

1 Date of preparation: May 27, 2009

2

3

4

5

6

7

8

9

10

11

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

13

14

15 **Differential effects of insect herbivory on arbuscular mycorrhizal colonization**

16

17

18

19

20

21

22

23

24

25

26 A. C. Gange (✉) · E. Bower

27 School of Biological Sciences,

28 Royal Holloway University of London,

29 Egham, Surrey TW20 0EX, UK

30 Tel. +44 (0) 1784 443188

31 Fax. +44 (0) 1784 470756

32 e: a.gange@rhul.ac.uk

33

34 V.K. Brown

35 Centre for Agri-Environmental Research,

36 Department of Agriculture,

37 University of Reading,

38 Earley Gate,

39 PO Box 237,

40 Reading RG6 6AR, UK

41

42 Correspondence author: A.C. Gange, address above

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

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

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

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

64

65 **Introduction**

66 Arbuscular mycorrhizal (AM) fungi form associations with the roots of a wide variety of
67 vascular plants. The consequences of this association for the host plant vary along a
68 continuum from positive (most common) to negative (Francis and Read 1995; Johnson et al.
69 1997). Traditionally, it has been assumed that positive effects on plants are brought about by
70 the enhanced nutrient supply to a mycorrhizal plant, compared with non-mycorrhizal
71 conspecifics. However, it has now been shown that plants may benefit from being
72 mycorrhizal in other ways. The presence of the fungal associates may lead to improved
73 performance in times of stress, for example when water is limiting (Smith and Read 1997), or
74 if the plant is attacked by pathogenic fungi (e.g. Newsham et al. 1995; West 1997) or insect
75 herbivores (Gange and Bower 1997; Gange 2001).

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

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

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

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

126

127 **Materials and methods**

128

129 Plant and insect species

130 *P. lanceolata* is a perennial forb, which can flower in its first year from seed. It is attacked by
 131 a range of generalist insects, none of which usually cause substantial defoliation (Scorer
 132 1913). Larvae of *Arctia caja* (Lepidoptera: Arctiidae) frequently feed upon it in the UK. This
 133 species hibernates as larvae in cold winters, but will feed intermittently if the weather is
 134 warm. This loose diapause can be simulated in the laboratory, where larvae will feed slowly
 135 for a long period, given adequate temperature (Friedrich 1986). *P. lanceolata* is strongly
 136 mycorrhizal and has a well-studied defensive chemistry consisting of carbon-based iridoid
 137 glycosides (Bowers and Stamp 1992). Colonization by AM fungi can increase glycoside
 138 content of leaves, leading to a reduction in the growth of *A. caja* (Gange and West 1994).

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

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

146

147 Field surveys of established plants

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

Deleted: b

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

163

164 Manipulative field experiments

165 Two field sites were established, one at Imperial College at Silwood Park, Berkshire, UK and
 166 one on the campus of Royal Holloway, University of London, UK. Both sites were of sandy
 167 loam soils, overlying Bagshot Sands. The site at Silwood Park was used for the *P. lanceolata*
 168 experiment and was adjacent to that described in the experiment of Gange and West (1994).
 169 Here, the soil was acidic (pH 5.4) and nutrient levels were $2.1 \mu\text{g NO}_3^- \text{g}^{-1}$ and $3.9 \mu\text{g P g}^{-1}$
 170 (bicarbonate extractable). The *S. jacobaea* experiment was at Royal Holloway and was very
 171 similar, with a pH of 5.7, $2.6 \mu\text{g NO}_3^- \text{g}^{-1}$ and $3.1 \mu\text{g P g}^{-1}$.

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

177 insecticide 'BioLonglast'® (P.B.I., Waltham Cross Herts, UK), containing the contact
178 permethrin (53.2 g l⁻¹) and systemic dimethoate (8.6 g l⁻¹), diluted to 4.5 ml l⁻¹, was applied at
179 50 ml m⁻²); fungicide-treated (in which the granular contact soil fungicide 'Rovral'
180 (containing 40% w/w iprodione) was applied at the rate of 2g m⁻² formulated product) and
181 insecticide- and fungicide-treated. The experiment was thus a 2 x 2 factorial, with 15
182 replicates of each treatment. Insecticide was applied with a hand-held sprayer, while
183 fungicide was applied with a granular dispenser. Both treatments took place at fortnightly
184 intervals. The insecticide used had contact and systemic action, thus controlling external and
185 internal feeders.

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

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

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

206

207 Laboratory experiments

208 Regular defoliation of *P. lanceolata*

209 Seeds of *P. lanceolata* were germinated in sterile sand and transplanted at the two true leaf
210 stage into 13 cm diameter pots, each containing 450 g of John Innes number 2 compost (Gem
211 Gardening). Initially, 400 g of compost was placed in each pot and AM inoculum added by
212 placing a 2g layer of inert clay granules containing hyphae and spores from a culture of
213 *Glomus intraradices*, previously isolated from the field site, on top of the compost. The
214 remaining 50g of compost were placed on top of the inoculum and one seedling planted into
215 the centre of each pot. One hundred and sixty replicate pots were established. Plants were
216 maintained in a Constant Environment Room at 15°C with a light regime of 16:8 L:D and
217 75% RH.

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

236

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

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

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

248

249 Statistical analysis

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

258

259 Results

260 Field surveys of established plants

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

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

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

280

281 Manipulative field experiments

282 *P. lanceolata*

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

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

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

300 *S. jacobaea*

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

309 The pattern of regrowth in colonized and uncolonized plants was very different (Fig. 4),
 310 leading to a significant interaction between mycorrhizal treatment and time ($F_{4,232} = 3.28$, $P <$
 311 0.05). Plants without the AM association appeared to produce regrowth leaves faster than
 312 those which were colonized, suggestive that immediately after defoliation, the AM
 313 association was detrimental to the plant. After three weeks, mycorrhizal plants had caught up
 314 with non-mycorrhizal individuals and after five weeks, AM plants had nearly twice the
 315 number of leaves of uncolonized plants.

316

317 Laboratory experiments

318 *P. lanceolata*

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

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

330

331 *S. jacobaea*

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

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

342

343 Discussion

344

345 Mycorrhizal phenology

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

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

369

370 Interactions between insects and AM fungi in mycotrophic plants

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

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

396 To our knowledge, this is the first study to show that insect herbivory can reduce AM
397 colonization in field and laboratory conditions. Several authors have examined the effects of
398 large mammal grazing, with mixed results. Bethlenfalvai and Dakessian (1984) and Trent et
399 al. (1988) found that grazing reduced AM colonization of grasses, while Wallace (1987)
400 could find no effect of ungulates (mainly bison) on several species of prairie grasses.
401 Meanwhile, Wallace (1981) found a positive correlation between grazing intensity and AM
402 colonization of plant species in a Serengeti grassland. Other studies have examined the
403 effects of manual defoliation on mycorrhizas in which foliage removal has reduced
404 colonization (Daft and El-Giahmi 1978; Allsopp 1998) or had little or no effect (Borowicz
405 1993; Hartley and Amos 1999). However, interpretation of all these studies in terms of plant
406 performance is difficult, because the reverse interaction (effect of mycorrhiza on the
407 herbivore) is absent in manually defoliated plants or unknown in vertebrates (Gange and
408 Bower 1997).

409 When reductions in AM colonization have been found, the explanation usually given is
410 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

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

450

451 Interactions between insects and AM fungi in non-mycotrophic plants

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

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

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

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

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

499
 500 **Acknowledgements** We are grateful to the Natural Environment Research Council for
 501 funding these studies.

502 503 **References**

- 504 Allsopp N (1998) Effect of defoliation on the arbuscular mycorrhizas of three perennial
 505 pasture and rangeland grasses. *Plant Soil* 202: 117-124
- 506 Beena KR, Raviraja NS, Sridhar KR (2000) Seasonal variations of arbuscular mycorrhizal
 507 fungal association with *Ipomoea pes-caprae* of coastal sand dunes, Southern India. *J Env*
 508 *Biol* 21: 341-347
- 509 Bethlenfalvay GJ, Dakessian S (1984) Grazing effects on mycorrhizal colonization and
 510 floristic composition of the vegetation on a semiarid range in northern Nevada. *J Range*
 511 *Manage* 37: 312-316

- 512 Black KG, Mitchell DT, Osborne BA (2000) Effect of mycorrhizal-enhanced leaf phosphate
513 status on carbon partitioning, translocation and photosynthesis in cucumber. *Plant Cell*
514 *Env* 23: 797-809
- 515 Borowicz VA (1993) Effects of benomyl, clipping and competition on growth of
516 prereproductive *Lotus corniculatus*. *Can J Bot* 71: 1169-1175
- 517 Borowicz VA, Fitter, AH (1990) Effects of endomycorrhizal infection, artificial herbivory,
518 and parental cross on growth of *Lotus corniculatus* L. *Oecologia* 82: 402-407
- 519 Bower E (1997) Interactions between arbuscular mycorrhizal fungi and foliar-feeding insects.
520 PhD thesis, University of London
- 521 Bowers MD, Stamp NE (1992) Chemical variation within and between individuals of
522 *Plantago lanceolata* (Plantaginaceae). *J Chem Ecol* 18: 985-995
- 523 Crawley MJ (1997) Plant-herbivore dynamics. In Crawley MJ (ed) *Plant Ecology*. Blackwell
524 Science, Oxford, pp. 401-474.
- 525 Daft MJ, El-Ghiahmi AA (1978) Effect of arbuscular mycorrhiza on plant growth VII. Effects
526 of defoliation and light on selected hosts. *New Phytol* 80: 365-372
- 527 DeMars BG, Boerner RJ (1995) Mycorrhizal dynamics of three different woodland herbs of
528 contrasting phenology along topographic gradients. *Am J Bot* 82: 1426-1431
- 529 Duff RB, Bacon JSD, Mundie CM, Farmer VC, Russell JD, Forrester AR (1965) Catalpol and
530 methylcatalpol: naturally occurring glycosides in *Plantago* and *Buddleia* species. *Biochem*
531 *J* 96: 1-5
- 532 Fay P, Mitchell DT, Osborne BA (1996) Photosynthesis and nutrient-use efficiency of barley
533 in response to low arbuscular mycorrhizal colonization and addition of phosphorus. *New*
534 *Phytol* 132: 425-433
- 535 Francis R, Read DJ (1995) Mutualism and antagonism in the mycorrhizal symbiosis, with
536 special reference to impacts on plant community structure. *Can J Bot* 73(Suppl): 1301-
537 1309
- 538 Friedrich E (1986) *Breeding Butterflies and Moths. A practical Handbook for British and*
539 *European Species*. Harley Books, Colchester
- 540 Gange AC (2001) Species-specific responses of a root- and shoot-feeding insect to arbuscular
541 mycorrhizal colonization of its host plant. *New Phytol* 150: 611-618
- 542 Gange AC, Ayres RL (1999) On the relation between arbuscular mycorrhizal colonization
543 and plant 'benefit'. *Oikos* 87: 615-621

- 544 Gange AC, Bower E (1997) Interactions between insects and mycorrhizal fungi. In Gange
545 AC, Brown VK (eds) *Multitrophic Interactions in Terrestrial Systems*. Blackwell Science,
546 Oxford, pp. 115-131
- 547 Gange AC, Bower E, Stagg PG, Aplin DM, Gillam AE, Bracken M (1999b) A comparison of
548 visualization techniques for recording arbuscular mycorrhizal colonization. *New Phytol*
549 142: 123-132
- 550 Gange AC, West HM (1994) Interactions between arbuscular-mycorrhizal fungi and foliar-
551 feeding insects in *Plantago lanceolata* L. *New Phytol* 128: 79-87
- 552 Gehring CA, Whitham TG (1994) Interactions between aboveground herbivores and the
553 mycorrhizal mutualists of plants. *TREE* 9: 251-255
- 554 Harley JL, Harley EL (1987) A check-list of mycorrhizas in the British flora. *New Phytol*
555 (Suppl) 105: 1-102
- 556 Hartley SE, Amos L (1999) Competitive interactions between *Nardus stricta* L. and *Calluna*
557 *vulgaris* (L.)Hull: the effect of fertilizer and defoliation on above- and below-ground
558 performance. *J Ecol* 87: 330-340
- 559 Herms DA, Mattson WJ (1992) The dilemma of plants: to grow or defend. *Quart Rev Biol*
560 67: 283-335
- 561 Hetrick BAD, Wilson GWT, Owensby CE (1990) Mycorrhizal influences on big bluestem
562 rhizome regrowth and clipping tolerance. *J Range Manage* 43: 286-290
- 563 Ietswaart JH, Griffioen WAJ, Ernst WHO (1992) Seasonality of VAM infection in three
564 populations of *Agrostis capillaris* (Gramineae) on soil with or without heavy metal
565 enrichment. *Plant Soil* 139: 67-73
- 566 Islam Z, Crawley MJ (1983) Compensation and regrowth in ragwort (*Senecio jacobaea*)
567 attacked by cinnabar moth (*Tyria jacobaea*). *J Ecol* 71: 829-843
- 568 Johnson NC, Graham JH, Smith FA (1997) Functioning of mycorrhizal associations along the
569 mutualism-parasitism continuum. *New Phytol* 135: 575-585
- 570 McGonigle TP (1988) A numerical analysis of published field trials with vesicular-arbuscular
571 mycorrhizal fungi. *Func Ecol* 2: 473-478
- 572 McGonigle TP, Miller MH, Evans DG, Fairchild GL, Swan JA (1990) A new method which
573 gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal
574 fungi. *New Phytol* 115: 495-501
- 575 Merryweather JM, Fitter AH (1995) Phosphorus and carbon budgets: mycorrhizal
576 contribution in the obligately mycorrhizal *Hyacinthoides non-scripta* (L.) Chouard ex
577 Rothm. under natural conditions. *New Phytol* 129: 619-627

- 578 Newsham KK, Fitter AH, Watkinson AR (1995) Multi-functionality and biodiversity in
579 arbuscular mycorrhizas. *TREE* 10: 407-411
- 580 Prins AH, Vrieling K, Klinkhamer PGL (1990) Flowering behaviour of *Senecio jacobaea*:
581 effects of nutrient availability and size-dependent vernalization. *Oikos* 59: 248-252
- 582 Scorer AG (1913) *The Entomologist's Log-book and Dictionary of the Life Histories and*
583 *Food Plants of the British Macrolepidoptera*. Routledge, London
- 584 Smith SE, Read DJ (1997) *Mycorrhizal Symbiosis*. Academic Press, San Diego
- 585 Staddon, PL, Fitter AH, Robinson D (1999) Effects of mycorrhizal colonization and elevated
586 atmospheric carbon dioxide on carbon fixation and below-ground carbon partitioning in
587 *Plantago lanceolata*. *J Exp Bot* 50: 853-860
- 588 Tinker PB, Durall DM, Jones MD (1994) Carbon use efficiency in mycorrhizas: theory and
589 sample calculations. *New Phytol* 128: 115-122
- 590 Trent JD, Wallace LL, Svejcar TJ, Christiansen S (1998) Effect of grazing on growth,
591 carbohydrate pools and mycorrhizae in winter wheat. *Can J Plant Sci* 68: 115-120
- 592 Vrieling K, van Wijk CAM (1994) Cost assessment of the production of pyrrolizidine
593 alkaloids in ragwort (*Senecio jacobaea* L.). *Oecologia* 97: 541-546
- 594 Wallace LL (1981) Growth, morphology and gas exchange of mycorrhizal and
595 nonmycorrhizal *Panicum coloratum* L., a C₄ grass species, under different clipping and
596 fertilization regimes. *Oecologia* 49: 272-278
- 597 Wallace LL (1987) Mycorrhizas in grasslands: interactions of ungulates, fungi and drought.
598 *New Phytol* 105: 619-632
- 599 Wardle DA (1999) How soil food webs make plants grow. *TREE* 14: 418-420
- 600 West HM (1997) Interactions between arbuscular mycorrhizal fungi and foliar pathogens:
601 consequences for host and pathogen. In: Gange AC, Brown VK (eds) *Multitrophic*
602 *Interactions in Terrestrial Systems*. Blackwell Science, Oxford, pp 79-89
- 603 Wright DP, Scholes JD, Read DJ (1998) Effects of VA mycorrhizal colonization on
604 photosynthesis and biomass production of *Trifolium repens* L. *Plant Cell Env* 21: 209-216
- 605 Zar JH (1996) *Biostatistical Analysis*. Prentice Hall Inc, Upper Saddle River, NJ
- 606

607 **Table 1** Summary of Analysis of Variance results testing for the effects of insecticide (I),
 608 fungicide (F) and the interaction between them (I*F) on insect damage, mycorrhizal
 609 colonization and plant biomass in field-grown *P. lanceolata*. All degrees of freedom 1,56.
 610

	Leaf damage		AM colonization		Plant foliar biomass	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
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

611

612

613

614 **Table 2** Summary of Analysis of Variance results testing for the effects of insecticide (I),
 615 fungicide (F) and the interaction between them (I*F) on insect damage, mycorrhizal
 616 colonization and plant biomass in field-grown *S. jacobaea*. All degrees of freedom 1,56.
 617

	Leaf damage		AM colonization		Plant foliar biomass	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
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.

618

619 **Figure legends**

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

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

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

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

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

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

639