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Alan C. Gange · Erica Bower · Valerie K. Brown

Differential effects of insect herbivory on arbuscular mycorrhizal colonization

A. C. Gange (✉) · E. Bower
School of Biological Sciences,
Royal Holloway University of London,
Egham, Surrey TW20 0EX, UK
Tel. +44 (0) 1784 443188
Fax. +44 (0) 1784 470756
e: a.gange@rhul.ac.uk

V.K. Brown
Centre for Agri-Environmental Research,
Department of Agriculture,
University of Reading,
Earley Gate,
PO Box 237,
Reading RG6 6AR, UK

Correspondence author: A.C. Gange, address above

Abstract A series of field and laboratory experiments were conducted to examine whether natural levels of insect herbivory affect the arbuscular mycorrhizal colonization of two plant species. The plant species were the highly mycorrhizal (mycotrophic) *Plantago lanceolata*, which suffers small amounts of insect damage continuously over a growing season and the weakly mycorrhizal (non-mycotrophic) *Senecio jacobaea*, which is frequently subject to rapid and total defoliation by moth larvae.

Herbivory was found to reduce AM colonization in *P. lanceolata*, but had no effect in *S. jacobaea*. Similarly, AM colonization reduced the level of leaf damage in *P. lanceolata*, but had no such effect in *S. jacobaea*. AM fungi were found to increase growth of *P. lanceolata*, but this effect was only clearly seen when insects were absent. AM fungi reduced the growth of *S. jacobaea* irrespective of whether insects were present.

It is concluded that the reduction of AM fungal colonization by herbivory in *P. lanceolata* is due to the reduced amount of photosynthate available to the symbiont. This may only become apparent at threshold levels of insect damage and, below these, increased photosynthesis elicited by the mycorrhiza is able to compensate for foliage loss to the insects. However, in *S. jacobaea*, the mycorrhiza appears to be an aggressive parasite and insect attack only exacerbates the reduction in biomass. In mycotrophic plants, insect herbivores may be responsible for poor functioning of the symbiosis in field conditions and there is a symmetrical interaction between insects and fungi. However, in non-mycotrophic plants, the interaction is strongly asymmetrical, being entirely in favour of the mycorrhiza.

Keywords insect herbivory, arbuscular mycorrhiza, *Plantago lanceolata*, *Senecio jacobaea*

Introduction

Arbuscular mycorrhizal (AM) fungi form associations with the roots of a wide variety of vascular plants. The consequences of this association for the host plant vary along a continuum from positive (most common) to negative (Francis and Read 1995; Johnson et al. 1997). Traditionally, it has been assumed that positive effects on plants are brought about by the enhanced nutrient supply to a mycorrhizal plant, compared with non-mycorrhizal conspecifics. However, it has now been shown that plants may benefit from being mycorrhizal in other ways. The presence of the fungal associates may lead to improved performance in times of stress, for example when water is limiting (Smith and Read 1997), or if the plant is attacked by pathogenic fungi (e.g. Newsham et al. 1995; West 1997) or insect herbivores (Gange and Bower 1997; Gange 2001).

It has been suggested that, for any plant, there exists a curvilinear relation between the extent of AM fungal colonization and the degree of benefit the plant exhibits (Gange and Ayres 1999). For some plants, there may be a positive effect over a wide range of colonization densities, while for others, even very low levels of colonization can result in a decrease in plant performance. Excellent experimental examples of these effects are given by Francis and Read (1995). The reasons for the apparent negative effect of some mycorrhizal species on some plant species are unclear, but include loss of photosynthate to the mycorrhiza, nutrient immobilization, altered root exudation leading to allelopathy and effects on other components of the rhizosphere microflora (Gange and Ayres 1999). It has been estimated that losses of photosynthate to the AM association are in the order of 6-10% per annum (Tinker et al. 1994). Therefore any other factor, such as herbivory, which also results in photosynthate loss could mean that a plant that is mycorrhizal and attacked by herbivores exhibits no benefit from the mycorrhiza, because the loss of carbon to fungi and herbivores outweighs any advantage from increased nutrient uptake.

It is a fair assumption that in field situations, any plant colonized by AM fungi is also likely to be attacked by foliar-feeding insects. There is an extensive literature showing how foliage loss to insects can result in decreased individual plant yield, altered population dynamics and community structure (Crawley 1997). Gehring and Whitham (1994) reviewed the interactions between above-ground herbivores and mycorrhizal fungi. In their paper, 'herbivory' was taken to include manual defoliation as well as grazing by large mammals. For those plants which formed an AM association, herbivory reduced mycorrhizal colonization in 66% of cases. However, a feature of this review is that there were no studies involving insect herbivores, a situation that had not changed by the time of the review by Gange and Bower (1997). In the latter paper, evidence is given of a reduction in AM colonization of *Plantago lanceolata* L due to foliage removal by *Arctia caja* L., but to our knowledge, this remains the only example of insect herbivory affecting AM colonization. The availability of carbon is likely to be a critical factor in understanding the multitrophic interactions between subterranean fungi and foliar insects, because both are competitors for this resource. It is therefore surprising that, while there are a number of studies that have examined whether the presence of AM fungi can affect foliar-feeding insect performance, those that have asked whether foliage removal by insects has an effect on the mycorrhiza are conspicuous by their absence. If much leaf area is lost to foliar-feeding insects, there may be either of two possible consequences for the mycorrhiza: (1) if the carbon supply to the AM association is maintained, then the mycorrhiza could become a carbon parasite, leading to

strong negative effects of AM colonization on plant growth or (2) if loss of leaf area means a reduced carbon supply to the roots, the mycorrhiza may decline in abundance, also resulting in lowered plant performance, though not to the extent as in (1). Scenario (1) would have the effect of lowering the curvilinear relation of Gange and Ayres (1999) down the y axis, while scenario (2) would move the curve towards zero along the x axis.

Assuming that the curvilinear response of plants to AM colonization density is valid, and that foliar-feeding insects can reduce AM colonization, we hypothesised that the effect of herbivory may differ in plants that are positively affected by AM fungi, compared with those which are antagonised. Thus, in a mycotrophic plant which benefits from colonization at virtually any density, a lowering of AM abundance as a result of herbivory should have little effect plant performance. However, in a plant which is antagonised by virtually any colonization density (non-mycotrophic), herbivory may actually benefit the plant to a degree, because the 'parasitic' effect of the mycorrhiza is reduced. We tested this hypothesis using a series of laboratory and field experiments with *P. lanceolata*, a species that benefits greatly from AM colonization (Gange and West 1994) and *Senecio jacobaea* L., which does not (Bower 1997).

Materials and methods

Plant and insect species

P. lanceolata is a perennial forb, which can flower in its first year from seed. It is attacked by a range of generalist insects, none of which usually cause substantial defoliation (Scorer 1913). Larvae of *Arctia caja* (Lepidoptera: Arctiidae) frequently feed upon it in the UK. This species hibernates as larvae in cold winters, but will feed intermittently if the weather is warm. This loose diapause can be simulated in the laboratory, where larvae will feed slowly for a long period, given adequate temperature (Friedrich 1986). *P. lanceolata* is strongly mycorrhizal and has a well-studied defensive chemistry consisting of carbon-based iridoid glycosides (Bowers and Stamp 1992). Colonization by AM fungi can increase glycoside content of leaves, leading to a reduction in the growth of *A. caja* (Gange and West 1994).

S. *jacobaea* produces a rosette of leaves in its first year and will only flower having reached a threshold size and received adequate vernalization (Prins et al. 1990). It is weakly mycorrhizal (Harley and Harley 1987) and has a defensive chemistry based on nitrogen-containing pyrrolizidine alkaloids. This chemistry has been very well studied (e.g. Vrieling and van Wijk 1994), particularly in relation to the Cinnabar moth (*Tyria jacobaeae* L.), larvae

of which frequently cause 100% defoliation in summer. Plants can regrow some foliage and even flower after the defoliation event (Islam and Crawley 1983).

Field surveys of established plants

Two field sites were chosen on the campus of Royal Holloway, University of London, Surrey, UK. The site used for sampling of *P. lanceolata* was a meadow, mown in spring and autumn with the dominant vegetation being *Agrostis stolonifera* L., *Holcus lanatus* L., *Leucanthemum vulgare* L., *Trifolium pratense* L., and *P. lanceolata*. Ten plants of *P. lanceolata* were chosen at random at monthly intervals over the course of one calendar year. Before each plant was disturbed, the insect fauna was removed manually, counted and identified. Total leaf number and the number damaged by insects was recorded. Each plant was carefully dug up, ensuring that the root system remained as intact as possible. Roots were washed free of soil and arbuscular mycorrhizal colonization of each plant recorded using autofluorescence microscopy (Gange et al. 1999). Arbuscules were quantified using the cross-hair eyepiece method of McGonigle et al. (1990).

Deleted: b

The second site was a similar meadow, close to the other site, in which the dominant vegetation was *A. stolonifera*, *Luzula campestris* L., *Rumex acetosella* L., and *S. jacobaea*. Ten plants of *S. jacobaea* were selected at random at monthly intervals. Insect damage and mycorrhizal colonization were recorded in the same way as for *P. lanceolata*.

Manipulative field experiments

Two field sites were established, one at Imperial College at Silwood Park, Berkshire, UK and one on the campus of Royal Holloway, University of London, UK. Both sites were of sandy loam soils, overlying Bagshot Sands. The site at Silwood Park was used for the *P. lanceolata* experiment and was adjacent to that described in the experiment of Gange and West (1994). Here, the soil was acidic (pH 5.4) and nutrient levels were 2.1 μg NO_3^- g^{-1} and 3.9 μg P g^{-1} (bicarbonate extractable). The *S. jacobaea* experiment was at Royal Holloway and was very similar, with a pH of 5.7, 2.6 μg NO_3^- g^{-1} and 3.1 μg P g^{-1}.

Each site was treated with weedkiller ('Roundup', containing 360 g l^{-1} glyphosate) in autumn, shallow ploughed in winter and hand raked the following spring. Sixty plots, each 30 cm x 30 cm and separated by 50 cm buffer zones, were arranged in a randomized block design, with four plots in a block each allocated to one of four treatments. These were control (natural levels of AM colonization and insect herbivory); insecticide-treated (where the foliar

insecticide 'BioLonglast'® (P.B.I., Waltham Cross Herts, UK), containing the contact permethrin (53.2 g l^{-1}) and systemic dimethoate (8.6 g l^{-1}), diluted to 4.5 ml l^{-1}, was applied at 50 ml m^{-2}); fungicide-treated (in which the granular contact soil fungicide 'Rovral' (containing 40% w/w iprodione) was applied at the rate of 2g m^{-2} formulated product) and insecticide- and fungicide-treated. The experiment was thus a 2 x 2 factorial, with 15 replicates of each treatment. Insecticide was applied with a hand-held sprayer, while fungicide was applied with a granular dispenser. Both treatments took place at fortnightly intervals. The insecticide used had contact and systemic action, thus controlling external and internal feeders.

Seeds of *P. lanceolata* and *S. jacobaea* were germinated in sterilized compost and planted out one per plot at the second true leaf stage. Rabbits were excluded from both sites by 2 cm wire mesh fencing and molluscs were reduced in number by the application of 'Mifaslug' (containing 6% w/w metaldehyde) pellets around each plant at fortnightly intervals. Treatment plots were hand-weeded, but surrounding vegetation in the buffer zones was left intact.

After 16 weeks, plants of *P. lanceolata* had finished flowering and were harvested. Each plant was carefully removed from the sandy soil and the shoot and root system separated. Leaf number and the number of insect-damaged leaves were counted. The shoot material was dried at 80°C for one week and weighed. Roots were washed free of soil and arbuscular mycorrhizal colonization recorded as previously described, with autofluorescence microscopy and the cross-hair eye piece method. At this stage, *S. jacobaea* plants had formed rosettes and so were maintained for a further year, being harvested after 68 weeks, when all plants had finished flowering. The same procedures and measurements were undertaken as for *P. lanceolata*.

In order to assess the effect of AM colonization of the regrowth of *S. jacobaea*, a separate experiment was conducted in which 40 plants (20 with and 20 without fungicide) were grown, in a field site adjacent to the one described above. After defoliation by *T. jacobaeae*, the plants were maintained for a period of five weeks and total leaf number on each counted at weekly intervals.

Laboratory experiments

Regular defoliation of *P. lanceolata*

Seeds of *P. lanceolata* were germinated in sterile sand and transplanted at the two true leaf stage into 13 cm diameter pots, each containing 450 g of John Innes number 2 compost (Gem Gardening). Initially, 400 g of compost was placed in each pot and AM inoculum added by placing a 2g layer of inert clay granules containing hyphae and spores from a culture of *Glomus intraradices*, previously isolated from the field site, on top of the compost. The remaining 50g of compost were placed on top of the inoculum and one seedling planted into the centre of each pot. One hundred and sixty replicate pots were established. Plants were maintained in a Constant Environment Room at 15°C with a light regime of 16:8 L:D and 75% RH.

Larvae of *Arctia caja* were reared from a single egg batch obtained from a female adult captured at Mercury Vapour light at Silwood Park. Larvae were reared on a mixed diet consisting of leaves of *Taraxacum* sect. *Ruderalia* Kirschner, Oellgaard & Stepanek (*T. officinale* Wigg. Group), *Rumex obtusifolius* L. and *Rubus fruticosus* L. agg. When they reached second instar, a single larva was placed on half of the 3 week old plants and allowed to feed for one week. Plants were enclosed in a muslin cage to prevent the escape of each larva; control (no herbivory) plants were also placed in identical cages. After the week, cages and larvae were removed and plants maintained insect-free for two weeks. After this time, ten randomly selected plants from each treatment (herbivory and control) were harvested and mycorrhizal colonization of each measured as described above. Foliar and root material were separated and dried to constant weight. The herbivory event was then repeated on the remaining 70 plants that had been previously attacked, with each herbivory plant again receiving a larva for a week. Once larvae had been used in the experiment they were not used again. In total, eight one-week herbivory events were performed, each followed by a two-week insect-free period. A total of eight harvests were performed and the experiment was terminated after 24 weeks. No plant mortality occurred during the experiment and no insects died during the herbivory events. By week 12, larvae had moulted to the third instar, but no other moulting took place.

Variation in the extent of defoliation on *S. jacobaea*

Plants of *S. jacobaea* were produced as for *P. lanceolata* (above) and a total of 120 plants were inoculated with *G. intraradices.* To simulate the nature of herbivory in the field, when plants were eight weeks old, they were exposed to a single herbivory event, of varying intensity. Third instar larvae of the polyphagous moth *Phlogophora meticulosa* L. were introduced at the rate of 0, 3 or 6 larvae per plant and allowed to feed for a twelve hour

period. Preliminary experiments had indicated that these rates and duration of feeding would produce defoliation rates of 0, 50% and 100%. Eight replicates of each treatment were harvested on day one of the experiment (immediately after the herbivory event) and four further harvests took place at ten day intervals over a period of 40 days. At each harvest, dry shoot biomass was recorded and AM colonization measured as above.

Statistical analysis

The seasonal change in AM colonization and insect herbivory of each plant species was examined with one way ANOVA, employing date as the main effect. All percentage data were subjected to the angular transformation prior to analysis (Zar 1996). The manipulative field experiments were analyzed with two-factor ANOVA, after testing for normality and homogeneity of variances, employing insecticide and fungicide as the main effects in the UNISTAT® statistical package. The effect of AM colonization on regrowth of *S. jacobaea* was examined with a repeated measures ANOVA. The laboratory experiments were analyzed with two-factor ANOVA, employing herbivory and date as main effects.

Results

Field surveys of established plants

There was a significant change in AM colonization levels of established *P. lanceolata* over the course of one calendar year ($F_{11,109} = 6.97$, $P < 0.001$; Fig. 1A). Colonization by arbuscules was highest at about 27% (root length colonized) in winter and spring, falling to about a third of this level during summer. No plants suffered 100% defoliation (total foliage loss), but the proportion of leaves damaged rose to 100% during summer (Fig. 1B). Insect damage also showed a distinct seasonal trend ($F_{11,109} = 7.11$, $P < 0.001$), with the pattern being almost a mirror image of that of AM colonization. Leaf damage consisted of edge chewing by Lepidoptera and non-edge (i.e. laminar holes) chewing by Coleoptera. Lepidopteran damage occurred mostly in early autumn, while Coleopteran damage occurred during April – June.

S. jacobaea had far lower levels of AM colonization than *P. lanceolata* (Fig. 1C), but there was still a significant seasonal change in colonization ($F_{11,109} = 2.48$, $P < 0.05$) that was similar to *P. lanceolata*. Colonization fell to virtually zero between June and September and peaked at about 6% root length colonized in mid winter. The pattern of insect damage was also the opposite of that seen in colonization (Fig. 1D), with 100% damage occurring in

August, falling to about 10% damage in mid winter ($F_{11,109} = 5.87$, $P < 0.001$). The spring peak of damage was caused almost entirely by *Longitarsus jacobaeae* Wat. (Coleoptera: Chrysomelidae) while the August peak was exclusively due to *T. jacobaeae.* At this time, many plants were completely defoliated by larvae of this insect.

Manipulative field experiments

P. lanceolata

Application of insecticide was very effective in reducing insect damage (Fig. 2A) while fungicide application significantly increased the proportion of leaves attacked (Table 1). Although there was a statistical interaction between the treatments, this is of little relevance, as it is caused by there being no such fungicide-induced increase in damage in plants treated with both compounds, due to the insecticide being applied.

Application of fungicide was successful in reducing AM colonization (Fig. 2B) while insecticide significantly increased it (Table 1). Again, there was a significant interaction between the treatments. This was caused by the fact that, in the presence of insects, fungicide had little effect on colonization, while if insects were reduced, the effect of fungicide application could be clearly seen.

Application of insecticide significantly increased dry foliar biomass, while fungicide decreased it (Fig. 2C, Table 1). However, of more interest was the significant interaction between the treatments, as the effect of fungicide was only clearly seen when insects were excluded. Therefore, in this experiment, AM fungi gave a growth benefit to plants only when insects were rare and not when they were common, suggestive of the fact that insect herbivory was having a negative effect on the abundance (Fig. 2B) and functioning (Fig. 2C) of the mycorrhiza.

S. jacobaea

Insecticide application was extremely effective in reducing damage in this species (Fig. 3A), but fungicide application had no effect (Table 2). Meanwhile, colonization was reduced by fungicide, but unaffected by insecticide (Fig. 3B, Table 2). Perhaps the most interesting fact was that application of either compound significantly increased dry foliar biomass of this species (Fig. 3C, Table 2). Therefore, reducing mycorrhizal colonization and/or insect herbivory led to a positive growth benefit for the plant, suggesting that both were detrimental for this plant species. There were no interactions between the treatments, with the largest plants being those treated with both insecticide and fungicide (Fig. 3C).

The pattern of regrowth in colonized and uncolonized plants was very different (Fig. 4), leading to a significant interaction between mycorrhizal treatment and time ($F_{4,232} = 3.28$, $P < 0.05$). Plants without the AM association appeared to produce regrowth leaves faster than those which were colonized, suggestive that immediately after defoliation, the AM association was detrimental to the plant. After three weeks, mycorrhizal plants had caught up with non-mycorrhizal individuals and after five weeks, AM plants had nearly twice the number of leaves of uncolonized plants.

Laboratory experiments

P. lanceolata

Mycorrhizal colonization was virtually zero at the start of the experiment, when plants were three weeks old (Fig. 5A). However, this increased rapidly and after 24 weeks, plants without herbivory had about 36% root length colonized. Herbivory caused a significant reduction in AM colonization ($F_{1,144} = 8.04$, $P < 0.01$), although this did not become apparent until five 'events' had taken place, on week 18. At the end of the experiment, AM colonization of plants subject to herbivory was only 20%.

The effect of herbivory was manifest in shoot (Fig. 5B) and root biomass (Fig 5C). The effect on root biomass was particularly dramatic ($F_{1,144} = 39.79$, $P < 0.001$) with a 58% reduction in this parameter. After 21 weeks, root production had virtually ceased in attacked plants, while that of control plants was increasing rapidly. This led to a significant interaction between herbivory and time ($F_{7,144} = 5.72$, $P < 0.001$).

S. jacobaea

Colonization of all plants was very similar at the start of the experiment (Fig. 6). However, after 10 days, 100% defoliation had caused a significant reduction ($F_{2,81} = 8.71$, $P < 0.001$). After 20 days, colonization was decreased dramatically by total defoliation, although it had recovered after 40 days. The 50% defoliation treatment had no significant effect on colonization and in this and the control (no herbivory) treatment, colonization remained at about 4% throughout the experiment.

The efficacy of the treatments can be seen in Fig. 6B, in which the three larvae treatment reduced foliar biomass by 52% while the six larval treatment reduced it by 95%. Biomass slowly recovered in each treatment, but by the end of the experiment, it was still significantly lower in attacked plants compared with the undefoliated controls ($F_{2,81} = 9.45$, $P < 0.001$).

Discussion

Mycorrhizal phenology

These relatively simple, but realistic, experiments have shown that insect herbivores can affect the mycorrhizal colonization of plants, but in a complex way. The effects were different in the two plant species studied, because mycorrhizal colonization appeared to be of great benefit to *P. lanceolata*, but detrimental to *S. jacobaea*. Both plant species exhibited a seasonal change in AM colonization level, with relatively high levels from autumn through to spring with a decrease during summer. Throughout the year, *P. lanceolata* was much more heavily colonized than *S. jacobaea*, with the lowest level for *P. lanceolata* of 6% being similar to that of the highest recorded for *S. jacobaea*, of 5.8%. *S. jacobaea* also exhibited much plant to plant variation, with many individuals being uncolonized, while one specimen (in November) had a colonization level of 21%. Seasonal changes in AM colonization are typical of herbaceous plants growing in temperate ecosystems, although the patterns we observed are different to several other studies. For example, Ietswaart et al. (1992) found that colonization of *Agrostis capillaris* L. peaked in summer and was lowest in winter, as did DeMars and Boerner (1995) who studied three different woodland herbs. Indeed, our data resemble those obtained by Merryweather and Fitter (1995) with the vernal *Hyacinthoides non-scripta* (L.) Chouard ex Rothm.

No previous study of mycorrhizal phenology has examined simultaneously the incidence of insect herbivory. It is therefore tempting to suggest that the phenologies of AM colonization recorded were direct results of foliage damage, as when damage was high, colonization was low, (and vice versa), in both plant species. However, AM phenology is also affected by environmental factors, such as soil temperature and water availability (e.g. Beena et al. 2000), though our data do suggest that foliage-feeding insects are another factor causing seasonality of mycorrhizas.

Interactions between insects and AM fungi in mycotrophic plants

In *P. lanceolata*, insect herbivory reduced AM colonization in the manipulative field experiment by 56%. However, reducing mycorrhizas by fungicide application increased the proportion of leaves damaged by 38%. In a similar experiment, in an adjacent field site, Gange and West (1994) also found that fungicide application increased the proportion of damaged leaves by 58%. We found that when insects were abundant, AM fungi had no effect on plant biomass, but when insects were reduced, mycorrhizas were seen to have a positive

effect. These results suggest that foliage removal by insects reduces the functioning of the mycorrhiza, over the course of a season. It is therefore likely that the failure to detect a mycorrhizal response in many field trials (McGonigle 1988) has been due to the lack of insect control in such experiments. Conversely, when AM fungi were abundant, insects had a large negative effect on biomass, but if AM fungi were reduced, insects had no effect. The latter result is more surprising, because one may expect that plants in the fungicide treatment would have greatly reduced biomass, by having the lowest colonization level, through a combination of fungicide application and increased insect herbivory. However, this did not occur and suggests that *P. lanceolata* is a plant that benefits from AM presence at virtually any colonization density, thus confirming our original hypothesis for mycotrophic plants. According to Gange and Ayres (1999), since there is a curvilinear response of plants to AM colonization, it is possible to reduce AM levels very considerably, but still detect no effect on the host plant. These data also suggest that the negative effect of AM fungi on chewing insects in *P. lanceolata* (Gange and West 1994) is of relatively less importance than the negative effect of insects on the fungal association. Insecticide-treated plants therefore grew best because they had least herbivory and highest colonization levels. One would not expect the dual chemical treatment plants to show higher biomass than the fungicide-treated plants, because any potential increase in colonization resulting from reduced herbivory would be cancelled out by the application of fungicide.

To our knowledge, this is the first study to show that insect herbivory can reduce AM colonization in field and laboratory conditions. Several authors have examined the effects of large mammal grazing, with mixed results. Bethlenfalvay and Dakessian (1984) and Trent et al. (1988) found that grazing reduced AM colonization of grasses, while Wallace (1987) could find no effect of ungulates (mainly bison) on several species of prairie grasses. Meanwhile, Wallace (1981) found a positive correlation between grazing intensity and AM colonization of plant species in a Serengeti grassland. Other studies have examined the effects of manual defoliation on mycorrhizas in which foliage removal has reduced colonization (Daft and El-Giahmi 1978; Allsopp 1998) or had little or no effect (Borowicz 1993; Hartley and Amos 1999). However, interpretation of all these studies in terms of plant performance is difficult, because the reverse interaction (effect of mycorrhiza on the herbivore) is absent in manually defoliated plants or unknown in vertebrates (Gange and Bower 1997).

When reductions in AM colonization have been found, the explanation usually given is that loss of photosynthetic tissue impairs the ability of plants to support the carbon demand of

411 the mycorrhiza (Gehring and Whitham 1994; Gange and Bower 1997). Such an hypothesis,
412 based on carbon limitation, is consistent with other situations of reduced AM levels when
413 photosynthesis is reduced, such as low irradiance (generally shading) (Smith and Read 1997).
414 When carbon allocation has been measured, it has been found that clipping of foliage reduces
415 the availability of carbon to the roots, resulting in poorer functioning of the mycorrhiza
416 (Borowicz and Fitter 1990). It is possible that carbon limitation is the explanation for reduced
417 AM colonization in insect-attacked *P. lanceolata,* particularly as this plant has a defensive
418 chemistry involving carbon-based iridoid glycosides (Duff et al. 1965). In this respect, a
419 plant species likely to be colonized by AM fungi, but also attacked by insects, faces the
420 classic problem of whether to 'grow or defend' (Herms and Mattson 1992). 'Growth' in this
421 case needs to be interpreted not just as plant biomass, but the construction and maintenance of
422 the mycorrhizal association as well.

423 There are many studies showing that AM fungi can increase photosynthesis, particularly
424 when nutrients are limiting (Fay et al. 1996; Black et al. 2000). Indeed, this has been shown
425 for *P. lanceolata* (Staddon et al. 1999), but in this and other species, the extra carbon fixed is
426 allocated to the mycorrhiza, rather than the plant itself (Wright et al. 1998; Staddon et al.
427 1999). Such increases in carbon allocated to the fungus may explain why some studies
428 involving manual defoliation of plants appear to show no effect on the mycorrhiza. However,
429 there must be a limit to the extent of defoliation, beyond which the mycorrhizally-induced
430 increase in C fixation is no longer possible, with a resulting decrease in colonization as carbon
431 supply is impaired. There are very few studies that have examined whether the degree of
432 foliage removal affects AM colonization. Perhaps the clearest is one of the first, by Daft and
433 El-Giahmi (1978). In that study, there was a suggestion of a linear relation between intensity
434 of defoliation and AM colonization in maize (*Zea mays* L.) and tomato (*Lycopersicon*
435 *esculentum* Miller), with 60% defoliation of each species reducing colonization to about 40%
436 of the value on undefoliated plants.

437 We examined the effect of the degree of defoliation in *P. lanceolata* by allowing damage
438 to accumulate on potted plants, in a manner that mimics the pattern of attack in the field. In
439 this experiment, a reduction in AM colonization was not seen immediately, but only became
440 clear after 18 weeks, when plants had been attacked five times, for a total of five weeks. By
441 the end of the experiment, herbivory had reduced AM colonization by 40%, a similar situation
442 to that seen in the experiment reported by Gange and Bower (1997), in which cumulative
443 herbivory reduced the colonization of *P. lanceolata* by *Glomus mosseae* (Nicol. & Gerd.) by
444 33%. These data are strongly suggestive that for a time, the plants in these experiments were

able to maintain the mycorrhiza, through a mycorrhizal-enhanced availability of C. However, by about week 18 a threshold value of herbivory may have been exceeded, meaning that the carbon supply to the mycorrhiza began to be impaired, resulting in a loss of arbuscular colonization. Therefore, in field conditions, plants that are mycorrhizal may only lose the benefits from their mutualists if insect herbivory exceeds certain levels.

Interactions between insects and AM fungi in non-mycotrophic plants

In the mycotrophic *P. lanceolata*, there is a virtually symmetrical interaction between insects and fungi, with the advantage being in favour of the insects. However, we found quite the reverse situation in the non-mycotrophic *S. jacobaea*. In this species, insect herbivory had no effect on AM colonization in the manipulative field experiment, even though many of the plants in non-insecticide treatments were completely defoliated by *T. jacobaeae*. AM colonization had no effect on herbivory, with both control and fungicide-treated plants suffering about 80% of their leaves damaged. Perhaps the most interesting result was that irrespective of whether insects were present or absent, AM fungi had a detrimental effect on plant growth, as application of fungicide increased biomass, relative to the control. Fungicide application can be a relatively crude tool with which to manipulate mycorrhizal fungi, as other root-inhabiting fungi may also be killed. If these were pathogenic, then chemical application might be seen to increase plant growth. The roots of both *P. lanceolata* and *S. jacobaea* from the field experiments were subjected to staining, to reveal all fungal structures, but very little non-mycorrhizal material could be found, an identical situation to that reported by Gange et al. (1999). We are confident that the treatment effect thus observed is real, and that if AM fungi colonize *S. jacobaea*, they are parasitic on this plant. Therefore, plants in control plots were smallest, being attacked by insects and a parasitic mycorrhiza.

We hypothesized that if insect herbivory reduces AM colonization, then such a parasitic effect of a mycorrhiza may disappear. This, however, did not happen in the field experiment. In the case of *S. jacobaea* colonization levels were low, variable, and similar to those of established plants. The overriding conclusion is that in natural situations, the majority of plants of *S. jacobaea* are uncolonized by AM fungi. Of the remainder, the vast majority exhibit low levels of colonization, but even these levels are detrimental to the growth of the plants. One can only assume that the fungi which do colonize this plant have a strong demand for carbon and are thus parasitic, being unaffected by even total foliage loss.

S. jacobaea suffers regularly from defoliation by *T. jacobaeae* larvae in southern England, but most plants appear to possess powers of regrowth and can even flower in the weeks

following such a catastrophic herbivory event (Islam and Crawley 1983). Further evidence for the detrimental effect of AM colonization in this plant was seen in our experiment on regrowth of mycorrhizal and non-mycorrhizal plants. Here, we found that mycorrhizal plants appeared to be at a distinct disadvantage immediately following defoliation. The regrowth of these plants was slower for the first three weeks, suggesting that energy resources which might have been used by the plant were being commandeered by the mycorrhiza. After six weeks, mycorrhizal plants were slightly larger, an effect that may have been the result of improved photosynthesis, if the mycorrhiza elicits a similar effect in this plant as it does in *P. lanceolata*. This result is in direct contrast to the study of Hetrick et al. (1990) where AM fungi were beneficial in aiding the regrowth of the mycotrophic grass *Andropogon gerardii* Vit. following severe defoliation.

It is perhaps surprising that a plant can suffer 100% defoliation and yet still have no measurable loss in AM colonization. To investigate this problem, we again attempted to mimic the pattern of damage seen in the field, in which plants received 50% or 100% defoliation by Lepidopteran larvae. Colonization was significantly reduced by total defoliation, but this effect was transient and mycorrhizal levels had recovered by 40 days after the event. However, biomass levels had not, again suggesting that the mycorrhiza was acting as a hindrance to plant growth. Therefore, in non-mycotrophic plants such as *S. jacobaea*, there is a highly asymmetrical interaction between insect and fungus, with the advantage being purely in favour of the fungus.

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Table 1 Summary of Analysis of Variance results testing for the effects of insecticide (I), fungicide (F) and the interaction between them (I*F) on insect damage, mycorrhizal colonization and plant biomass in field-grown *P. lanceolata*. All degrees of freedom 1,56.

	Leaf damage		AM colonization		Plant foliar biomass	
	F	*P*	*F*	*P*	*F*	*P*
I	109.9	< 0.001	26.68	< 0.001	13.19	< 0.001
F	96.51	< 0.001	48.35	< 0.001	10.61	< 0.001
I*F	53.97	< 0.001	17.09	< 0.001	3.32	< 0.05

Table 2 Summary of Analysis of Variance results testing for the effects of insecticide (I), fungicide (F) and the interaction between them (I*F) on insect damage, mycorrhizal colonization and plant biomass in field-grown *S. jacobaea*. All degrees of freedom 1,56.

	Leaf damage		AM colonization		Plant foliar biomass	
	F	*P*	*F*	*P*	*F*	*P*
I	95.19	< 0.001	1.75	N.S.	23.81	< 0.001
F	0.062	N.S.	4.31	< 0.05	7.66	< 0.01
I*F	0.91	N.S.	0.21	N.S.	0.039	N.S.

Figure legends

Fig. 1 Naturally-occurring seasonal changes in arbuscular mycorrhizal colonization (A) and associated insect damage (proportion of leaves attacked) (B) of *Plantago lanceolata* and colonization (C) and damage (D) in *Senecio jacobaea*. Values are means ! one standard error.

Fig. 2 Proportion of leaves damaged by insects (A), arbuscular mycorrhizal colonization (B) and dry foliar biomass (C) of field-grown *Plantago lanceolata*. Key: control: natural levels of insects and mycorrhizas; F: application of soil fungicide; I: application of foliar insecticide; FI: application of both compounds. Values are means ! one standard error.

Fig. 3 Proportion of leaves damaged by insects (A), arbuscular mycorrhizal colonization (B) and dry foliar biomass (C) of field-grown *Senecio jacobaea*. Key as in Fig 3.

Fig. 4 Regrowth of mycorrhizal (↓) and non-mycorrhizal (🡻) *Senecio jacobaea* plants, after total defoliation by larvae of *Tyria jacobaeae*. Values are means ! one standard error.

Fig. 5 Changes in arbuscular mycorrhizal colonization (A), shoot (B) and root (C) biomass of *Plantago lanceolata* attacked one week in every three by larvae of *Arctia caja*. Herbivory events occurred in weeks 1,4,7,10,13,16,19 and 22 of the experiment and the first harvest was on week three. Key: (🡻) no herbivory; (↓) herbivory. Values are means ! one standard error.

Fig. 6 Changes in arbuscular mycorrhizal colonization (A) and shoot biomass (B) of *Senecio jacobaea*, following zero (🡐), 50% (↖) or 100% (↓) defoliation of foliar tissues. Values are means ! one standard error.