycorrhizal plants might be the presence of defence compounds in the phloem of *Phleum pratense*, indicating increased plant resistance against aphids induced by AMF (Pozo and Azcón-Aguilar 2007). Bezemer et al. (2005) have recently shown that *R. padi* might be sensitive to phenolic compounds encountered in the phloem, which may be synthesized at higher rate upon mycorrhizal inoculation (Zhu and Yao 2004). However, elicitation of defence compounds by repeated cutting of *Phleum pratense* is not likely, as plant response mechanisms triggered by wounding and by phloem feeding insects (i.e. aphids) follow different signaling pathways (Pozo and Azcón-Aguilar 2007).

The missing effect of parasitoid presence on aphid numbers is not surprising as the proportion of parasitized aphids was generally low (<16%) and aphid populations encountered parasitoids only once during a 12-h period.

AMF effects on parasitoids

First studies concerning the effects of AMF on parasitoid wasps focused on parasitoid preferences. Gange et al. (2003) provided data on AMF species-dependent variations in the rates of parasitism of the ichneumon *Diglyphus isaea* parasitizing the leaf mining fly *Chromatomyia syngenesiae*. Guerrieri et al. (2004) showed that non-mycorrhizal tomato plants infested with aphids were as attractive to *Aphidius ervi* as mycorrhizal, non-infested plants. In our study, we revealed that the performance of parasitoid wasps is also influenced by the presence and identity of AMF, as *Aphidius rhopalosiphi* got heavier and developed faster when their host *R. padi* was reared on mycorrhizal plants (Fig. 2). Changes in weight at eclosion and developmental time are highly correlated to several fitness traits, such as longevity, number of hosts attacked (in case of females), and number of matings achieved (in case of males; Godfray 1994). These changes were rather uniform for male and female parasitoids in the case of the *G. intraradices* treatment, but they varied substantially between sexes in the *G. mosseae* treatment. While we cannot discern the underlying reasons for the sex-specific difference, one possibility is that females adjusted their behavior when plants were infested by *G. mosseae* such that fertilized eggs resulting in females were laid in hosts that differed in size from those of unfertilized eggs. Alternatively, the observed sex-specific pattern in the *G. mosseae* treatment may indicate that larger female parasitoids enjoy a proportionally greater increase in fitness than larger males (Godfray 1994). Therefore, female parasitoids may have invested additional resources in an increased weight rather than

decreased development time, which is also reflected by the significant influence of the ratio between sexes within pots on parasitoid dry weight (Table 4). Despite this sex-specific difference in the *G. mosseae* treatment, the observed differences in parasitoid dry weight and development time between mycorrhizal and control treatments were not due to changes in sex ratio, as this variable was relatively constant in all AMF treatments (proportion of females 44.8 ± 7.1%). Alternatively, it might be the case that male and female parasitoids can use the resources provided by *G. mosseae* infection in different ways. This hypothesis needs to be addressed in further studies.

Parasitoid developmental time was correlated with aphid density, i.e., parasitoids developed faster when more aphids were available for oviposition. One possible explanation for this relationship is that at higher aphid densities, the parasitoids encounter more aphids of different larval instars and hence are able to carry out more ovipositions in more suitable aphid stages. In the pea aphid (*Acyrthosiphon pisum*), oviposition in intermediate instars reduced the developmental time of *Aphidius ervi* relative to ovipositions in younger instars or adults (Sequeira and Mackauer 1992). While we did not directly study parasitoid oviposition, such selection behavior is conceivable. More generally, there is little information about density dependence in parasitoid host selection behavior and the consequences for offspring fitness.

Sequeira and Mackauer (1992) showed that the values of weight and developmental time covary and are furthermore highly dependent on the age of the parasitized aphids. This association was not present in our study, as the proportion of aphids that died as adults was the same in the two AMF inoculation treatments and the control, indicating that aphids were parasitized at comparable larval stages in all fungal treatments.

In accordance to the study by Gange et al. (2003), we found changes in the rates of parasitism, expressed as a significant higher proportion of parasitized aphids in the *G. intraradices* treatment (Fig. 3). Gange et al. (2003) partially attributed their observed mycorrhizal effects on the rates of parasitism to a decreased parasitoid searching efficiency due to changes in plant architecture. However, the limited space under the cellophane bags in our experiment surely interfered with this effect. Additional effects, such as the induction of volatiles influencing parasitoid activity, are also likely, as these can be AMF species-specific (Bezemer and van Dam 2005).

Interactions of belowground organisms with plant roots resulting in contrasting reactions on aboveground aphids and parasitoids were also reported by Bezemer et al. (2005). They attributed increasing parasitoid performance to a visually observed increase in aphid size, although they did not explicitly quantify this parameter. Another possible explanation for the observed effects on aphid and parasitoid level would be a decrease in the aphid immune answer against parasitoid eggs on mycorrhizal plants, which could have led to an increase in parasitoid performance (W. Völkl, personal communication; see also Godfray 1994). However, all of these hypothetical mechanisms do not seem to follow a linear relation, as aphid population growth rates were highest on control plants, intermediate on plants from the *G. mosseae* treatment, and worst on *G. intraradices*-inoculated plants. In contrast, parasitoid weight and development time were best on *G. mosseae*-inoculated plants, worst on control plants, and intermediate with *G. intraradices*.

Conclusion

Our results show that three interacting trophic levels are significantly affected by both the presence and the species identity of AMF. Bottom–up effects of AMF influenced plants, aphids, and their parasitoids differently, with a positive impact on plants and parasitoids and a negative impact on aphids. However, changes in plant nutrient contents C, N, and P) were not driving the observed performance alterations, as these values were equal between mycorrhizal and non-mycorrhizal plants. Therefore, food choice experiments (Prince et al. 2004) and stable isotope probing (Langellotto et al. 2006) would be useful approaches for monitoring changes in preferences and nutrient fluxes. The observed changes in the trophic interactions due to AMF inoculation emphasize that belowground interactions can have strong implications for aboveground food webs (van der Putten et al. 2001). Models of trophic interactions (Hoover and Newman 2004; van der Putten et al. 2004) should include the impact of a symbiosis as widespread as arbuscular mycorrhiza (Treseder and Cross 2006).

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