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Testing for mycorrhizal fungi-plant-ant indirect effects

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Recent work has demonstrated indirect effects between mycorrhizal fungi and insect herbivores and pollinators. The existence of indirect effects between mycorrhizal fungi and protection-for-food mutualists, such as extrafloral nectar-foraging 'bodyguard ants', is unknown. In this study, we examined the potential for indirect effects of arbuscular mycorrhizal fungi on aggressive ant bodyguards, mediated by changes in the expression of extrafloral nectaries of a shared host plant. We found that mycorrhizal plants grew larger and produced more extrafloral nectaries compared to their non-mycorrhizal counterparts. The difference in the number of nectaries between mycorrhizal and non-mycorrhizal plants, however, was too small to elicit differences in ant attendance. In spite of the lack of a significant indirect effect of mycorrhizal fungi on ant attendance, mycorrhizal plants suffered damage to a significantly greater proportion of their leaves compared to non-mycorrhizal plants. This result likely stems from other (non-ant-mediated) indirect effects of mycorrhizal fungi on herbivores.

Keywords: arbuscular mycorrhizal fungi; *Formica ravida*; herbivory; indirect effects; *Vicia faba*

Abbreviations: AMF, arbuscular mycorrhizal fungi; DDF, denominator degrees of freedom; EF, extrafloral; M+, mycorrhizal plants; M−, non-mycorrhizal plants; NDF, numerator degrees of freedom.

Introduction

Arbuscular mycorrhizal fungi (AMF) form a phylum of fungi that engage in symbioses with a majority of plant species (Bever et al. 2001; Schüßler et al. 2001). The relationship is usually considered to be beneficial to both partners (i.e., mutualism: Boucher 1985), with the plant receiving nutrients that mycorrhizal hyphae collect from the soil, and the fungus receiving carbohydrates that the plant produces during photosynthesis (Bonfante-Fasolo and Scannerini 1992). This exchange can greatly affect characteristics of the host plant that may subsequently affect the attendance and/or performance of aboveground insects, including herbivores (e.g., Gange et al. 2002, 2003, 2005; Wamberg et al. 2003; Guerrieri et al. 2004; Koschier et al. 2007; earlier studies reviewed in Gehring and Whitham 2002) and mutualists (Gange et al. 2003; Guerrieri et al. 2004; Gange and Smith 2005; Wolfe et al. 2005; Cahill et al. 2008). These indirect effects can, in turn, produce feedbacks to the host plant, such as altered levels of damage by herbivores. Of the five previous studies of the indirect effects of AMF on insect mutualists, in three cases AMF resulted in increased pollinator visitation due to increased inflorescence size and/or improved quality and quantity of floral nectar (Gange and Smith 2005; Wolfe et al. 2005), or due to changes in floral display at the patch level (Cahill et al. 2008). In the other two cases AMF altered the action of insects that acted as mutualists to plants by parasitizing the plants' herbivores (Gange et al. 2003; Guerrieri et al. 2004).

Importantly, AMF also have the potential to indirectly affect other types of mutualisms, such as protection-for-food mutualisms. In these mutualisms, the host plant provides 'bodyguard' insects (usually ants) with food rewards, such as extrafloral (EF) nectar. And, in turn, while foraging for nectar, the bodyguards protect their host plants from herbivores (see Bentley 1977; Rogers 1985; Koptur 1992; Beattie and Hughes 2002; Arimura et al. 2005). If AMF alter floral nectar-mediated plant-insect interactions (Gange and Smith 2005; Wolfe et al. 2005; Cahill et al. 2008), it is likely that they also alter EF nectar-mediated plant-insect interactions. AMF-bodyguard indirect effects are likely to be very common in nature because: (i) AMF are common and diverse (Bever et al. 2001); (ii) EF nectaries are expressed by many plant species across more than 90 families (Koptur 1992); and (iii) ants are typically abundant and opportunistic foragers, and they defend many different EF nectary-bearing plants in a wide range of habitats (Schupp and Feener 1991).

The way in which AMF-plant and plant-ant mutualisms interact with each other may be complex. For example, in one experiment *Vicia faba* (broad bean) plants inoculated with AMF produced fewer EF nectaries compared to non-inoculated plants

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(Laird and Addicott 2007). On the other hand, in many cases AMF increase plant growth (Lekberg and Koide 2005), which, given that EF nectary number is likely closely tied to plant size (Laird and Addicott 2007), means that AMF should often positively affect the number of EF nectaries produced by their host plant. Whether AMF increase or decrease EF nectary expression in terms of nectary number, nectar production or sugar concentration, EF nectary expression itself positively affects both ant attendance and the efficacy of protection by ants in a variety of plants (e.g., Koptur 1979; Ness 2003, Rudgers 2004), including *Vicia faba* (Katayama and Suzuki 2004).

Thus, in *Vicia faba*, AMF have been found to affect EF nectary expression (Laird and Addicott 2007), which in turn has been found to affect ant attendance and plant protection (Katayama and Suzuki 2004). Therefore, it is likely that AMF indirectly alter the outcome of protection-for-food mutualisms in *Vicia faba*, as a model for the many other plant species that are both mycorrhizal and ant-tended. Here, we report on a field study in semi-natural conditions designed to test this hypothesis using *Vicia faba* plants whose mycorrhizal status was experimentally manipulated, and the aggressive ant species *Formica ravida*. We predict that any effects of AMF on EF nectary expression will translate into corresponding indirect effects on ant attendance and feedback effects on plant protection.

Methods

Study site and species

Our study took place in a badlands habitat in Dinosaur Provincial Park, Alberta, Canada. The areas of the park where our study took place are dominated by sage (*Artemesia* spp.) and needle-and-thread grass (*Stipa comata*).

Our focal plant species was *Vicia faba* cv 'Broad Windsor' (Fabaceae), a species that forms symbiotic associations with AMF (e.g., Kucey and Paul 1982). Additionally, *V. faba* plants produce conspicuous, ant-tended EF nectaries on their stipules (Bugg and Ellis 1990; Engel et al. 2001), the construction of which can be negatively affected by mycorrhizal fungi under some experimental conditions (Laird and Addicott 2007). Furthermore, experimentally reducing the expression of EF nectaries in *V. faba* leads to reductions in ant attendance, and the efficiency with which those ants remove herbivores (Katayama and Suzuki 2004). Therefore, *V. faba* was a highly suitable species with which to test the indirect effects of AMF on mutualistic ants.

We used 25 nests of *Formica ravida* ants (Formicidae). In our study area, *F. ravida* tend aphids on sage plants, and feed on EF nectar of native EF nectary-bearing plants, such as *Helianthus annuus*. Thus, even though *V. faba* is not found naturally in our study area, the ant colonies we studied commonly engaged in EF nectary-mediated interactions, and similar ant-aphid interactions. Moreover, a previous study reported that members of the *Formica* genus routinely feed at EF nectaries on *V. faba* (Bugg and Ellis 1990). Therefore, we considered the possibility of an AMF-*Vicia*-*Formica* indirect effect to be quite likely. Also, *F. ravida* is a very aggressive species of ant, and vigorously defends EF nectary-bearing host plants from herbivores. Because of this, we also expected to observe any AMF-mediated variation in EF nectary production to not only result in variation in ant attendance, but also in variation in the presence of non-*Formica* insects, and, subsequently, in the level of plant damage.

Mycorrhizal treatments

Plants were randomly assigned to one of two mycorrhizal treatments, 'mycorrhizal (M+)' and 'non-mycorrhizal (M–)'. There were 25 M+ plants and 25 M– plants. The preparation of the treatments is described in detail in Laird and Addicott (2007). Briefly, *V. faba* seeds in both treatments were surface sterilized in 2% sodium hypochlorite (20 min, followed by five 5-min rinses in distilled water), germinated in the dark on moist filter paper, and planted six days later in 150×180 mm pots containing an AMF-free, soil-less mixture of peat moss, perlite and crushed clay (4:3:3 by volume).

Before planting, the emerging radicles of M+ seedlings were dipped in a mycorrhizal inoculant (Bio/Organics, Inc., LaPine, OR, USA) that was composed of crushed rock and the spores of several AMF species (*Glomus aggregatum*, *G. clarum*, *G. deserticola*, *G. intraradices*, *G. monosporus*, *G. mosseae*, *Gigaspora margarita*, and *Paraglomus brasilianum*), some of which (*G. mosseae*, *G. intraradices*, and *G. clarum*) are known to participate in mycorrhizal symbioses with *V. faba* (Kucey and Paul 1982; Vieweg et al. 2004; Rabie and Almadini 2005). During planting, M+ plants were placed directly atop 5 ml of the inoculant. In a simultaneous and complementary experiment, using the same method of selective inoculation, we found these techniques to be highly effective in ensuring that inoculated (M+) plants formed mycorrhizal associations, while non-inoculated (M–) plants did not (Laird and Addicott 2007). This was later verified (see Verifying mycorrhizal treatments, below).

Initially, the plants were placed in a growth chamber. They were watered daily with deionized water while receiving 13 h of light (at 20°C) and 11 h of darkness (at 16°C). On 27 May 2005, 15 days after planting, the pots were removed from the growth chamber and taken to Dinosaur Provincial Park. Before the pots were placed on the ground, squares of 1-micron nylon mesh were affixed over each pot's drainage holes. This was done to prevent mycorrhizal fungi from colonizing the plants' roots from below, while still allowing excess water to drain from the

pots. In addition, six vertically-oriented strips of cloth tape were attached at uniform intervals to the sides of the pots; this was done to increase the traction for foraging ants on the smooth, plastic pots.

Ant foraging experiment

On 29 May 2005, one M+ plant and one M– plant were randomly assigned to each of the 25 *Formica* nests. We placed the pots in each pair side-by-side and directly adjacent to their corresponding nest. The orientation of the pots with respect to the nest was opportunistic (i.e., they were placed on the flattest side of the nest to reduce the likelihood of tipping), and their orientation with respect to one another was random.

We collected data on 13 occasions between 1 June and 1 July 2005. On three sampling dates (12, 19 and 24 June), we watered the plants with 375 ml of well water. The rest of the time the plants received sufficient rain water. For each plant on each sampling occasion, we counted the number of *Formica* individuals foraging for EF nectar, as well as the number of non-*Formica* insects and spiders that were present, hereafter referred to as 'number of non-*Formica*' for simplicity. We identified insects to order. The most frequently observed insects on the plants were Dipterans, Hymenopterans, Coleopterans, Hemipterans and Homopterans, with the latter three likely to be particularly important as herbivores. However, there were too few individuals from each order at each sampling event to analyze orders separately, so these data were combined. We also recorded a number of plant traits: plant height, number of EF nectaries, and number of fully-expanded leaves, distinguishing between leaves that had been damaged and those left undamaged by herbivory. Damaged leaves were those that had lost area due to leaf-chewing, or were injured by phloem-suckers (i.e., aphids). By 1 July 2005, most of the plants were visibly senescent, and the experiment was terminated.

Verifying mycorrhizal treatments

A previous experiment indicated that our method of selective inoculation was effective for creating mycorrhizal and non-mycorrhizal *V. faba* plants (Laird and Addicott 2007). Nevertheless, we used root staining and microscopy techniques to determine whether M+ plants were mycorrhizal and M– plants were not. The techniques were those of Brundrett (1994) and Brundrett and McGonigle (1994). We rinsed the roots of 10 randomly chosen M+ plants and 10 randomly chosen M– plants with distilled water and placed them in vials of 50% ethanol. Later, we rinsed the roots again, and cleared them by autoclaving them in 10% KOH for 15 min at 121°C. We then rinsed the roots a third time, and stained them by autoclaving them in 0.03% Chlorazol Black E for 15 min at 121°C. We stored the roots in 50% glycerol in preparation for microscopic analysis. We examined the roots at 400 × and scored the percentage of line intersections with evidence of AMF: arbuscules, vesicles, or mycorrhizal hyphae. The maximum percent colonization of M+ roots was significantly greater than the maximum percent colonization of M– roots (means ± SEMs: M+: 37.1 ± 5.4%; M–: 2.2 ± 1.0%; Wilcoxon rank sum test: $Z = 3.77$, $p = 0.0002$, $n_{M+} = n_{M-} = 10$). Staining additional roots with Vierheilig et al.'s (1998) ink-vinegar method gave qualitatively similar results: evidence of AMF colonization in M+ roots, and a lack of evidence of AMF colonization in M– roots.

Analysis

All statistical analyses were performed using SAS software (SAS Institute, Cary, NC), except for the Wilcoxon rank sum test, which was performed using JMP software (SAS Institute, Cary, NC). We used repeated-measures analysis of variance to determine the effect of AMF on the height of the main shoot of *V. faba* plants. Mycorrhizal treatment (M+ and M–), sampling date, and nest, as well as the three two-way interactions, were included in the model. However, the three-way interaction was not included in the model, because there was no replication at that level, similar to standard 'blocked' designs. We used a similar repeated-measures ANOVA to determine the effect of AMF on the number of EF nectaries per plant. We used analogous generalized linear models to determine the effect of AMF on the number of foraging *Formica* per plant, the number of non-*Formica* per plant, and the proportion of leaves damaged by herbivory. The generalized linear models for number *Formica* and the number of non-*Formica* employed Poisson distributions; the proportion of leaves damaged by herbivory employed a binomial distribution. For the number of *Formica* and the number of non-*Formica*, it was necessary to omit nest and its interactions from the models. For the proportion of leaves damaged by herbivory, nest was retained in the model, but its interactions were omitted. If these factors were included, SAS's PROC GENMOD was unable to estimate the models' parameters. An alternative solution would be to use the same type of repeated-measures ANOVA we used for plant height and the number of EF nectaries. However, unlike those dependent variables, the distributions of the number of *Formica*, the number of non-*Formica*, and the proportion of leaves damaged were highly non-normal and/or heteroscedastic, and resistant to correction by data transformation. We considered it more important that our models use appropriate distributions rather than include nest, which was merely a by-product of our experimental design (which was itself a consequence of the colonial nature of ants), and not a factor of interest per se.

Results

There was a significant effect of sampling date on the height of *Vicia faba* plants – plants became taller over the course of the experiment (Figure 1, Table 1). More interestingly, however, plants in the M+ treatment were significantly taller than those in the M– treatment (Figure 1, Table 1). There were also significant effects on plant height of ant nest and the interaction of nest and mycorrhizal treatment (Table 1). Post hoc contrasts indicated that M+ plants had a significantly greater average height than their M– 'nestmate' at six nests, while M– plants had a significantly greater average height than M+ plants at just two nests (α for post hoc contrasts adjusted for multiple comparisons using the Dunn-Šidák procedure).

Similar to plant height, there was a significant effect of sampling date on the number of EF nectaries on *Vicia faba* plants (Figure 2, Table 2). There was also a small but significant effect of mycorrhizal treatment, with M+ plants having significantly more EF nectaries than M– plants (Figure 2, Table 2). The effect of AMF on the number of EF nectaries was mainly a function of plant size, as the number of EF nectaries per unit plant height did not differ significantly between M+ and M– plants (not shown). As with plant height, there were significant effects of nest and the interaction of nest and mycorrhizal treatment on the number of EF nectaries produced (Table 2). For each of the four nests that post hoc contrasts indicated a significant effect of mycorrhizal treatment, the M+ plant averaged more EF nectaries than the corresponding M– plant (again using an adjusted α).

Although there were large and significant fluctuations in ant attendance across sampling dates, ant attendance was not significantly affected by mycorrhizal treatment (Figure 3, Table 3). Rather, both M+ and M– plants tracked similar ant attendance trends through time (Figure 3). Additionally, there was no significant mycorrhizal treatment by date interaction (Figure 3, Table 3).

The number of non-*Formica* insects and spiders varied significantly over the course of the experiment (Figure 4, Table 4), peaking in mid-June. However, there was no significant effect of mycorrhizal

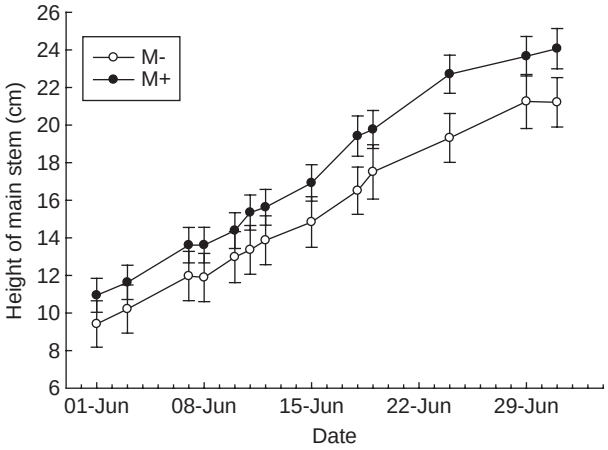

Figure 1. Plant height across the 13 sampling dates for M– plants (open symbols) and M+ plants (closed symbols). Symbols indicate means ± SEMs. Table 1 shows the statistical test results.

Table 1. Repeated measures ANOVA using plant height as the dependent variable. Included in the ANOVA were mycorrhizal treatment (MYC), sampling date (DATE), ant nest (NEST), and all two-way interactions. The variance-covariance structure that yielded the lowest AIC_c was 'first order autoregressive'. See Figure 1.

Source	NDF [a]	DDF [b]	*F*	*p*
MYC	1	11.4	23.25	0.0005
DATE	12	241	57.76	<0.0001
NEST	24	11.4	10.92	<0.0001
MYC × DATE	12	241	1.13	0.33
MYC × NEST	24	12	14.00	<0.0001
DATE × NEST	288	143	0.87	0.83

[a]NDF, numerator degrees of freedom; [b]DDF, denominator degrees of freedom; calculated using the Kenward-Roger method.

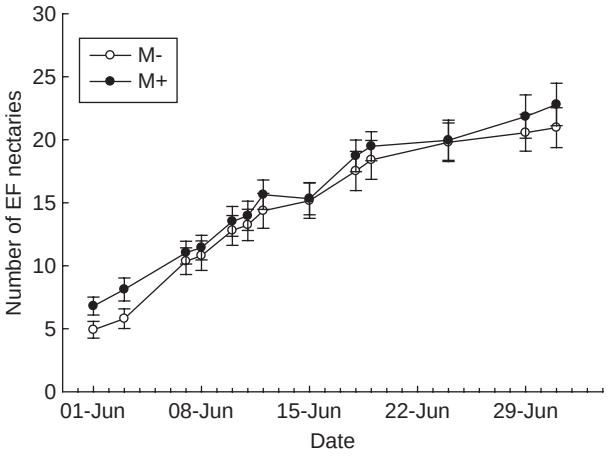

Figure 2. Number of EF nectaries across the 13 sampling dates for M– plants (open symbols) and M+ plants (closed symbols). Symbols indicate means ± SEMs. Table 2 shows the statistical test results.

Table 2. Repeated measures ANOVA using the number of EF nectaries as the dependent variable. Included in the ANOVA were mycorrhizal treatment (MYC), sampling date (DATE), ant nest (NEST), and all two-way interactions. The variance-covariance structure that yielded the lowest AIC_c was 'first order antedependence'. See Figure 2.

Source	NDF [a]	DDF [b]	*F*	*p*
MYC	1	24.2	4.79	0.038
DATE	12	51.3	32.78	<0.0001
NEST	24	24.2	6.42	<0.0001
MYC × DATE	12	51.3	0.67	0.77
MYC × NEST	24	2.67	33.32	0.011
DATE × NEST	288	64.1	1.03	0.46

[a]NDF, numerator degrees of freedom; [b]DDF, denominator degrees of freedom; calculated using the Kenward-Roger method.

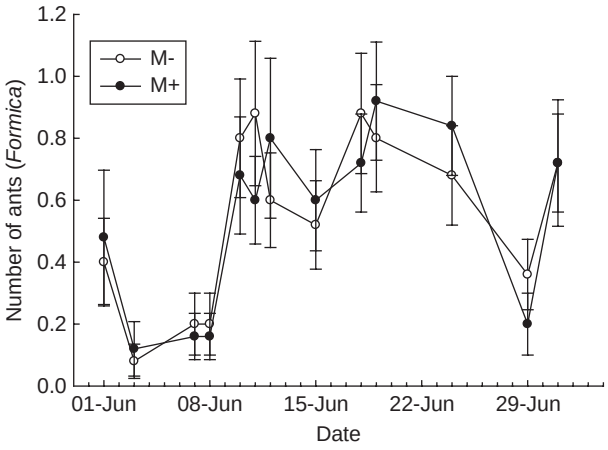

Figure 3. Number of *Formica* individuals across the 13 sampling dates for M– plants (open symbols) and M+ plants (closed symbols). Symbols indicate means ± SEMs. Table 3 shows the statistical test results.

Table 3. Generalized linear model using the number of *Formica* individuals as the dependent variable. The model employed a Poisson distribution. Included in the model were mycorrhizal treatment (MYC), sampling date (DATE), and their interaction. The variance-covariance structure 'compound symmetry' yielded a model with a deviance-to-degrees of freedom ratio of 1.06. See Figure 3.

Source	DF [a]	χ^2	p
MYC	1	0.01	0.91
DATE	12	32.14	0.0013
MYC × DATE	12	8.85	0.72

[a]DF, degrees of freedom.

treatment or the mycorrhizal treatment by date interaction (Figure 4, Table 4).

The proportion of leaves showing herbivore damage increased over most of the course of the experiment (Figure 5, Table 5). In addition, there was a significant effect of mycorrhizal treatment, with

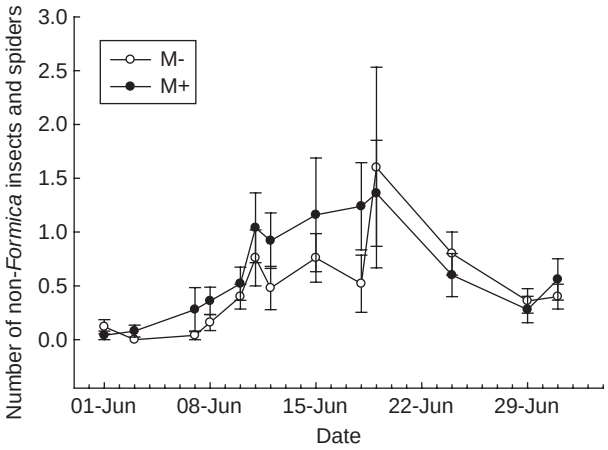

Figure 4. Number of non-ant insects and spiders across the 13 sampling dates for M– plants (open symbols) and M+ plants (closed symbols). Symbols indicate least squares means ± SEMs. Table 4 shows the statistical test results.

Table 4. Generalized linear model using the number non-*Formica* individuals as the dependent variable. The model employed a Poisson distribution. Included in the model were mycorrhizal treatment (MYC), sampling date (DATE), and their interaction. The variance-covariance structure 'compound symmetry' yielded a model with a deviance-to-degrees of freedom ratio of 1.54. See Figure 4.

Source	DF [a]	χ^2	p
MYC	1	1.32	0.25
DATE	12	24.74	0.016
MYC × DATE	12	17.55	0.13

[a]DF, degrees of freedom.

M+ plants experiencing more damage compared to M– plants (Figure 5, Table 5). Neither the mycorrhizal treatment by date interaction nor nest was significant (Figure 5, Table 5).

Discussion

In this experiment, mycorrhizal (M+) *Vicia faba* plants produced significantly more EF nectaries compared to non-mycorrhizal (M–) plants (Figure 2). Yet, in an earlier study that used the same batch of inoculant, we showed the opposite effect – a reduction in the construction of EF nectaries in M+ plants (Laird and Addicott 2007). Therefore, EF nectary production in *V. faba* appears to be highly context-dependent (Mondor et al. 2006; Laird and Addicott 2007). Specifically, the variable plant response in EF nectary production reflects variable responses in plant growth. In our previous study, there was no effect of AMF on plant height (Laird and Addicott 2007). Here, however, there was a strong positive effect of AMF on plant height (Figure 1), leading to more EF nectaries on M+ than on M– plants (Figure 2). Increased plant growth is a common effect of AMF colonization (Lekberg and Koide 2005), an effect that is sometimes more likely in situations when plants, including *Vicia faba* (Ishac

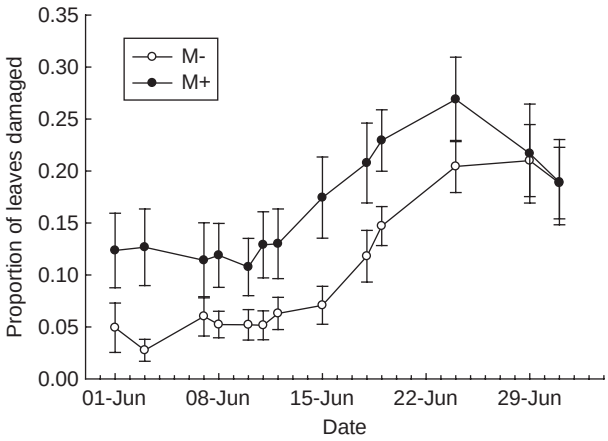

Figure 5. Proportion of leaves damaged across the 13 sampling dates for M– plants (open symbols) and M+ plants (closed symbols). Symbols indicate means ± SEMs. Table 5 shows the statistical test results.

Table 5. Generalized linear model using the proportion of leaves damaged as the dependent variable. The model employed a binomial distribution. Included in the model were mycorrhizal treatment (MYC), sampling date (DATE), and their interaction, and nest (NEST). The variance-covariance structure 'independent' yielded a model with a deviance-to-degrees of freedom ratio of 2.05. See Figure 5.

Source	DF[a]	χ^2	p
MYC	1	7.96	0.0048
DATE	12	36.92	0.0002
NEST	24	30.66	0.16
MYC × DATE	12	11.20	0.51

[a]DF, degrees of freedom.

et al. 1994), are grown under stressful conditions such as drought stress (Augé 2001; but see Lekberg and Koide 2005). The field conditions in this experiment were likely more stressful for plants compared to the tightly controlled and favorable conditions of our previous experiment, which took place in a growth chamber (Laird and Addicott 2007). In particular, *Vicia faba* is sensitive to high temperature (McDonald and Paulsen 1997), a situation common in the Alberta badlands where summer temperatures are frequently above 30°C, but not in our growth chamber experiment, where the maximum temperature was set at 20°C. Hence, the plants in this experiment were more likely to be stressed, and therefore more likely to benefit from AMF in terms of plant height, and by extension, EF nectary production. Alternatively, the differences between the two studies may be attributed to the fact that plants grown in the field received more light compared to plants grown in the growth chamber, leading to a greater availability of carbon for both AMF and EF nectaries in the current study.

The difference in EF nectary production between M+ and M– plants did not translate into significant AMF-mediated variation in ant attendance (Figure 3) or the number of non-ant insects on the *V. faba* plants (Figure 4). This is perhaps not surprising, given that the difference in average EF nectary number between M+ and M– plants was less than two on all but one sampling date (Figure 2). Previous studies that reported a positive effect of EF nectary expression on ant attendance typically had a much greater difference in EF nectary number than the one reported here. For example, experimentally removing *all* the EF nectaries from plants can significantly reduce ant attendance in *V. faba* (Katayama and Suzuki 2004) and other species of *Vicia* (Koptur 1979). Further, the advantage of discriminating between food sources should decrease as the difference in their quantity or quality shrinks. Thus, we surmise that in our study, the size of the effect of AMF on EF nectary production, while statistically significant, was not biologically significant. Ants are either not capable of discriminating between plants with slightly different numbers of EF nectaries or it is not profitable for them to do so. These results emphasize the fact that effects between members of one link in an interaction chain do not necessarily propagate to the next link, even when the first effect is a potentially important mediator of the second (as is the case with extrafloral nectaries and ant attendance in *V. faba* (Bugg and Ellis 1990; Katayama and Suzuki 2004). Rather, indirect effects can attenuate in a similar manner to the decreasing strength of a trophic cascade that is sometimes observed across trophic levels (Schmitz et al. 2000).

The fact that other studies have found significant nectar-mediated indirect effects between AMF and pollinators (Gange and Smith 2005; Wolfe et al. 2005) suggests that AMF-ant indirect effects might be possible in other plant species or under different ecological circumstances. For example, if specialist AMF species were used, rather than the generalists found in the commercial inoculant, the positive effects of AMF on plant height and EF nectary production might have been more pronounced (see Helgason et al. 2007), perhaps leading to greater discrimination by ants.

Despite the absence of significant effects of AMF on ant attendance or the number of non-*Formica*, M+ plants experienced significantly more herbivore damage compared to M– plants, in terms of the proportion of leaves damaged (Figure 5). The main focus of this experiment was on the potential for AMF to alter plant damage via indirect (i.e., ant-based) defences. However, AMF can also affect characteristics of plants that are directly relevant to herbivorous insects (Gehring and Whitham 2002), such as nutritional value, toxicity, and palatability. Which if any of these factors was responsible for the increase in damage in M+ plants is unknown, but reduced toxicity and/or increased palatability are less likely, because mycorrhizal plants are typically able to mount more effective chemical defences to generalist herbivores compared to non-mycorrhizal plants (reviewed in Gehring and Whitham 2002). For example, the presence of mycorrhizal fungi leads to the increased production of the feeding deterrents aucubin and catalpol in *Plantago lanceolata* plants (Gange and West 1994).

Conclusions

AMF can affect the production of the rewards that plants offer to their insect bodyguards in exchange for protection from herbivores. These effects appear to be highly variable, and can range from situations in which AMF result in a decrease in EF nectary production (Laird and Addicott 2007) to situations in which they result in an increase in EF nectary production (Figure 2). These AMF-mediated changes in EF nectary production should result in corresponding changes in ant attendance, insofar as the foraging patterns of ants are sensitive to these changes. In this

study, however, the size of the effect of AMF on EF nectary number was too small to alter the abundance of ants foraging on M+ versus M– plants. Nevertheless, we observed a significant difference between M+ and M– plants in plant damage, suggesting that other, non-ant-mediated AMF-herbivore indirect effects are at play in this system.

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