Effects of mycorrhizal fungi on insect herbivores: a meta-analysis

JULIA KORICHEVA,1 ALAN C. GANGE, AND TARA JONES

School of Biological Sciences, Royal Holloway University of London, Egham, Surrey TW20 0EX United Kingdom

Abstract. Mycorrhizal status of the host plant is often ignored in studies on plant–herbivore interactions, but mycorrhizal colonization is known to induce many morphological, physiological, and biochemical changes in host plants, which in turn may alter plant quality as a host for insect herbivores. Both positive and negative effects of mycorrhizal colonization of the host plant on performance and density of insect herbivores have been reported in previous studies. We have conducted a meta-analysis of 34 published and unpublished studies on this topic in order to find out the sources of variation in mycorrhizae effects on insect herbivores. Effects of mycorrhizae on chewing insects depended upon the parameter measured and the degree of herbivore feeding specialization. Density and consumption of chewing insects were higher on mycorrhizal plants, but this did not lead to greater plant damage, presumably because herbivore survival tended to be lower on mycorrhizal plants. Mono- and oligophagous chewers benefited from mycorrhizal colonization of their host plants, whereas performance of polyphagous chewers was reduced on mycorrhizal plants. Among sucking insects, phloem feeders benefited from mycorrhizal infection, but performance of mesophyll feeders was lower on mycorrhizal plants. The type of mycorrhiza was not important for chewing insects, but performance of sucking insects was increased more by arbuscular mycorrhizal fungi (AM) than by ectomycorrhizae (ECM). Among AM inoculation studies, the most commonly used fungal species, Glomus intraradices, tended to have a negative effect on chewer performance, whereas all other fungal species tended to have a positive effect. There was no significant difference in results between studies using inoculation and fungicides, field and laboratory studies, and published and unpublished studies. Mycorrhizal status of the host plant thus influences insect herbivore performance, but the magnitude and direction of the effect depend upon the feeding mode and diet breadth of the insect and the identity of fungi.

Key words: arbuscular mycorrhizae; ectomycorrhizae; feeding guilds; insect herbivores; meta-analysis; multitrophic interactions.

INTRODUCTION

The vast majority of vascular plants in terrestrial ecosystems form a mycorrhizal association with soil fungi. The most common is the arbuscular mycorrhiza (hereafter AM), which occurs in ~70% of the world’s flowering plants, mostly forbs and grasses, and is formed by ~150 different fungal species (Hodge 2000). Ectomycorrhizal fungi (ECM) typically associate with woody plants, and while the proportion of the world’s flora that is ECM is relatively small (~3%), the number of fungal species is much larger than AM, at ~5000 (Smith and Read 1997). Both AM and ECM function in a similar manner, by providing plants with mineral nutrients (principally phosphorus and nitrogen) and water through their hyphal network in return for receiving carbohydrates from their host plants. Mycorrhizae are found in every terrestrial ecosystem on the planet and are important in many ecological and agricultural processes, such as nutrient cycling, plant growth, crop yield, land revegetation, and plant community structure.

Every mycorrhizal plant in the world is likely to have at least one (often many) species of insect herbivores that attack it. Plant quality for insect herbivores is determined by many morphological, physiological, and biochemical factors (Schoonhoven et al. 2005), many of which can be affected by the mycorrhizal status of the plant. Mycorrhizal plants benefit from improved water and nutrient uptake, which increases plant size, vigor, and nutrient levels (Smith and Read 1997), which are important indicators of plant quality for herbivores (Mattson 1980, Price 1991). In addition, mycorrhizal colonization triggers a variety of molecular and biochemical responses in host plants, including differential expression of plant defense-related genes and changes in production of many allelochemicals (reviewed by Bi et al. 2007). It is likely, therefore, that the presence of a mycorrhiza will affect insect herbivore performance and, ultimately, insect population densities and the extent of herbivore damage received by plants.

Over the last two decades, many studies have been conducted on the effects of the mycorrhizal status of the plant on insect herbivores, encompassing a variety of insect herbivores, host plants, and mycorrhizal fungi.
Mycorrhizal colonization, this would be suggestive of a defensive chemical explanation. The largest changes in plant chemistry might be expected at the site of mycorrhizal colonization, i.e., in root tissue, than in aboveground plant parts. Therefore, we predicted that root-feeding herbivores will be affected by mycorrhizal colonization more than herbivores feeding on aboveground plant parts.

In addition to changes in plant chemical composition, mycorrhizal colonization is known to affect plant size, with mycorrhizal plants usually being larger than their non-mycorrhizal conspecifics (Smith and Read 1997). Insect herbivores most likely to benefit from increased plant size are those that require large and vigorously growing plant organs for their development (e.g., gallers; Price 1991) as well as those specialized to feeding on particular plant organs and limited in their growth by organ size (e.g., leafminers and seed feeders). Furthermore, mycorrhizal colonization has been shown to increase the size of vascular bundles in some plants (Krishna et al. 1981), and this may increase the chance of successful phloem location by phloem feeders (Tjallingii and Esch 1993). Therefore, positive responses of the above groups of insects to mycorrhizal colonization would be suggestive of the role of changes in plant size and vigor.

Insect–mycorrhizal experiments have either been conducted under controlled conditions in constant environment rooms or glasshouses or in natural field situations. Laboratory experiments are usually designed using sterilized soil, to which a non-mycorrhizal microbial inoculum is returned (Koide and Li 1989) and one to two (seldom three) species of mycorrhizal fungi are added to some plants. Insect performance is then compared on mycorrhizal and non-mycorrhizal plants. Three major factors could impinge upon the realism of such experiments. Firstly, the mycorrhizal fungus (or fungi) used is not always isolated from the chosen host plant in the field (for an exception, see Gange et al. 2005a), thus one has no knowledge whether the insect studied would ever encounter a plant colonized by the particular fungus used. This is important, given the fact that different mycorrhizal species have differing effects on plant growth and herbivory (Bennett and Bever 2007). Secondly, in any natural situation, it is remarkably rare for any individual host plant to be mycorrhiza-free, although colonization levels often vary by orders of magnitude, even within one population (Gange and Ayres 1999). Thirdly, plants in the field appear to be colonized by many different fungi at once (e.g., Vandenkooornhuys et al. 2002), and combinations of fungi seem to have different effects on insects compared with single species (Gange et al. 2005a, Bennett and Bever 2007). Therefore, we predicted that the results of laboratory studies and studies using a single fungal inoculant may differ from those of field studies and studies using fungal mixtures.
A problem with field studies is that mycorrhizal manipulation can only be performed with fungicides. The merits of this approach have been debated many times (e.g., Smith et al. 2000, Allison et al. 2007). Because there is no chemical specific to mycorrhizae, fungicides may disrupt the non-mycorrhizal fungal and bacterial community within roots and in the rhizosphere (Chen et al. 2001), as well as have some direct or indirect effects on herbivores, making the interpretation of such studies difficult. However, there is really no alternative to fungicides if one is to perform manipulations of mycorrhizal abundance in natural situations, and the best approach is probably to use a chemical that has the minimum amount of recorded side effects (Gange et al. 1992, Smith et al. 2000). We therefore tested for fungicides may disrupt the non-mycorrhizal fungal and bacterial community within roots and in the rhizosphere (Chen et al. 2001), as well as have some direct or indirect effects on herbivores, making the interpretation of such studies difficult. However, there is really no alternative to fungicides if one is to perform manipulations of mycorrhizal abundance in natural situations, and the best approach is probably to use a chemical that has the minimum amount of recorded side effects (Gange et al. 1992, Smith et al. 2000). We therefore tested for differences in experiments that had taken the inoculation approach and those that used the fungicide method, to determine whether previous experimental results may have been compromised by the method employed.

**Methods**

**Inclusion criteria and compilation of the database**

In order to be included in the analysis, any study had to satisfy the following criteria. (1) The degree of mycorrhizal colonization had been experimentally manipulated. In the majority of studies this was achieved by inoculating plants grown in sterile soil or by reducing mycorrhizal colonization with fungicides. We excluded studies that examined correlations between natural levels of mycorrhizal colonization and insect herbivory (e.g., Mueller et al. 2005), because it is impossible to discriminate between potential effects of mycorrhizae on herbivores and reciprocal effects of herbivores on mycorrhizae. (2) Plants had been randomly allocated to mycorrhizal treatments and mycorrhizal effects were not confounded by other factors. Based on this criterion, we excluded the study by Palermo et al. (2003) in which the comparison between mycorrhizal and non-mycorrhizal trees was confounded by differences in plant genotypes (resistant or susceptible to herbivory). (3) Insect herbivore performance, density, and/or degree of damage were assessed on control and experimental plants. (4) Means, standard deviations, and sample sizes for the control and treatments were available in the paper or from the author, either in numerical or graphical form.

Relevant published papers were retrieved by conducting searches in Web of Science using combination of the keywords “mycorrhiza* AND herbivor*.” In addition, we checked all the papers citing some of the early seminal papers on the topic (Rabin and Pacovsky 1985, Gange and West 1994, Gehring and Whitham 1994) and examined reference lists of previous narrative reviews and individual studies on the topic. This search yielded 28 studies published between 1985 and 2007 that satisfied our inclusion criteria (see Appendix).

Meta-analysis based exclusively on published studies may produce biased results if the probability of the study to be published depends upon the statistical significance, magnitude, and/or direction of research findings. Therefore, it is recommended (Møller and Jennions 2001) that whenever possible a meta-analysis include unpublished studies and gray literature (e.g., dissertations). We have attempted to obtain unpublished data sets on the topic by searching the abstracts of the recent Ecological Society of America meetings and Google using the same keywords as in Web of Science and by contacting individual researchers. We obtained six unpublished data sets containing 86 comparisons of insect performance on mycorrhizal vs. non-mycorrhizal plants that have satisfied our inclusion criteria. Four of them represent data from MSc or PhD theses conducted in Germany, the UK, and Canada, while two were unpublished studies conducted by Alan Gange and Stefan Hempel.

We included several comparisons per study when data from different individual experiments were reported or when several herbivore species, plant species, or performance parameters were studied. When insect performance or density/damage was measured on repeated occasions, we used the last datum. The resulting data set included 238 measurements of the performance of 40 species of insect herbivores on 41 species of host plants.

**Response variables**

Insect herbivore performance parameters were classified as growth rate, mass (larval mass, pupal mass, or adult mass), fecundity (number of eggs laid or number of live offspring produced), development time, consumption, oviposition preference, and survival. Measurements of herbivore density and the degree of plant damage were included as well.

**Explanatory variables**

To compare the effects of mycorrhizae on herbivores with different feeding modes, we classified insects into four feeding guilds: chewing, sucking, galling, and mining. The degree of herbivore feeding specialization was categorized as monophagous (feeding on plants within the same genus), oligophagous (feeding on plants within the same family), or polyphagous (feeding on plants from different families). The part of the plant on which the herbivore fed was categorized as leaves, stems, seeds, roots, phloem sap, mesophyll, or meristem (the latter three categories were used for sucking insects only). The type of host plant was classified as herbaceous annual, herbaceous perennial, woody deciduous, or woody evergreen. Some experiments varied nutrient addition, and so the level of nutrients (low vs. high) was used.

The location of the experiment, as laboratory vs. field, and the type of treatment, either inoculation or fungicide use, were also examined. Fungal effects were examined by using the type of mycorrhiza (AM vs. ECM), the number of fungal species used (one vs.
mixture), and the identity of fungal species as explanatory variables. Finally, we searched for biases in the literature, by comparing studies by Gange et al. (38% of all published studies) vs. others and by comparing results of published vs. unpublished studies.

Meta-analysis

The meta-analysis was carried out by using the MetaWin 2.0 statistical program (Rosenberg et al. 2000). To measure the effect size we used Hedges $d$, which is a standardized mean difference between treatment and control means (Gurevitch and Hedges 2001). As a control we always selected the treatment with the lowest mycorrhizal colonization (either plants grown in sterile soil or plants treated with fungicide). Therefore, a positive effect size indicates a positive effect of mycorrhizae on herbivore performance, density, or degree of damage. The signs of effect sizes for herbivore development time were reversed, so that positive effect sizes indicate shortened development time. Effect sizes were combined across studies using the mixed effects model, which assumes that differences among studies within a class are due to both sampling error and random variation. In ecological data synthesis, the assumptions of mixed models are more likely to be met than those of fixed effects models, and the former are thus preferred (Gurevitch and Hedges 2001). Bias-corrected bootstrap 95% confidence intervals around the effect size were generated from 4999 iterations (Adams et al. 1997). Estimates of the effect size were considered to be significantly different from zero if their 95% confidence intervals did not include 0. If the mean effect size was found to be significantly different from 0, we calculated a fail-safe number ($n_0$) using the weighted method of Rosenberg (2005). This number indicates the number of new studies of null effect and mean weight that have to be added to the analysis in order to reduce the significance level of the observed mean effect to 0.05. A fail-safe number greater than $5n_0 + 10$, where $n$ is the original number of studies included in the analysis, is considered robust against publication bias (Rosenthal 1991).

To test the importance of different sources of variation in determining the sign and magnitude of the treatment effect, we subdivided studies into groups on the basis of the explanatory variables listed above and examined between-group heterogeneity using a $\chi^2$ test statistic, $Q_b$ (Gurevitch and Hedges 2001). The potential problem with using several study characteristics as explanatory variables is that they may not be independent from one another, and this may confound the results of analysis. Therefore, we examined the associations between explanatory variables by conducting the $\chi^2$ tests of independence. If significant association between two variables was found, effects of each variable were examined separately at each level of the other variable, wherever sample sizes permitted (cf. Koricheva 2002).

Results

Overall, mycorrhizal colonization of the host plant had a marginally significant positive effect on herbivore performance ($E_+ = 0.227, 95\% CI = -0.053$ to $0.495, n = 238$). However, herbivore responses varied significantly depending on the performance parameter measured ($Q_b = 43.96, df = 8, P < 0.001$). The parameters measured differed between herbivore feeding guilds; for example, fecundity was measured only for sucking insects, while plant damage was estimated only for chewing insects (Table 1). Therefore, we examined herbivore responses to mycorrhizal colonization within each guild separately, in order to avoid confounding variation in responses of different performance parameters by variation between guilds.

Variation among insect herbivores

Mycorrhizal colonization of the host plant had no overall effect on chewing insects ($E_+ = 0.137, 95\% CI = -0.230$ to $0.494, n = 150$), but there was significant variation between the effects on various performance parameters ($Q_b = 17.80, df = 6, P = 0.0067$). Density and consumption were significantly higher on mycorrhizal plants, whereas survival tended to be lower than on non-mycorrhizal plants or plants with mycorrhizal colonization reduced by fungicides (Table 1). Note, however, that density was measured for seed-feeding insects only, and it is thus impossible to separate the effects of plant part and performance parameter in this case. Chewing-insect mass, growth rate, development time, and the resulting degree of plant damage were not significantly affected by mycorrhizal colonization. Similar results were obtained when analysis was restricted to leaf-feeding insects only (results not shown).

Performance of sucking insects was overall positively affected by mycorrhizal infection of their host plants ($E_+ = 0.525, 95\% CI = 0.255$ to $0.799, n = 40, n_0 = 501$), but the magnitude of the effect tended to vary between different performance parameters ($Q_b = 9.76, df = 5, P = 0.082$). Mass, growth rate, and fecundity were significantly higher on mycorrhizal plants than on non- or reduced-mycorrhizal plants, whereas density, development time, and oviposition preference were not significantly affected (Table 1). Performance of leaf miners was not significantly affected by mycorrhizal colonization of their hosts plants ($E_+ = 0.415, 96\% CI = -0.426$ to $1.214, n = 39$), but there was highly significant variation between the effects of mycorrhizae on different performance parameters ($Q_b = 63.45, df = 4, P < 0.001$). Oviposition preference for mycorrhizal plants was much higher, but miner density on these plants was significantly lower than on non-mycorrhizal plants (Table 1). Mass, survival, and consumption of miners were not significantly affected by mycorrhizal infection. Note, however, that the above results are based on studies examining responses to mycorrhizae of a single species of leafminer, Chromatomyia syngenesiae.
Finally, gall-forming insect performance was significantly negatively affected by mycorrhizal colonization of their hosts ($E_s = -0.459$, 95% CI = −0.663 to −0.154, $n = 6$, $n_{fs} = 19$), with both mass and survival being lower on mycorrhizal plants (Table 1). There was no significant effect on density and no significant difference between mycorrhize effects on different performance parameters ($Q_b = 0.96$, df = 2, $P = 0.618$). However, these results should be treated with caution, as they are based on a single study (Gange and Nice 1997) and a single species of galleral ($Urophora cardui$).

Among chewing insects, mycorrhize effects were significantly modified by herbivore feeding specialization ($Q_b = 19.01$, df = 2, $P < 0.001$). For instance, mass of mono-and oligophagous chewers was significantly higher on mycorrhizal plants ($E_s = 1.005$, 95% CI = 0.655–1.312, $n = 10$, $n_{fs} = 138$ for monophagous insects and $E_s = 1.199$, 95% CI = 0.566–1.790, $n = 7$, $n_{fs} = 76$ for oligophagous insects), whereas mass of polyphagous chewers was higher on control plants ($E_s = -0.385$, 95% CI = −0.617 to −0.139, $n = 34$, $n_{fs} = 168$). Similar results were obtained when survival of chewers was analyzed (results not shown). However, among sucking insects was there was no difference in responses of mono- and polyphagous herbivores to mycorrhizal colonization ($Q_b = 0.55$, df = 1, $P = 0.457$). Performance of both groups of suckers was higher on mycorrhizal plants (monophagous, $E_s = 0.400$, 95% CI = 0.0931–0.7478, $n = 12$, $n_{fs} = 49$; polyphagous, $E_s = 0.604$, 95% CI = 0.272–0.985, $n = 29$, $n_{fs} = 255$).

A comparison of responses of mono- and polyphagous herbivores could not be conducted for miners and gall formers, as the only species of leaf miner studied was polypagous and the only species of gall former was monophagous.

There was no significant difference in responses to mycorrhizal colonization between chewing insects belonging to orders Lepidoptera, Coleoptera, Hymenoptera, and Orthoptera ($Q_b = 0.23$, df = 3, $P = 0.972$).

### Variation among plant parts

Overall, the plant part on which the herbivore fed did not significantly influence responses to mycorrhizal colonization in chewing insects ($Q_b = 2.26$, df = 2, $P = 0.324$). However, densities of seed feeders were positively affected ($E_s = 0.797$, 95% CI = 0.387–1.247, $n = 8$, $n_{fs} = 70$), while performance of root feeders tended to be lower on mycorrhizal plants ($E_s = -0.197$, 95% CI = −0.702 to 0.414, $n = 17$). Meanwhile, performance of leaf feeders was unaffected ($E_s = 0.138$, 95% CI = −0.281 to 0.571, $n = 126$). Similar results were obtained when the comparison between leaf feeders and root feeders was restricted to studies measuring herbivore mass only (results not shown).

For sucking insects, the plant tissue on which herbivores fed significantly affected responses to mycorrhiza ($Q_b = 10.24$, df = 2, $P = 0.006$). Only insects feeding on phloem sap significantly benefited from mycorrhizal colonization ($E_s = 0.689$, 95% CI = 0.401–0.975, $n = 32$, $n_{fs} = 546$), whereas performance of insects feeding on mesophyll was significantly lower on mycorrhizal plants ($E_s = -0.294$, 95% CI = −0.762 to −0.028, $n = 4$). Performance of sucking insects feeding on meristematic tissue also tended to be lower on mycorrhizal plants ($E_s = -0.263$, 95% CI = −1.00 to 0.322, $n = 6$).

Similar analysis could not be conducted for miners and gall formers, as the only examined species of miners fed on leaves and the only species of galleral fed on stems.

### Variation among mycorrhizal fungi

The type of mycorrhiza was significantly associated with the type of the host plant: the majority of experiments with AM were conducted on herbaceous plants, whereas the majority of ECM studies were conducted on woody plants ($\chi^2 = 107.58$, df = 1, $P < 0.0001$ for chewing insects and $\chi^2 = 36.00$, df = 1, $P < 0.0001$ for sucking insects), which reflects the distribution pattern of the above types of mycorrhiza between different types of plants. Therefore, to control for confounding effects of plant type on mycorrhiza type, for chewing insects we compared effects of AM and ECM across all plants and for woody evergreen plants only. Since the results of both analyses were similar, but the first one was based on a much larger number of studies, we report the results for the analysis based on all types of plants (Table 2). Mycorrhize type had no significant effect on performance of chewing insects ($Q_b$,
= 0.573, df = 2, P = 0.750), although inoculating plants with both AM and ECM appeared to have stronger positive effect than either AM or ECM alone (Table 2). However, the combined effects of AM and ECM on herbivores have so far been tested only in a single study (Gange et al. 2005b).

With sucking insects, the effects of type of mycorrhiza and type of host plant could not be separated because all studies using ECM were conducted on woody evergreen plants and all but one study using AM were conducted on herbaceous perennials. Keeping the potential confounding effect of plant type in mind, the type of mycorrhiza had a weakly significant effect on performance of sucking insects ($Q_b = 3.12, \text{df} = 1, P = 0.077$). Only AM had a significant positive effect on sucking-insect performance ($E_i = 0.668, 95\% \text{ CI} = 0.324–1.068, n = 26, n_{bs} = 238$), while ECM had no significant influence ($E_i = 0.186, 95\% \text{ CI} = -0.111 to 0.433, n = 16$).

Within AM, the species of fungi used for inoculation had a significant effect on performance of chewing insects ($Q_b = 10.13, \text{df} = 4, P = 0.038$). Interestingly, the most commonly used fungal species, *Glomus intraradices*, tended to have a negative effect on chewing performance, whereas all other fungal species tended to have a positive effect (Table 2). The result remained the same when the analysis was restricted to leaf feeders only or when survival was excluded from the list of performance parameters (results not shown).

A similar comparison could not be conducted for sucking insects because *Glomus intraradices* was used in nearly all studies. Too few studies in each group prevented species comparisons for ECM studies.

Among AM experiments, using a mixture of fungal species for inoculation tended to have a stronger positive effect on chewing-insect performance than inoculation with a single fungal species or with the natural AM fungal community isolated from soil (Table 2), although the difference between groups was not statistically significant ($Q_b = 3.46, \text{df} = 2, P = 0.177$). When this comparison was restricted only to studies within which the effects of single species vs. species mixture inoculates were tested, the difference between species mixture and single species effects became smaller (species mixture, $E_i = 1.201, 95\% \text{ CI} = -0.116 to 3.551, n = 11$; single species, $E_i = 0.769, 95\% \text{ CI} = -0.137 to 1.867, n = 28$) and not significant ($Q_b = 0.43, \text{df} = 1, P = 0.511$).

**Variation among host plants**

The type of host plant had no significant effect on responses of chewing insects to mycorrhizae. Among AM studies, there was no significant difference between responses of herbivores feeding on annual and perennial forbs ($Q_b = 1.13, \text{df} = 1, P = 0.288$). Similarly, among ECM studies, chewing herbivore responses differed neither between conifers and angiosperm trees ($Q_b = 0.04, \text{df} = 1, P = 0.847$) nor between evergreen vs. deciduous trees ($Q_b = 0.15, \text{df} = 1, P = 0.696$). For sucking insects, effects of type of plant and type of mycorrhiza could not be separated (see Variation among mycorrhizal fungi).

**Variation in experimental design**

Mycorrhizal colonization in the majority of reviewed studies was manipulated either by inoculation of sterile soil with fungi or by applying fungicide. Inoculation was most commonly used in laboratory experiments, whereas fungicide treatment was usually applied in the field (chewing insects, $\chi^2 = 21.49, \text{df} = 1, P < 0.0001$; sucking insects, $\chi^2 = 27.48, \text{df} = 1, P < 0.0001$). Therefore, to exclude confounding effects of experiment type on treatment type and vice versa, we compared the effects of treatment type within field studies only. Similarly, we compared laboratory and field experiments only within inoculation treatment. The above comparisons could be conducted for chewing insects only, because in sucking insects inoculation method was used in all laboratory experiments, but only in two field experiments. Responses of chewing insects did not differ significantly between the inoculation and fungicide experiments in field studies ($Q_b = 1.99, \text{df} = 1, P = 0.391$), although insect responses to mycorrhizae tended to be negative in fungicide experiments and positive in inoculation experiments (fungicide, $E_i = -0.609, 95\% \text{ CI} = -1.395 to 0.093, n = 28$ and not statistically significant).

**Table 2. Effects (mean $E_i$ and 95\% CI) of different types of mycorrhizae on the performance of chewing insects.**

<table>
<thead>
<tr>
<th>Mycorrhizal type</th>
<th>$n$</th>
<th>$E_i$</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM</td>
<td>122</td>
<td>0.132</td>
<td>-0.188 to 0.484</td>
</tr>
<tr>
<td>ECM</td>
<td>22</td>
<td>0.006</td>
<td>-1.196 to 1.206</td>
</tr>
<tr>
<td>AM + ECM</td>
<td>6</td>
<td>0.578</td>
<td>-3.867 to 5.081</td>
</tr>
<tr>
<td>AM inoculant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single species</td>
<td>86</td>
<td>0.140</td>
<td>-0.279 to 0.546</td>
</tr>
<tr>
<td>Species mixture</td>
<td>11</td>
<td>1.041</td>
<td>-0.137 to 2.982</td>
</tr>
<tr>
<td>Natural fungal community</td>
<td>8</td>
<td>0.262</td>
<td>0.027 to 0.505</td>
</tr>
<tr>
<td>AM species</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Glomus intraradices</em></td>
<td>46</td>
<td>-0.315</td>
<td>-0.770 to 0.073</td>
</tr>
<tr>
<td><em>Glomus mosseae</em></td>
<td>15</td>
<td>0.372</td>
<td>-0.492 to 1.728</td>
</tr>
<tr>
<td><em>Glomus fasciculatum</em></td>
<td>11</td>
<td>0.341</td>
<td>-0.415 to 1.489</td>
</tr>
<tr>
<td><em>Glomus caledonium</em></td>
<td>8</td>
<td>1.348</td>
<td>-0.931 to 3.702</td>
</tr>
<tr>
<td><em>Glomus etunicatum</em></td>
<td>4</td>
<td>0.343</td>
<td>-0.662 to 1.580</td>
</tr>
</tbody>
</table>

*Note:* Abbreviations are: AM, arbuscular mycorrhiza; ECM, ectomycorrhiza.
11; inoculation, $E_s = 0.578$, $95\% \text{ CI} = -1.086$ to 2.128, $n = 29$). Similarly, there was no significant difference between the results of field and laboratory experiments with chewing insects using inoculation treatment ($Q_b = 0.042$, $df = 1$, $P = 0.989$) or sucking insects ($Q_b = 0.129$, $df = 1$, $P = 0.720$). However, manipulating levels of P significantly affected responses of sucking insects ($Q_b = 12.49$, $df = 1$, $P < 0.001$), which were significantly greater at low P levels ($E_s = 1.516$, $95\% \text{ CI} = 1.151–2.092$, $n = 4, n_{06} = 30$) than at high P ($E_s = 0.311$, $95\% \text{ CI} = 0.219–0.452$, $n = 4$). Responses of chewing insects were not significantly affected by P levels ($Q_b = 0.04$, $df = 1$, $P = 0.842$). Finally, in a study by Rieske (2001), where NPK levels were manipulated, performance of gypsy moth larvae on oak was negatively affected by mycorrhizae at high NPK level ($E_s = -0.425$, $95\% \text{ CI} = -0.637$ to $-0.307$, $n = 3$), but effects were not significant at low NPK level ($E_s = 0.095$, $95\% \text{ CI} = -0.013$ to 0.169, $n = 3$), and the difference between the two levels was statistically significant ($Q_b = 8.00$, $df = 1$, $P = 0.005$).

Since 38% of all studies included in this analysis (and all but one study of AM fungi effects in the field) were conducted by A. C. Gange and colleagues, we compared the results of studies by Gange et al. with those conducted by other researchers. There was no significant difference in responses of chewing insects to mycorrhizae reported by Gange et al. and others ($Q_b = 0.524$, $df = 1$, $P = 0.469$). However, responses of sucking insects reported by Gange et al. were significantly larger than those of other researchers ($Q_b = 12.82$, $df = 1$, $P < 0.001$), and the difference remained significant when the analysis was restricted only to AM studies and phloem-feeders. This appears to be due to differences in the performance parameters recorded ($\chi^2 = 25.08$, $df = 7$, $P = 0.0007$). In most of the studies by Gange et al., performance of sucking insects was measured in terms of mass and fecundity, which responded strongly to mycorrhizal colonization (Table 1), while in studies by others, responses were often measured in terms of density, which was not significantly affected by mycorrhizal colonization (Table 1).

Finally, the results of published and unpublished studies differed neither for chewing insects ($Q_b = 0.014$, $df = 1$, $P = 0.906$) nor for sucking insects ($Q_b = 0.006$, $df = 1$, $P = 0.939$), suggesting an encouraging lack of publication bias.

**DISCUSSION**

The results of meta-analysis of 34 studies conducted on 40 insect species from six orders feeding on 41 different host plants show that mycorrhizae are a significant factor affecting the performance and density of phytophagous insects. While much variation in response to mycorrhizal presence is apparent, some clear generalizations can be made that enable us to suggest mechanisms by which mycorrhizae affect the performance of herbivorous insects.

Our first hypothesis was that if mycorrhizae alter the nutritive quality of plant tissues, then ECM, which increase plant N uptake, would affect insects more than AM. Contrary to this prediction, we found no difference between the effects of the two types of mycorrhiza for chewing insects, and for sucking insects only AM had a positive effect on their performance, providing no evidence that mycorrhizal effects on insects are due to changes in N availability. Moreover, no differences were found between the results of studies in which effects of mycorrhizal colonization were tested at different nitrogen levels. However, insect herbivores respond to a wide variety of nutrients within plants, and it may be that to date, authors have concentrated upon plant N content, when other nutritional explanations may be more relevant. For instance, sugars might be important determinants of plant quality for phloem feeders, and in our analysis these insects showed increases in mass, growth rate, and fecundity on mycorrhizal plants compared with those on which the mycorrhiza was absent or significantly reduced. The mechanism of plant resistance to phloem feeders such as aphids is often one in which the insect’s failure to locate the phloem elements leads to reduced feeding time and reduced growth rate (Caillaud et al. 1995). Aphid stylet pathways follow a sucrose concentration gradient, created by the diffusion of sucrose out of phloem elements (Lei and Xu 2008). When the diffusion gradient is weak, the probability of finding the phloem is reduced, and the plant is described as “resistant.” Arbuscular mycorrhizal fungi can increase leaf sucrose concentrations dramatically (e.g., Caglar and Bayram 2006), thus it is possible that phloem sucrose concentrations are greater in mycorrhizal plants, the diffusion gradients stronger, and the aphid’s degree of success in phloem location higher. Coupled with the increase in the size of the vascular bundle observed in some plants (Krishna et al. 1981), these changes would provide an explanation for increased success in phloem location and the consistent enhancement of aphid performance seen on mycorrhizal plants (Ponder et al. 2000). To date, there has been no analysis of the effects of mycorrhizal colonization on phloem sap contents, and this would be a useful exercise to undertake.

In addition to sugars, mycorrhizae, particularly AM, are known to increase uptake of phosphorus (Smith and Read 1997), which is an important element for insect growth (Schoonhoven et al. 2005), and responses of sucking insects to mycorrhizae were significantly greater at low phosphorus level. There is also abundant evidence that mycorrhizae can affect the water balance.
of plants, usually alleviating drought stress (Augé 2001), but to date no study has considered whether this might also be a mechanism by which mycorrhizal fungi affect herbivore performance. Goverde et al. (2000) found that the lipid content of *Polyommatus icarus* butterflies was increased if their larvae fed on mycorrhizal plants. Insects lack the ability to synthesize sterols that are required for lipid synthesis (Behmer and Nes 2003), but mycorrhizae have the potential to alter phytosterol content (Fontaine et al. 2001). We suggest therefore that future insect–mycorrhizal studies should include a consideration of other factors, such as sterols, essential amino acids, proteins, carbohydrates, vitamins, or fatty acids, all of which may be food-limiting for certain insects (Nation 2008). Moreover, dual mycorrhizal plants such as *Eucalyptus, Populus,* and *Salix* provide an ideal system for comparing the effects of AM and ECM on herbivore performance within the same species of the host plant (Gehring and Whitham 2002, Gange et al. 2005b), and more experiments in these systems should be encouraged.

The second potential explanation for modified herbivore performance on mycorrhizal plants is changes in plant defensive chemistry. Most of the main groups of plant defenses have been shown to be altered by mycorrhizal colonization, including alkaloids (Abu-Zeyad et al. 1999), monoterpenoids (Toussaint et al. 2008), diterpenoids (Manninen et al. 2008), simple phenolics (e.g., flavonoids, Larose et al. 2002), complex phenolics (e.g., tannins, Beyeler and Heyser 1997), and glucosinolates (Vierheilig et al. 2008). However, the magnitude of the mycorrhiza-induced changes in plant defensive chemistry is often smaller than that in response to pathogen or herbivore attack (Pozo and Azco´n-Aguilar 2007), and it is rare to find a study in which mycorrhiza-induced reductions in herbivore growth have been linked to secondary chemistry (Gange and West 1994). A more important mycorrhiza-induced defense mechanism appears to be priming, i.e., preconditioning of plant tissues for a more effective activation of defenses upon herbivore or pathogen attack (Pozo and Azcón-Aguilar 2007). When priming occurs, concentrations of defensive chemicals are not significantly affected by mycorrhiza itself, but are induced only in response to herbivore feeding, which might explain why few studies have established causal links between mycorrhizal colonization, changes in secondary chemistry, and herbivore performance (but see Gange and West 1994). Finally, mycorrhizae can also affect plant indirect defenses against herbivores by changing attractiveness of plants to parasitoids (Guerrieri et al. 2004), presumably through altering the volatile profiles of plants.

Several results of the meta-analysis are suggestive of mycorrhizae altering plant defenses in host plants. For instance, among chewing insects, we found that mycorrhizae generally have negative effects on polyphagous insects, but positive effects on monophages and oligophages. Moreover, among sucking insects, phloem-feeders were positively affected, while performance of mesophyll feeders was negatively affected by mycorrhizal colonization. Insects with a narrow diet breadth may be immune or even require the chemicals present in their host that will otherwise deter generalist species (Schoonhoven et al. 2005). It is therefore likely that the main reason why mycorrhizae affect the growth of phytophagous insects is the activation of plant defenses. Furthermore, chewing and sucking insects feeding on cell contents are generally more susceptible to the presence of secondary metabolites, which are commonly stored inside cells, than sucking insects feeding on phloem, in which defensive chemicals are largely absent (Larsson 1989). Consistent with this explanation, we found no difference between effects of mycorrhizal colonization on mono- and polyphagous sucking insects. Interestingly, we also found no significant difference in mycorrhizae effects on root-feeding herbivores and herbivores associated with aboveground parts of the plants, which supports the notion that mycorrhizae-induced changes in plant defenses are systemic and not only restricted to root tissues (Bi et al. 2007, Pozo and Azcón-Aguilar 2007).

Mycorrhizae can also influence the performance of herbivorous insects by affecting plant size and vigor, with mycorrhizal plants usually being much larger than conspecific non-mycorrhizal ones (Smith and Read 1997). Several results of the meta-analysis are consistent with effects of mycorrhizae on herbivores being mediated by changes in plant size. For instance, ovipositing females of leafminers often lay more eggs on larger leaves (e.g., Faeth 1991, Gripenberg et al. 2007), which might explain the observed strong oviposition preference by miners for mycorrhizal plants. Furthermore, increased performance of seed feeders on mycorrhizal plants could be due to positive effects of mycorrhizae on seed production (Koide 2000, Gange et al. 2005a). However, performance of gall makers, which usually benefit from plant vigor (Price 1991), was reduced on mycorrhizal plants. It should be noted, however, that the results of this analysis come from a single study (Gange and Nice 1997), and thus more research on mycorrhizal effects on gall makers is needed before generalizations for this feeding guild can be developed.

We found no significant differences between laboratory and field experiments or those in which fungicide had been used, compared with the inoculation approach. These results are encouraging, since they suggest that the controlled studies are representative of more natural situations in the field. However, we found that the species of AM inoculated influences the outcome of the experiment. *Glomus intraradices* was the most commonly used AM fungus, probably because it is easy to culture. This fungus seems to have much more of a negative effect on insects than do other species of AM, and use of it in single inoculations is probably most unrepresentative of the fungal community that exists within the roots of most plants (Vandenkoornhuyse et al. 2002). Gange (2000)
highlighted a similar problem with experiments involving mycorrhizae and Collembola, so we suggest that experiments involving controlled combinations of mycorrhizae are useful, but that the fungi used should be those that are found naturally associated with the plant in question.

Manipulation of mycorrhizae in the field is problematic. Although widely used, the fungicide method is potentially fraught with problems, due to non-target effects on other members of the soil microbial community (Smith et al. 2000, Allison et al. 2007). This becomes more important when one considers that this microbial community can itself have significant effects on the growth of aboveground insect herbivores (Bezemer et al. 2005). Perhaps surprisingly, we found no significant difference between the outcome of fungicide and inoculation experiments, although responses of chewing insects tended to be negative in fungicide experiments and positive in inoculation experiments. Further experiments need to be conducted to establish whether this result is valid and to disentangle the direct and indirect effects of chemical application (Allison et al. 2007).

In conclusion, we found that mycorrhizae have significant effects on all aspects of insect herbivore performance, including consumption, growth rate, mass, fecundity, and survival, as well as insect herbivore density. Negative effects are most commonly seen with generalist chewing insects, while positive effects are observed with specialist chewing insects and phloem feeders. The above patterns are suggestive of plant community can itself have significant effects on the growth of aboveground insect herbivores (Bezemer et al. 2005). Perhaps surprisingly, we found no significant difference between the outcome of fungicide and inoculation experiments, although responses of chewing insects tended to be negative in fungicide experiments and positive in inoculation experiments. Further experiments need to be conducted to establish whether this result is valid and to disentangle the direct and indirect effects of chemical application (Allison et al. 2007).

In conclusion, we found that mycorrhizae have significant effects on all aspects of insect herbivore performance, including consumption, growth rate, mass, fecundity, and survival, as well as insect herbivore density. Negative effects are most commonly seen with generalist chewing insects, while positive effects are observed with specialist chewing insects and phloem feeders. The above patterns are suggestive of plant community can itself have significant effects on the growth of aboveground insect herbivores (Bezemer et al. 2005). Perhaps surprisingly, we found no significant difference between the outcome of fungicide and inoculation experiments, although responses of chewing insects tended to be negative in fungicide experiments and positive in inoculation experiments. Further experiments need to be conducted to establish whether this result is valid and to disentangle the direct and indirect effects of chemical application (Allison et al. 2007).

In conclusion, we found that mycorrhizae have significant effects on all aspects of insect herbivore performance, including consumption, growth rate, mass, fecundity, and survival, as well as insect herbivore density. Negative effects are most commonly seen with generalist chewing insects, while positive effects are observed with specialist chewing insects and phloem feeders. The above patterns are suggestive of plant community can itself have significant effects on the growth of aboveground insect herbivores (Bezemer et al. 2005). Perhaps surprisingly, we found no significant difference between the outcome of fungicide and inoculation experiments, although responses of chewing insects tended to be negative in fungicide experiments and positive in inoculation experiments. Further experiments need to be conducted to establish whether this result is valid and to disentangle the direct and indirect effects of chemical application (Allison et al. 2007).

Acknowledgments

We are grateful to the Natural Environment Research Council for funding experimental work and to Marcel Goverde, Stefan Hempel, Anne Kempel, Abby Kula, Robert Laird, and Stuart Wooley for providing additional or unpublished data from their studies.

Literature Cited


APPENDIX

Published studies included in the meta-analysis (Ecological Archives E090-145-A1).