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Multitrophic links between arbuscular mycorrhizal fungi and insect parasitoids

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Abstract

The effects of arbuscular mycorrhizal colonisation of *Leucanthemum vulgare* on parasitism of a leaf-mining insect was studied in a field and a laboratory experiment. In the field, parasitism of *Chromatomyia syngenesiae* by *Diglyphus isaea* was lower on mycorrhizal plants, compared with plants where the association was reduced. A laboratory experiment, in which *L. vulgare* was inoculated with three species of AM fungi, showed that the effects on parasitism rates were mycorrhizal species dependent. Some fungal combinations increased parasitism, some decreased it, while others had no effect. It is concluded that the most likely cause of these differences is plant size, with parasitoid searching efficiency being reduced on the larger plants, resulting from certain mycorrhizal species combinations. However, a mycorrhizal effect on herbivore-produced plant volatiles cannot be ruled out.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi have been shown to have significant effects on the growth and/or survival of foliar-feeding insects in the majority of cases examined (Gehring & Whitham 2002). These effects may be positive or negative, depending on the mode of feeding and degree of specialism of the phytophage. Thus, generalist chewing insects seem to respond in a negative fashion to AM colonisation of their hosts (Rabin & Pacovsky 1985; Gange & West 1994), while specialist chewing or sap-feeding insects show increases in performance on mycorrhizal plants (Borowicz 1997; Goverde *et al*. 2000; Gange *et al*. 2002).

Recent reviews of multitrophic interactions involving insects, plants and fungi (van der Putten *et al*. 2001; van Dam *et al*. 2003) have highlighted the lack of studies involving higher trophic levels. Studies with foliar endophytes in grasses have shown that these fungi can produce chemical toxins that reduce the growth of the phytophage, and hence the parasitoid (Barker & Addison 1996). Furthermore, the community structure of parasitoids associated with foliar-feeding insects can be different on hosts with or without the fungus (Omacini *et al*. 2001). However, studies involving the effects of soil organisms on higher trophic levels are remarkably few, but critically important, if we are to understand community interactions in a multitrophic context (van der Putten *et al*. 2001). Masters *et al*. (2001) have shown that root-feeding insects can increase the parasitism rate of seed-feeding insects in *Cirsium palustre*, while Wurst & Jones (2003) found no effect of earthworm activity on aphid parasitism. To date, no study has examined the interactions between AM fungi and the parasitoid of a herbivorous insect. This paper demonstrates that such interactions occur and are detectable in both field and laboratory.

MATERIALS AND METHODS

Field experiment

An experimental wildflower meadow, 50 m x 50 m, was treated with a weedkiller (Glyphosate, Monsanto) in autumn of 1996, rotovated in winter and hand raked in spring 1997. It was sown with a wildflower seed mixture, suitable for acidic sandy soils (pH of soil 5.7). Twenty plots, each 3 m x 3 m, were established and allocated to one of two treatments: a) application of the fungicide Rovral (Bayer Environmental Science) (containing 40% w/w iprodione) to the soil to reduce levels of AM colonisation and b) control, untreated and therefore with natural levels of mycorrhizas. The fungicide was applied at a rate of 2 g m^{-2} of formulated product at six weekly intervals. Plots were arranged in a randomised block design. During the second year of growth, Ox-eye daisy, *Leucanthemum vulgare* Lam., began to establish in the sward, and by the spring of 1999 was the dominant species in the community. Observations in summer 1998 revealed that these plants were attacked commonly by leaf-miners of the generalist species *Chromatomyia syngenesiae* Hardy (Diptera: Agromyzidae), and these were attacked by the generalist parasitoid, *Diglyphus isaea* Walker (Hymenoptera: Eulophidae). Field observations of miner density and parasitism were conducted during four consecutive seasons, 1999-2002, during which time the host plant declined in density.

Plants were sampled in mid June of each season, when they had reached maximum height and leaf number, and leaf miners were mature. On each occasion, a minimum of five plants in each plot were selected at random, and the total number of leaves and mines of *C. syngenesiae* counted on each plant. Twenty leaves bearing fully-developed mines were randomly picked from each plot and maintained on moist tissue paper in a constant environment (CE) room (18:6 L:D) at 20°C until either an adult fly or parasitoid emerged. At this time in each season, three plants per plot were selected at random and excavated and their roots washed free of soil. AM colonisation was revealed by epifluorescence microscopy (Gange *et al*. 1999), and the percent of root length colonised (% RLC) quantified with the cross hair eyepiece method (McGonigle *et al*. 1990).

In each season, differences in miner density and rate of parasitism (percent mines attacked) between treatments were examined with one factor Analysis of Variance, using plot means as replicates. Percentage data were subject to the angular transformation prior to analysis.

Laboratory experiment

In June 1999, spores of AM fungi were isolated from 20 soil cores (4 cm x 10 cm deep), with two cores taken from each control plot. Spores were extracted by a combination of wet sieving and sucrose centrifugation (Brundrett *et al*. 1996) and identified using Schenck & Pérez (1990) and Yao *et al*. (1996). Single spore cultures of the three most common species (*Glomus caledonium* (T.H. Nicolson & Gerd.) Trappe & Gerd., *G. fasciculatum* (Thaxt.) Gerd. & Trappe and *G. mosseae* (Nicol. & Gerd.), (hereafter referred to as *G.c., G.f.* and *G.m.* respectively) were established on the roots of *L. vulgare* seedlings, grown in inert, expanded clay granules (Seramis®, (Pedigree Petfoods)) and each seedling maintained within a Sunbag® (Sigma Chemical Co) to prevent cross-contamination. Cultures were maintained in the CE room at 20 ºC for 12 months. At this time, plants were allowed to die by cessation of watering. Root samples were checked for AM colonisation and contamination using ink staining (Vierheilig *et al* 1998) and spore identity checked after sucrose extraction. Any cultures showing contamination (by non-mycorrhizal fungi or unidentified AM spores) or no colonisation were discarded. The dry granules, containing spores, hyphal fragments and roots were stored at 10 °C and used as AM inoculum.

In June 2001, cloned material of *L. vulgare* was produced by propagation of leaf cuttings. After 3 months of growth, plants were potted into 13 cm pots, containing 450 g John Innes No. 2 compost (Gem Gardening). At potting, plants were inoculated with AM fungi (see above) in all possible combinations, in a factorial design. Plants were given either 3 g of a single species AM inoculum, or 1.5 g of each of two species or 1 g of each of all three species. Control plants received 3 g of sterilised inoculum (consisting of 1g of each species). There were 20 replicate plants of each of the eight fungal treatments.

Plants were arranged in a randomised block in the CE room, within a large net cage (1 mm diameter mesh). One hundred freshly emerged adults of *C. syngenesiae* were placed in the cage and flies allowed to mate and oviposit freely. At 20 °C, the time from oviposition to the moult into the second larval instar is approximately 13 days (Merrett 1978). To allow parasitism to occur, 50 newly emerged adults of *D. isaea* were released into the cage 14 days after introduction of the flies.

Plants were maintained for a further three weeks (Merrett 1978), after which the leaf number and percentage of mined leaves on each plant were recorded. Mature mines were collected and parasitism rates recorded as before. The roots of each plant were washed free of soil and AM colonisation recorded as described above.

The effects of mycorrhizal colonisation on leaf number, miner density and percentage parasitism were examined with three-factor Analysis of Variance, with each fungal species as a main effect. Percentage data were transformed, as above.

RESULTS

Field experiment

In each year, there was a significant reduction in AM colonisation levels from application of fungicide, with control plants averaging about 36% Root Length Colonised (% RLC) and fungicide-treated plants 8.3%. Fungicide-treated plants were significantly smaller than control plants (Fig. 1a) in all four years. Leaf miners were most abundant in the first two years of the study (Fig 1b), but there was no significant effect of treatment on miner density in any year. Levels of parasitism declined over the four years (Fig. 1c) with parasitism in 1999 and 2000 being significantly lower on mycorrhizal plants ($F_{1,18}$ = 2.5, $P < 0.05$ and $F_{1,18}$ = 14.7, $P < 0.01$, respectively).

Laboratory experiment

Colonisation of roots occurred in every inoculated treatment, but was absent in control plants. There was no difference in % RLC of any of the inoculated treatments, the grand mean being 26%. Inoculation with *G.m.* produced a significant increase in leaf number (Fig. 2a; $F_{1,152}$ = 98.8, $P < 0.001$). There were significant interactions between *G.c* and *G.f.*, because single inoculations of these species reduced leaf number, while dual inoculations produced plants with more leaves than the controls (Fig. 2a).

Miner density also varied between treatments (Fig. 2b). Inoculation with *G.m.* colonisation reduced miner density ($F_{1,152}$ = 8.4, $P < 0.01$), but again there was an interaction between *G.c.* and *G.f.* ($F_{1,152}$ = 5.9, $P < 0.05$), with single species inoculations reducing mine density, whereas plants with both fungi had a mine density indistinguishable from that of the control.

Overall, *G.m.* caused a highly significant reduction in parasitism (Fig. 2c, $F_{1,152}$ = 15.3, $P < 0.001$), while *G.c.* increased it ($F_{1,152}$ = 5.8, $P < 0.05$). However, there was also a significant interaction between these two species ($F_{1,152}$ = 5.1, $P < 0.05$), as the antagonistic effect of *G.m*. was only clearly seen when *G.c.* was absent. Mines on plants colonised with the single inoculum of *G.f.* suffered greater parasitism than the controls (Fig. 2c).

DISCUSSION

AM fungi have clear effects on parasitism of herbivorous insects, detectable in both field and laboratory conditions. In two of the four years, when densities of the miner were higher, parasitism of *C. syngenesiae* in the field was lower on mycorrhizal plants. The fact that a consistent result was not found every year is important and may have been due to the abundance of the host plant and the miner. By 2001, the site was in its sixth year of succession and both plant and fly were considerably rarer than in the previous two years. This trend continued in 2002, when the fly was virtually absent. Multitrophic effects of AM fungi on parasitoids are therefore likely to be determined by the successional age of a community and future studies need to be set within a successional context.

AM effects on parasitoids may come about through some or all of three different mechanisms. These fungi have well known positive effects on plant size (Smith & Read 1997). However, as Gange & Ayres (1999) discuss, negative growth effects can often arise through certain host fungal combinations, high colonisation densities or particular abiotic situations (e.g. high soil P). Given that increased plant size or architecture can lead to decreased parasitoid searching efficiency (Cloyd & Sadof 2000; Gingras & Boivin 2002), one might predict that under 'normal' conditions of AM fungi increasing a host's stature, parasitoid attack rates may be lower on mycorrhizal plants, because these are larger. In the field, this certainly appeared to be so, with mycorrhizal plants being taller. However, the laboratory experiment showed that the situation is far more complex, with the mycorrhizal effect depending on the species of fungus colonising the root system. Certain fungal species or combinations reduced parasitism, while others increased it. In the three single species treatments, there was a pattern of decreased plant size leading to increased parasitism (*G.c* and *G.f.*) and vice versa (*G.m.*), a similar situation to that of Cloyd & Sadof (2002), where parasitism was negatively correlated with leaf number. However, dual or triple species inoculations of fungi tended to increase leaf number, but these did not always result in decreased miner abundance or parasitism.

A second mechanism is that parasitoids respond to volatiles emitted from the herbivore-damaged host plant (DeMoraes *et al*. 1998; Oppenheim & Gould 2002). Van Dam *et al*. (2003) point out that there are no known effects of AM fungi on induced defences in plants, as these fungi seem to suppress the defence response of their hosts (Mohr *et al.* 1998). However, AM fungi can have significant effects on the chemistry of host plant leaves, leading to alterations in herbivore performance (Gange & West 1994; Goverde *et al*. 2000). It is therefore possible that the chemistry of leaves on mycorrhizal plants may be altered to such

an extent as to affect the attractiveness of damaged leaves to a foraging parasitoid. Finidori-Logli *et al*. (1996) have identified several widely-occurring volatiles that are released in response to herbivore damage and that are attractive to foraging *D. isaea*. These occur within the closely-related genus *Chrysanthemum* (Storer *et* al. 1993) and so it is quite possible that they occur within *L. vulgare*. Furthermore, their production could be affected by mycorrhizally-determined carbon availability within the leaf (Gange & West 1994).

Parasitoids may respond to the density of their hosts in a positive, negative or null manner (Hassell 2000). While not explicitly tested for in this study, there appeared to be little evidence for density-dependent parasitism, with no relations between miner density and parasitism. The extremely limited evidence available in the literature suggests that AM effects on folivorous insects may translate into differences in population densities (Gehring & Whitham 2002). Thus, the potential exists for AM fungi to alter parasitism rates through changing the abundance of the phytophage if density-dependence occurs. These effects are likely to be confounded by effects of plant size or chemistry.

The community composition of AM fungi inhabiting the root system of a plant can change, both temporally and spatially (Helgason *et al*. 1999). We have shown that variations in AM fungal species within a root system can have different effects on higher trophic levels, mediated through changes in plant size and possibly chemistry. The nature of these effects needs to be understood, not just to enhance our knowledge of community structuring forces, but for the application of strategies involving natural enemies in pest control situations.

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FIGURE LEGENDS

Figure 1 (a) Mean height of *L. vulgare*, (b) Percent of leaves mined by *C. syngenesiae* and (c) Percent of mines parasitised in mycorrhizal (↓) and reduced-mycorrhizal (⬇) plots in a wildflower meadow over a four year period. Vertical lines represent one standard error.

Figure 2 (a) Mean leaf number of *L. vulgare*, (b) percent of leaves mined and (c) percent of mines parasitized when host plants were grown with different combinations of arbuscular mycorrhizal fungal species. Key to abbreviations: -: no mycorrhiza (control); c: *Glomus caledonium*, f: *G. fasciculatum* and m: *G. mosseae*. Vertical lines represent one standard error.

Figure 1

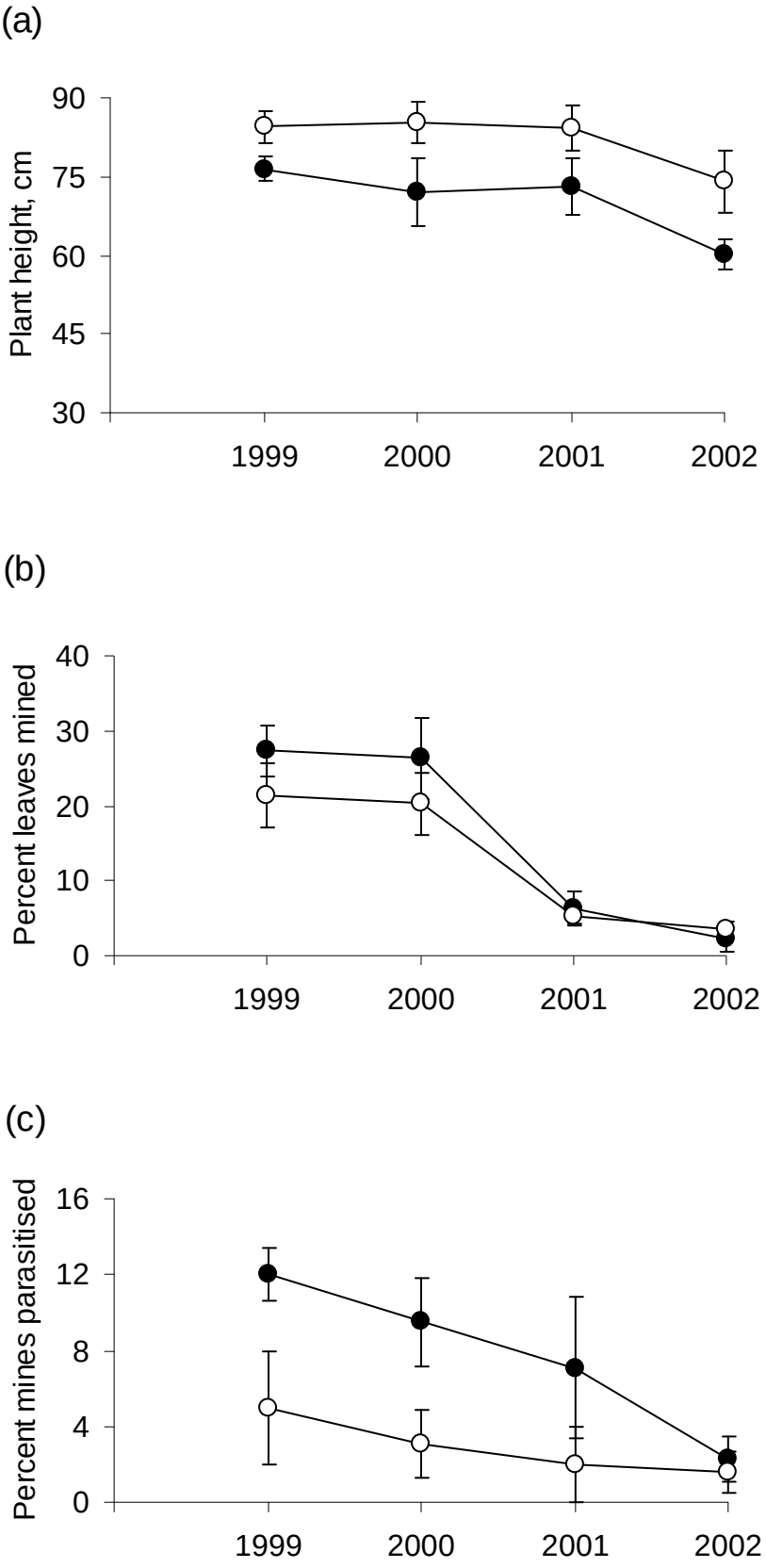
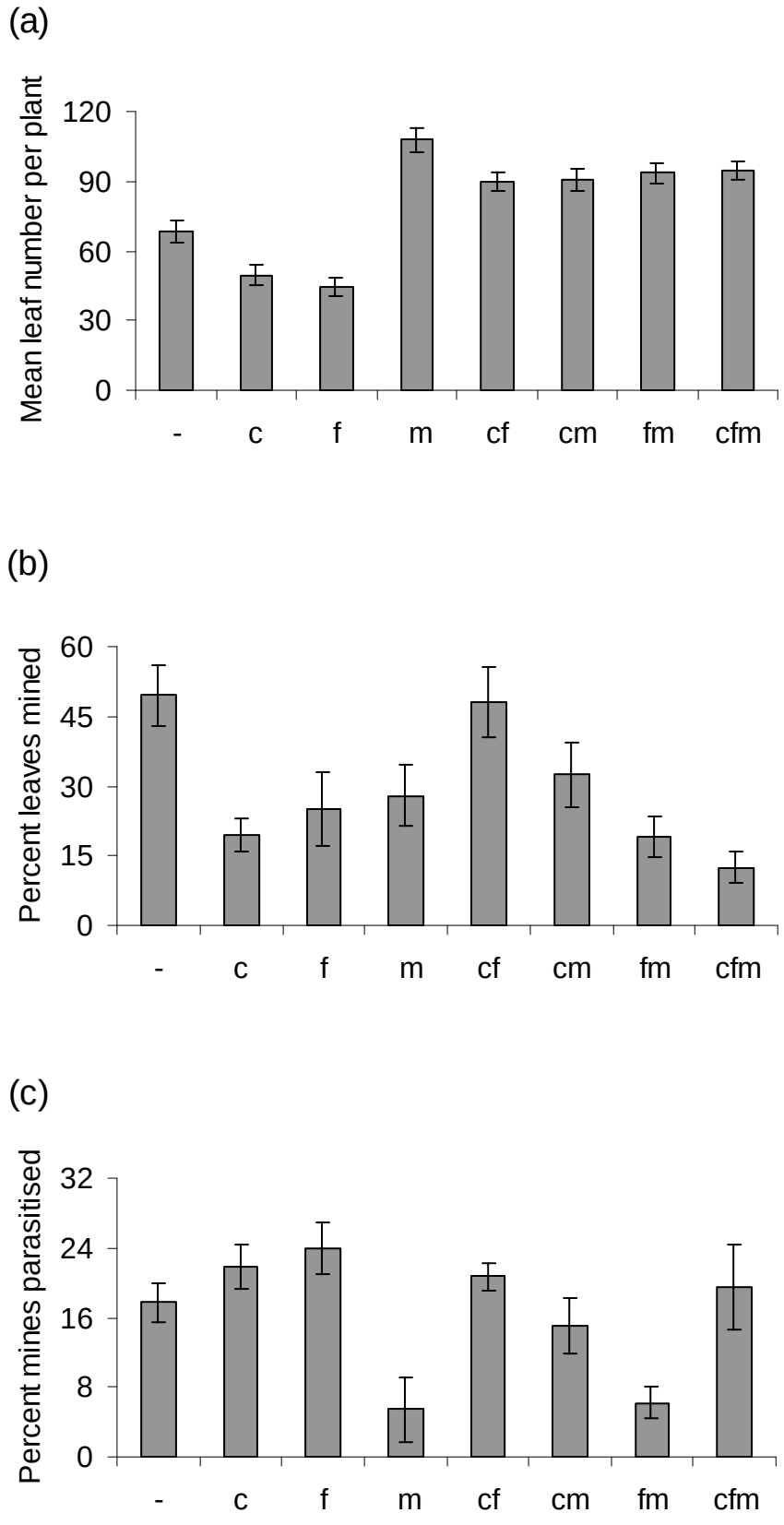

(a)
Plant height, cm
90
75
60
45
30
1999
2000
2001
2002
(b)
Percent leaves mined
40
30
20
10
0
1999
2000
2001
2002
(c)
Percent mines parasitised
16
12
8
4
0
1999
2000
2001
2002

Figure 2


(a)
Mean leaf number per plant
120
90
60
30
0
-
c
f
m
cf
cm
fm
cfm
(b)
Percent leaves mined
60
45
30
15
0
-
c
f
m
cf
cm
fm
cfm
(c)
Percent mines parasitised
32
24
16
8
0
-
c
f
m
cf
cm
fm
cfm