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COMBINED EFFECT OF FOLIAR AND MYCORRHIZAL ENDOPHYTES ON AN INSECT HERBIVORE

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Abstract. Many foliar endophytic fungi deter herbivores from feeding on their host plants, but a previous study has suggested that their deterrent effect may be reduced in the presence of arbuscular mycorrhizae. In our study, factorial experiments determined the effects of infection of perennial ryegrass, *Lolium perenne*, by the foliar endophyte *Neotyphodium lolii* [present (+E) or absent (−E)] and the mycorrhizal fungus *Glomus mosseae* [present (+M) or absent (−M)] on larvae of the noctuid moth *Phlogophora meticulosa*.

When host plants received adequate phosphorus (P), mycorrhizae and the foliar endophyte decreased the survivorship of larvae between their second and fifth instar in an additive manner. Sixth-instar larvae feeding on −M−E plants attained greater mass than those feeding on plants with one or both symbionts. There was an M × E interaction with the total amount of grass consumed per larva. The foliar endophyte increased relative consumption rate and reduced efficiency of conversion of ingested food of larvae, while the mycorrhizal fungus had no effect on insect nutritional indices.

Larvae preferred −E over +E leaf blades regardless of mycorrhizal infection status. They showed no preference with respect to plant mycorrhizal status. When foraging among mixed stands of intact plants, larvae inflicted more damage on −E than on +E plants. Mycorrhizae reduced feeding damage, but only in −E plants and to a lesser extent than the foliar endophyte. The relative difference between +E and −E plants, in terms of grazing damage, was decreased but not eliminated by mycorrhizae.

When P was limiting to plant growth, mycorrhizae still decreased the survivorship of larvae between their second and fifth instar, independently of the foliar endophyte. They accelerated larval development slightly. The foliar endophyte increased the survivorship of fifth-instar larvae, independently of mycorrhizae.

We concluded that both foliar endophytes and mycorrhizae affected larval growth and survivorship. Some effects were additive, while others were nonadditive and depended to some extent on host P nutrition. Our findings suggest that the beneficial effect of the foliar endophyte on the host plant, in terms of insect resistance, was reduced but not eliminated by mycorrhizae.

Key words: *antixenosis; arbusular mycorrhizae; endomycorrhizae;* Glomus mosseae; *herbivore defense hypothesis; interaction, multitrophic;* Lolium perenne; *mutualism; mycophyllas and mycorrhizae, effect on herbivores;* Neotyphodium lolii; Phlogophora meticulosa; *phytophagy.*

INTRODUCTION

The ability or not of fungal endophytes to protect their host plants from herbivory has become a focus for debate among plant–herbivore ecologists (Clay 1997, Saikkonen et al. 1998). Endophytic fungi (sensu Wilson 1995) have been isolated from a diverse range of herbaceous and woody plants (e.g., Rodrigues 1996, Wilson and Faeth 2001, Wirsel et al. 2001). At least some fungal endophytes are believed to be mutualistic symbionts of plants. *Neotyphodium* spp. Glenn, Bacon and Hanlin, which infect many C_3 grasses, are one such group. They do not produce fruit bodies and are believed to be strictly vertically transmitted (Schardl 1996; but see Moy et al. 2000). Despite this limitation, *Neotyphodium* spp. are often highly abundant in undisturbed grass populations, infecting up to 100% of tillers (e.g., White 1987, Clay and Leuchtmann 1989). Thus, infected plants likely have a selective advantage over uninfected conspecifics (Saikkonen et al. 1999).

According to the herbivore defense hypothesis (Saikkonen et al. 1999), that advantage stems primarily from negative effects of endophytes on herbivores (Carroll 1988, Clay 1988). Many *Neotyphodium* endophytes produce one or more secondary metabolites in vivo that negatively affect vertebrate and/or invertebrate herbivores (Bush et al. 1997). Laboratory experiments suggest that this may give infected hosts a competitive advantage in the presence of herbivory that they lack

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in its absence (Clay et al. 1993). However, field results are equivocal, with positive correlations (e.g., Bazely et al. 1997, Clay and Brown 1997), negative correlations (Saikkonen et al. 1999), or no correlation (e.g., Bazely et al. 1997) between the intensity of grazing and the proportion of infected tillers being reported. This, along with other evidence, has led to the alternative hypothesis that increased resistance to drought and/or some other mechanism leading to improved host vigor is the primary benefit of infection and endophyte-mediated resistance to herbivory is the exception rather than the rule (Saikkonen et al. 1998, 1999).

The findings of Barker (1987) seem to further weaken the herbivore defense hypothesis. The Argentine stem weevil, *Listronotus bonariensis* Kuschel—a major pest of *Lolium perenne* L. pastures in New Zealand—is sensitive to the presence of *Neotyphodium lolii*, the major *Neptyphodium* endophyte found in this host (Christensen et al. 1993). Barker (1987) showed that the arbuscular–mycorrhizal (AM) fungus *Glomus fasciculatum* (Thaxter sensu Gerd.) Gerd. and Trappe—one of the most abundant AM fungi in New Zealand—had no effect on weevil feeding behavior in *Neotyphodium*-free hosts; however, the tendency of weevils to avoid *Neotyphodium*-infected hosts (a response referred to as "antixenosis") was reduced when the host was also infected by the AM fungus.

AM fungi, of which there are over 150 species (Smith and Read 1997), are widespread in natural grasslands (Read et al. 1976, Sparling and Tinker 1978) and might be expected to commonly occur in the same hosts as *Neotyphodium*. Thus, if the effect of *G. fasciculatum* on *Neotyphodium* is a general property of AM fungi, the effect of *Neotyphodium* on herbivore resistance under natural conditions may be less than that observed in laboratory experiments using plants grown in sterilized soil. Experiments conducted in agricultural settings, where AM fungi may be adversely affected by fertilizers, pesticides, and/or soil disturbance, may also be unrealistic (Giovanetti and Ginaninazzi-Pearson 1994).

The aim of this investigation was to determine the effect of another AM fungus, *Glomus mosseae* Nicolson & Gerdemann, on both the antibiotic and antixenotic properties of *N. lolii* against an insect herbivore. Because the effect of AM fungi on their host plants is often dependent on phosphorus (P) availability to the host (Smith and Read 1997), we included host-plant P nutrition as a factor in part of our study.

Materials and Methods

Study organisms

Lolium perenne cv. "Express" (Pickseed West, Tangent, Oregon, USA) was used in all experiments. Endophytes isolated from this cultivar were identified as *Neotyphodium lolii* on the basis of isozyme phenotyping and in vitro conidial morphology (A. Leuchtmann, *personal communication*).

Inoculating with the arbuscular–mycorrhizal (AM) fungus.—*Plantago lanceolata* was used to prepare stock inoculant of *Glomus mosseae*, as this host is hardy and resistant to most greenhouse pests. Stock inoculant was prepared by infecting roots of *P. lanceolata* (grown in 1.12-L pots containing an autoclaved 10:1 volume : volume sand : soil [John Innes number 2 compost; Keith Singleton, Egremont, UK] mixture) using ~6.75 g of commercial inoculum (Agricultural Genetics Company, Royston, Herts, UK). Seeded pots were placed in a growth chamber (19–23°C, ~200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation [PAR] measured as moles of quanta) and plants were reared for 10–15 wk, at the end of which the potting medium, containing mycorrhizal roots, was harvested.

A sample of roots from each pot was examined microscopically to confirm its mycorrhizal status. Roots were cleared in 5% KOH and stained with trypan blue (Brundrett et al. 1994). The proportion of root length infected was determined using the magnified-intersections method of McGonigle et al. (1990).

Pots of experimental *Lolium* were inoculated with ~30 cm^3 of *Plantago* potting medium (including roots). Control "inoculum" was prepared in a similar manner, except that *Plantago* pots were inoculated with an aqueous suspension containing 6.75 g of commercial inoculum that had been poured through a 32-μm sieve to remove AM fungal propagules and then added after the seeds had been sown.

The moth.—Larvae of *Phlogophora meticulosa* L. (Noctuidae) were used to assess the effect of *Glomus* and *Neotyphodium* infection on insect resistance in *L. perenne*. *P. meticulosa* is a European generalist that feeds on a wide variety of herbaceous plants. Although grasses are not usually listed as hosts (e.g., Heath and Emmet 1983, Brooks 1991), in captivity the insect is capable of completing its development from first instar to imago on a diet of *Neotyphodium*-free *L. perenne* (M. Vicari, *personal observation*). Larvae typically undergo six instars before pupation.

Colonies of *P. meticulosa* were maintained using imagos captured on the Lancaster University campus (Lancaster, UK) and additional pupae provided by E. Bower (Royal Holloway College, University of London, UK). Larvae hatched in captivity were reared on excised leaves of wild *Rumex obtusifolius* or, if this was unavailable, green cabbage obtained from a supermarket. Nonsymbiotic *L. perenne* was not used in order to ensure that all larvae (as opposed to only those in the mycorrhizal and/or foliar endophyte-infected treatments) would experience a switch in diet at the start of each experiment.

Experiments

Four experiments were conducted to assess the direct and interactive effects of foliar endophytes and my-

corrhizae on the performance (experiments 1 and 2) and preference (experiments 3 and 4) of the herbivore. In experiment 1 we monitored the growth and survivorship of relatively large numbers of larvae from the beginning of their second to the end of their fifth instar. In experiment 2 we calculated nutritional indices for a smaller number of sixth-instar larvae, in an effort to shed light on the mechanism(s) by which larval performance is affected. In experiment 3 we conducted a preliminary assessment of larval preference by feeding larvae excised leaves in petri dishes. In experiment 4 we measured levels of damage inflicted by larvae on intact plants growing in mixed stands in outdoor enclosures.

Experiment 1: Survivorship and growth of second- to fifth-instar larvae

The interactive effects of foliar endophyte infection, mycorrhizal infection, and phosphorus nutrition of *L. perenne* on the growth and survivorship of larvae were assessed using a $2 \times 2 \times 2$ factorial bioassay with two levels of *Neotyphodium* infection: present (+E) and absent (−E); two levels of mycorrhizal infection: present (+M) and absent (−M); and two levels of phosphorus (P) nutrition: "low" (0.5 mg/kg (potting medium)$^{-1}$·wk^{-1}, or 0.5 ppm) and "high" (5.0 mg/kg (potting medium)$^{-1}$·wk^{-1}, or 5.0 ppm). The experiment was conducted in four trials (blocks) over a period of two years. Phosphorus nutrition levels were selected in order to produce a typical (e.g., Crush 1973) P × M interaction with plant growth; in a separate experiment, mycorrhizae increased shoot biomass of uncut −E plants at the low, but not at the high, P fertilization rate after 9 wk (M. Vicari, *personal observation*).

Plant growth.—Individual seeds of *Lolium* were planted in John Innes number 2 compost in 200-mL styrofoam cups. Plants were grown in a greenhouse with supplementary lights providing a 16-h photoperiod for one year prior to the study, during which time they were fertilized regularly with a 15:30:15 NPK (with trace elements) soluble fertilizer ("Miracle-gro" [Miracle Garden Care, Godalming, Surrey, UK]). In trials 1 and 2, five +E and five −E genotypes were used. In trials 3 and 4, +E and −E ramets of each of two genotypes (designated "I" and "II") were used. Endophyte-free ramets were obtained by transplanting infected ramets into sand containing 50 ppm propiconazole (contained in "Tumble Blite" [Fisons, Bramford, UK]) for four weeks, beginning one year prior to the start of trial 3.

Newly rooting tillers of genotypes used in the study were transferred to 200-mL styrofoam cups containing autoclaved horticultural sand ("MFC1" [Tarmac Quarry Products, Buxton, Derbyshire, UK]) pre-inoculated with mycorrhizal or non-mycorrhizal roots of *P. lanceolata*. Cups were then transferred to a growth room (14–18°C, 260 μmol quanta·m^{-2}·s^{-1} PAR with a 16:8 L:D photoperiod). Each cup immediately received one fertilization of 40 mg 26:0:26 Chempack number 6 phosphate-free fertilizer (Hoddesdon, Hertsfordshire, UK) and 1.10 mg KH_2PO_4 (=1.0 ppm P). After four weeks, cups allocated to high-P treatments received 5.50 mg KH_2PO_4 (=5.0 ppm P) weekly; those in low-P treatments received 0.55 mg (=0.5 ppm P). Twenty milligrams of P-free fertilizer was also given to all cups once per week.

A separate set of plants of genotypes I and II was reared for 10 wk and harvested to determine mycorrhizal infection levels and foliar nitrogen concentration. Nitrogen content of young fully expanded leaf blades, oven dried at 60°C for 72 h, was determined using the semi-micro Kjeldahl digestion procedure of Allen [1989:120; two-thirds of a ground selenium catalyst tablet (BDH; Poole, Dorset, UK) were substituted for HgO] followed by the indophenol-blue method of NH_4^+ quantification (Allen 1989:124).

Insect feeding.—Leaf blades were fed to larvae of *P. meticulosa* beginning eight weeks after planting. Newly molted second-instar larvae were placed in filter-paper-lined petri dishes and fed an excess daily until their fifth molt. Dishes were cleaned and remoistened daily, and the occurrence of molts and deaths was recorded. When larvae completed their fifth molt, their fresh masses were determined and the length of time required to complete the fifth molt (=development rate) was estimated to within the nearest 0.5 d.

Experiment 2: Growth, consumption, and assimilation efficiency of sixth-instar larvae

The effects of the two fungi on the feeding rate, growth, and nutritional indices of sixth-instar larvae were examined in a 2×2 factorial experiment with two levels of *Neotyphodium* infection and two levels of mycorrhizal infection as per experiment 1. All food plants received 5.0 ppm phosphorus. Surviving larvae from trials 3 and 4 of experiment 1 were used. Only larvae that had attained a minimum fresh mass of 200 mg following their fifth molt were included, since smaller larvae tended to undergo additional molts, and rarely survived to reach pupation. Every 24 h following their fifth molt, larvae were weighed and fed enough fresh grass to ensure that some would remain uneaten after 24 h. Dry-matter content of the grass was also determined, using an additional aliquot of grass. After 24 h, uneaten grass was collected from the dish and oven-dried to determine its dry-matter content. Feces were collected and dried to determine the amount of dry matter egested.

Larvae were frozen when they reached their maximum mass and were oven-dried at 60°C for 72 h and their dry mass determined. The gut contents of each weighed larva were then dissected out, and their mass subtracted from that of the larva to obtain the true larval dry mass. Larval mass gain was calculated by subtracting larval dry mass at the beginning of the sixth instar from the final dry mass. Larval dry mass at the

beginning of the instar was estimated as 0.0998 × fresh mass, as calculated for a separate aliquot of 12 newly molted final-instar larvae. Dry mass consumed and egested, and larval feeding period—defined as the time between completion of the fifth molt and maximum mass being attained—were calculated. Relative growth rate (RGR) was calculated as follows:

$$\text{RGR (g/g} \times \text{d)} = \frac{\text{Dry mass gain}}{\text{Mean body mass} \times \text{Feeding period}}$$

(Kogan 1986) where mean body mass was calculated as

Mean body mass

= Dry mass gain/ln(Final dry mass/Initial dry mass).

Relative consumption rate (RCR) and relative egestion rate (RER) were calculated in an analogous manner, using dry matter consumed or egested instead of dry mass gain. Efficiency of conversion of ingested food (ECI), approximate digestibility (AD) and efficiency of conversion of digested food (ECD) were also calculated according to Kogan (1986).

Experiment 3: Preliminary antixenosis bioassay

The effect of mycorrhizae on larval preference with respect to foliar endophyte infection status of leaves, and vice versa, was examined in a series of four experiments in which larvae were offered a choice between (1) +E−M and −E−M leaves; (2) +E+M and −E+M leaves; (3) +E+M and +E−M leaves; and (4) −E+M and −E−M leaves. Third- and fourth-instar larvae reared on *R. obtusifolius* were placed in individual petri dishes and starved for 8 h. Two 7.5-cm sections of leaf blade were placed in each dish, intersecting at a 90° angle. Larvae were allowed to feed in the dark for 6 h, at the end of which leaves were removed from the dishes and blind-scored for damage by visual assessment.

Experiment 4: Feeding behavior of larvae foraging among intact plants

In this experiment the effect of mycorrhizae on the amount of herbivore damage sustained by intact +E and −E plants growing in a mixed outdoor stand was assessed. Plants of a single genotype (number II) were used. Plants were grown in 11-cm pots containing 750 g sand, with or without mycorrhizal inoculum as in experiment 1.

On 1 August 1996, newly planted pots of all four E × M treatment combinations were placed in a greenhouse with natural light and given one fertilization of low-P solution (1.10-mg KH_2PO_4 and 40-mg P-free fertilizer). A second low-P fertilization was given on 21 August. Beginning 19 September, one fertilization of high-P solution (5.50-mg KH_2PO_4 with 20-mg P-free fertilizer) was applied every week until 23 October.

On 25 October the plants were moved to a pair of outdoor rectangular enclosures on the Lancaster University campus. Each enclosure consisted of two compartments 90 cm wide × 90 cm long × 50 cm deep. The basal 20 cm of each compartment was filled with fine washed gravel. The upper 30 cm was surrounded with muslin supported by a wood frame. Each compartment contained 64 pots that were buried up to their rims in gravel. Pots were arranged in an 8 × 8 checkerboard with +E and −E plants in an alternating pattern. One compartment within each enclosure contained only mycorrhizal plants; the other, only non-mycorrhizal plants.

Twenty-three fifth- and sixth-instar larvae were then released into each of the two compartments of one enclosure ("grazed"); the other enclosure ("ungrazed") received no larvae in either compartment and acted as a control. After 15 d, the 6 × 6 innermost plants from each of the grazed compartments were removed and assessed for damage. Each leaf on a grazed plant was scored as having (1) no damage; (2) minor nicks suggesting the leaf had been tasted and rejected (designated as "type A" damage); and (3) more extensive ("type B") feeding damage. The shoots were then harvested, oven-dried at 60°C for 96 h, and weighed. Shoots from the corresponding 36 plants from each of the control compartments were also oven-dried and weighed. The roots of eight randomly selected plants from each of the four ungrazed treatments were harvested, and mycorrhizal infection levels were determined as described above.

Statistical analyses

Statistical analyses were performed using SPSS version 10.0 (SPSS 1999), except for chi-square analyses, which were calculated by hand (Zar 1984). All noncategorical data were analyzed by ANOVA or MAN(C)OVA using type III sums of squares. All two- and three-way interactions between foliar endophytes, mycorrhizae, and phosphorus or herbivory (if included) were tested for. If significant interactions were detected, custom hypothesis tests were performed to determine the effect of foliar endophytes in the presence and in the absence of mycorrhizae, and (if appropriate) vice versa. Blocks (i.e., trials) were included as random main effects where appropriate. Ratio data were arcsine square-root transformed before analysis. Data that did not pass Levene's test for homogeneity of variance were square-root transformed or log transformed. Neither transformation was successful for larval fresh mass in Experiment 1; these data were analyzed using ANOVA by ranks (Zar 1984:249). Data analyzed by MAN(C)OVA passed Box's *M* test for homogeneity of covariance using the criteria of Tabachnick and Fidell (2001:330). Those analyzed by MANCOVA also passed tests of homogeneity of regression (Tabachnick and Fidell 2001:291).

In experiment 1, the overall proportion of larvae sur-

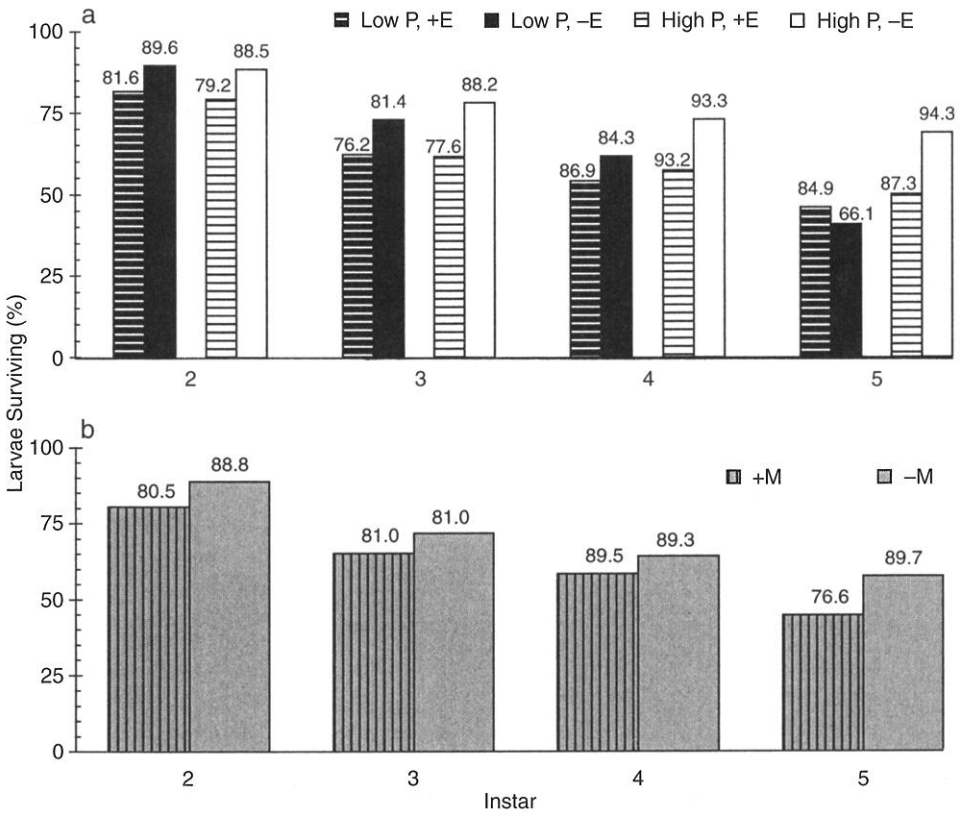

FIG. 1. Cumulative survivorship of *Phlogophora meticulosa* larvae feeding on *Lolium perenne* from the beginning of their second to the end of their fifth instar. (a) Effect of foliar endophytes (E) and phosphorus (P), pooled across mycorrhiza treatments; initial number of larvae at the beginning of the second instar: $N_0 = 98$ for both low (0.5 ppm) P treatments; $N_0 = 96$ for high (5.0 ppm) P, +E; and $N_0 = 94$ for high P, −E. (b) Effect of mycorrhizae (M), pooled across foliar endophyte and phosphorus treatments; $N_0 = 190$ for +M treatments, and N_0 196 for −M treatments. Bars indicate the percentage of larvae surviving from the beginning of the second to the end of the indicated instar; numbers above bars are the percentage survival within the indicated instar.

viving to the end of their fifth instar was analyzed by logistic regression using the Wald test statistic and backward conditional method of model building (Norusis 1993). Variables in the initial model included mycorrhizal infection (M), foliar endophyte infection (E), phosphorus fertilization rate (P), all two- and three-way interactions of the above, and trial. All variables were treated as categorical variables. Larval survivorship was also analyzed within each individual instar; statistical details are presented in the Appendix.

In experiment 2, cell sizes were too small to include all dependent variables in a single MANOVA (Tabachnick and Fidell 2001:329). Therefore the three primary measures of larval performance (larval dry mass, dry mass consumed, and dry mass egested) were analyzed by MANOVA; once significant effects were detected, the indices calculated from primary data (RGR, RCR, RER, ECI, AD, and ECD) were analyzed using separate ANOVAs. Larval feeding period was analyzed using ANOVA by ranks, since it was measured to within the nearest 0.5 d.

RESULTS

Experiment 1: Survivorship and growth of second- to fifth-instar larvae

There were significant main effects of mycorrhizae as well as M × P and E × P interactions (M = mycorrhizal infection, present [+] or absent [−]; P = phosphorus, nutrition level; E = endophyte [*Neotyphodium*], present or absent). Although there was an M × E interaction with larval survivorship in third-instar larvae (see the Appendix), the M × E and M × E × P interaction terms for the overall survivorship, fresh mass, and rate of development of larvae up to the end of their fifth instar were not significant.

Larval survivorship.—In general, larvae feeding on low-P plants had lower survivorship than those feeding on high-P plants (Fig. 1a). There was a significant E × P interaction with overall larval survivorship ($P < 0.001$). At high P, 19% fewer larvae survived to the end of their fifth instar when feeding on +E plants compared with those feeding on −E plants (pooled across mycorrhiza treatments; Fig. 1a). At low P, how-

TABLE 1. ANOVA results (P values) for development rate of *Phlogophora meticulosa* larvae between their second and fifth instar, and nonparametric ANOVA results (P values) for fresh mass of larvae immediately following their fifth molt.

Source of variation	df	Days to 5th molt (P)	Rank of larval fresh mass at 5th molt (P)
Mycorrhiza, M	1	0.706	<0.250
Foliar endophyte, E	1	0.600	<0.750
Phosphorus, P	1	**<0.001**†	**<0.001**‡
Trial number	3	**<0.001**	**<0.025**
M × E	1	0.169	<0.900
M × P	1	**0.027**	<0.250
E × P	1	0.389	<0.990
M × E × P	1	0.561	<0.750
Error df		187	197

Note: Boldface entries indicate $P < 0.05$.

† Dependent variable negatively correlated with P fertilization rate.

‡ Dependent variable positively correlated with P fertilization rate.

ever, survivorship of larvae was 5% higher on +E compared with −E plants. The beneficial effect of the foliar endophyte on larval survivorship at low P was manifested only in fifth-instar larvae; in second-instar larvae the fungus reduced survivorship at both high and low P (Fig. 1a and the Appendix).

There was a significant main effect of mycorrhizae on larval survivorship ($P = 0.007$). Overall survivorship of larvae was 13% lower when feeding on +M compared with −M plants (pooled across foliar endophyte and phosphorus treatments; Fig. 1b). The effect of mycorrhizae on overall larval survivorship was not P dependent (although there was an M × P interaction in third-instar larvae; Appendix). Mycorrhizae exhibited their strongest effect on fifth-instar larvae (Fig. 1b and the Appendix).

There were differences in overall larval survivorship between trials ($P < 0.001$). Larvae had the highest overall survivorship in trial 2 (66% of an initial 110 larvae survived to the end of their fifth instar), followed by trials 4 (58% of an initial 112 larvae), 1 (42% of an initial 109 larvae), and 3 (25% of an initial 55 larvae). If the two most "extreme" trials (2 and 3) are excluded from the analysis, the E × P interaction term remains in the model, although it is no longer statistically significant ($P = 0.069$). The mycorrhiza main effect is eliminated from the model and replaced by a nonsignificant M × P interaction ($P = 0.096$); mycorrhizae still tend to reduce survivorship, but do so more strongly in the low-P (15%) than in the high-P (6.1%) treatments.

Larval development rate and fresh mass.—Foliar endophytes had no effect on the development rate or fresh mass of larvae (Table 1). There was a significant M × P interaction with larval development rate (Table 1). In the low-P treatments, larvae that survived to the end of their fifth instar took 23.5 ± 0.5 d (mean ± 1 SE; $N = 35$ surviving larvae) to reach their fifth molt when feeding on +M plants, and 24.1 ± 0.6 d ($N = 49$ larvae) when feeding on −M plants. In the high-P treatments, however, larvae took longer to reach their fifth molt when feeding on +M (22.1 ± 0.6 d, $N = 50$ larvae) compared with −M (21.1 ± 0.5 d, $N = 64$ larvae) plants.

There were trial effects on larval development rate and fresh mass at fifth molt (Table 1). Larvae in trial 1 took the least time to reach their fifth molt (21.0 ± 0.4 d) and were the largest at their fifth molt (0.212 ± 0.014 g [mean ± 1 SE]). Those in trial 3 took the most time (28.4 ± 1.3 d) and were the smallest (0.129 ± 0.025 g). If these two trials are excluded, the M × P interaction with development time remains significant ($P = 0.003$). In addition, the M × P interaction with fresh mass becomes significant (ANOVA by ranks, $0.025 > P > 0.01$); at low P, mycorrhizae have little effect on larval fresh mass (−M: 0.103 ± 0.011 g, $N = 37$; +M: 0.107 ± 0.011 g, $N = 28$), whereas at high P they reduce larval fresh mass (−M: 0.246 ± 0.0128, $N = 40$; +M: 0.183 ± 0.016 g, $N = 33$).

Mycorrhizal infection levels and foliar N concentration.—The mean level of mycorrhizal infection of roots in the +M treatments was determined to be 14.9% of total root length. Phosphorus had no effect on mycorrhizal infection level. There was no main effect of the foliar endophyte on mycorrhizal infection level; however, there were E × plant genotype interactions (M. Vicari, *personal observation*).

The mean Kjeldahl N content of dried leaf blades of *Lolium* was 2.28%. There was no effect of mycorrhizae or foliar endophytes on foliar N. Genotype II leaf blades contained more N at low P (2.80%) than at high P (2.03%), while genotype I was unaffected by P availability (2.10% and 2.18%, respectively; P × genotype interaction $P < 0.001$). There were no other significant interactions.

Experiment 2: Growth and nutrition of sixth-instar larvae

Primary measures of larval performance and larval feeding period.—There were no significant differences between the initial fresh masses of larvae (all of which were 200 mg or more at the beginning of the instar) in the four treatments. A significant M × E interaction ($P = 0.022$) with the three primary measures of larval performance was detected by MANOVA. There were no significant main effects or interactions (ANOVA by ranks, all $P > 0.10$) of foliar endophytes or mycorrhizae with larval feeding period (median = 6.25 d, interquartile range = 2.125 d).

Univariate ANOVA detected a significant M × E interaction with final dry mass of larvae (Table 2). The foliar endophyte reduced larval dry mass in the absence of mycorrhizae (custom hypothesis test, $P = 0.012$), but not in their presence ($P = 0.640$). Mycorrhizae

TABLE 2. Univariate ANOVA results, (P values) and cell means, for primary measures of larval performance and calculated nutritional indices of sixth (final)-instar *Phlogophora meticulosa* larvae.

Dependent variable†	ANOVA (P)‡			Cell means (±1 SE) of untransformed data			
	M	E	M × E	−M−E (n = 8 larvae)	−M+E (n = 7 larvae)	+M−E (n = 6 larvae)	+M+E (n = 5 larvae)
Larval dry mass (g)	0.166	0.171	**0.044**	0.114 ± 0.006	0.089 ± 0.006	0.088 ± 0.010	0.094 ± 0.007
Dry mass eaten (g)§	0.151	0.900	**0.017**	0.696 ± 0.074	0.537 ± 0.056	0.443 ± 0.032	0.596 ± 0.035
Dry mass egested (g)	0.120	0.976	0.074	0.446 ± 0.048	0.364 ± 0.044	0.296 ± 0.029	0.375 ± 0.028
RGR ($g \cdot g^{-1} \cdot d^{-1}$)‖	0.970	0.900	0.904	0.188 ± 0.006	0.195 ± 0.020	0.194 ± 0.028	0.192 ± 0.023
RCR ($g \cdot g^{-1} \cdot d^{-1}$)‖,¶	0.763	**0.022**	0.640	1.476 ± 0.074	1.727 ± 0.132	1.379 ± 0.117	1.749 ± 0.196
RER ($g \cdot g^{-1} \cdot d^{-1}$)‖,¶	0.571	**0.035**	0.911	0.948 ± 0.052	1.158 ± 0.094	0.911 ± 0.072	1.101 ± 0.128
ECI (g/g)‖	0.856	**0.037**	0.631	0.130 ± 0.009	0.113 ± 0.007	0.137 ± 0.014	0.110 ± 0.005
AD (g/g)‖	0.567	0.840	0.095	0.357 ± 0.015	0.329 ± 0.020	0.336 ± 0.019	0.372 ± 0.018
ECD (g/g)‖	0.966	0.062	0.189	0.367 ± 0.031	0.345 ± 0.016	0.419 ± 0.059	0.300 ± 0.029

† AD = approximate digestibility; E = foliar endophyte (−, absent; +, present); ECD = efficiency of conversion of digested food; ECI = efficiency of conversion of ingested food; M = mycorrhiza (−, absent; +, present); RCR = relative consumption rate; RER = relative egestion rate; RGR = relative growth rate.

‡ Degrees of freedom = 1,22. Boldface entries indicate $P < 0.05$.

§ Data were square-root transformed to homogenize variances prior to analysis.

‖ Data were arcsine square-root transformed prior to analysis.

¶ Data were divided by 3 prior to arcsine square-root transformation to ensure all values <1.

reduced larval dry mass in the absence of the foliar endophyte (P = 0.015) but not in its presence (P = 0.636). Larvae feeding on +M−E or −M+E plants were similar in mass to those feeding on +M+E plants (Table 2).

There was also a significant M × E interaction with dry-matter consumption (Table 2). Mycorrhizae alone caused a reduction in larval consumption compared to −M−E controls (custom hypothesis test, P = 0.006). In the presence of the foliar endophyte, mycorrhizae had no effect (P = 0.459). The foliar endophyte alone showed a tendency to reduce total consumption, but it was not statistically significant (P = 0.071). In the presence of mycorrhizae, larvae feeding on +E plants actually tended to consume more than those feeding on −E plants, although again the tendency was not significant (P = 0.087). Thus, it appears that there was a strong mycorrhizal effect on dry-matter consumption, but only in the absence of the foliar endophyte (Table 2). A pattern similar to that described for dry-matter consumption was evident for dry-matter egestion, although the M × E interaction term was not significant (Table 2).

Calculated nutritional indices.—None of the calculated indices showed significant M × E interactions or mycorrhiza main effects (Table 2). However, the foliar endophyte significantly increased RCR and RER, and reduced ECI (Table 2). It had no significant effect on AD or ECD (Table 2).

Leaf dry-matter content.—The mean dry-matter content of *Lolium* leaf blades was 24.2% of fresh mass. There was no effect of mycorrhizae or foliar endophytes on arcsine square-root-transformed dry-matter proportion, nor were there any M × E or M × E × P interactions.

Experiment 3: Petri dish choice tests

When offered a choice between excised +E and −E leaf blades, a significant majority of larvae (50 out of 74 individuals) consumed more of the −E leaf blades (Table 3). Mycorrhizal infection of *Lolium* had no significant effect on larval preference with respect to foliar endophytes. When offered a choice between +M and −M leaf blades, larvae showed no preference, with 28 out of 68 individuals consuming roughly equal amounts of each leaf blade (Table 3). Infection by the foliar endophyte had no effect on (lack of) larval preference with respect to mycorrhizae.

Experiment 4: Foraging of larvae among intact plants

Shoot biomass.—In the outdoor enclosures, herbivory by *P. meticulosa* reduced shoot biomass by a mean of 6.4% across all treatments (P = 0.005; Fig. 2). There was a nearly significant (P = 0.056) three-way interaction between herbivory, the foliar endophyte, and mycorrhizae. Herbivory reduced biomass by 13.3% in −M−E plants (custom hypothesis test, $P < 0.001$) but by no more than 5.4% in +M−E, −M+E and +M+E plants (all $P > 0.10$).

Shoot biomass was reduced in symbiotic plants compared to −M−E controls. Pooled across foliar endophyte and herbivory treatments, mycorrhizae reduced plant biomass by 17.4% ($P < 0.001$); pooled across mycorrhiza and herbivory treatments, foliar endophytes reduced biomass by 9.7% ($P < 0.001$). There was a significant M × E interaction (P = 0.001): pooled across herbivory treatments, mycorrhizae reduced biomass by 22.7% in −E plants, but by only 11.3% in +E plants (Fig. 2).

Number of damaged leaves.—Mycorrhizae reduced the total number of leaves per plant (ANOVA, $P <$ 0.001), while foliar endophytes had no effect on total leaf number (P = 0.782). Because leaf number was affected by fungal treatment, the number of type-A damaged leaves (minor feeding scars) and type-B damaged leaves (moderate to severe feeding scars) per plant

TABLE 3. Numbers of third- and fourth-instar *Phlogophora meticulosa* larvae feeding preferentially on excised leaf blades of *Loloum perenne* from foliar endophyte-infected (+E) and endophyte-free (−E) plants, as influenced by mycorrhizae (+M or −M), and vice versa.

A) Effect of mycorrhizae on larval preference for −E over +E leaf blades

	Insect's two-way choice					
	−E	+E	No pref.	Total	χ^2_2	*P*
−M	26	2	9	37		
+M	24	7	6	37		
Total no. larvae†	50	9	15	74		
E × M interaction					3.46	$0.250 > P > 0.100$
E main effect					39.07	<0.001

B) Effect of foliar endophytes on larval preference for −M over +M leaf blades

	Insect's two-way choice					
	−M	+M	No pref.	Total	χ^2_2	*P*
−E	10	14	12	36		
+E	9	7	16	32		
Total no. larvae‡	19	21	28	68		
E × M interaction					2.73	$0.500 > P > 0.250$
M main effect					1.97	$0.500 > P > 0.250$

Note: Preferences were determined by visually estimating which leaf was most damaged.
† Pooled across M treatments.
‡ Pooled across E treatments.

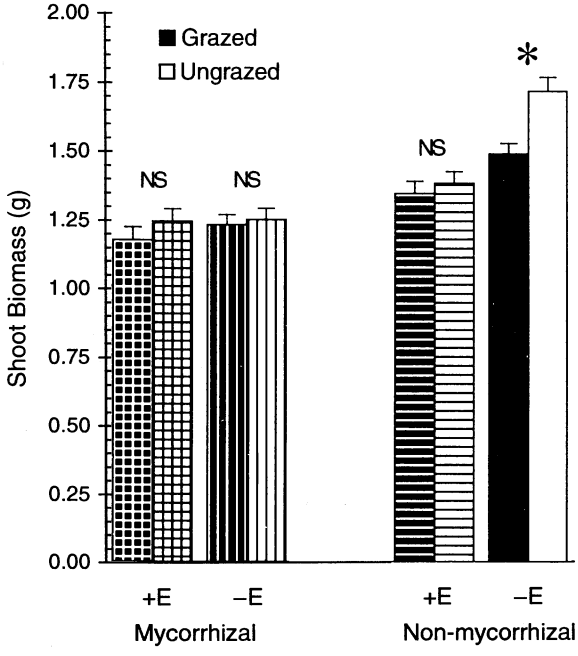

FIG. 2. Effect of mycorrhizal infection (M), foliar endophyte infection (E), and herbivory by fifth- and sixth-instar *Phlogophora meticulosa* larvae on shoot biomass of *Lolium perenne* in an outdoor enclosure. Data are means and 1 SE. The asterisk indicates a significant ($P < 0.05$) difference between grazed and ungrazed treatments. $N = 18$ plants for all treatments. Vertical hatching = +M treatments; horizontal hatching = +E treatments.

were analyzed by MANCOVA, using total leaf number as the covariate. A significant M × E interaction was detected ($P = 0.016$), as well as M ($P = 0.001$) and E ($P < 0.001$) main effects.

Foliar endophytes significantly reduced the number of type-A damaged leaves as a function of plant size (univariate ANOVA, $P < 0.001$) while mycorrhizae had no effect on this type of leaf damage ($P = 0.234$; Fig. 3). There was a significant M × E interaction ($P = 0.007$) with the number of type-B damaged leaves. When the latter is adjusted to compensate for differences in plant size, it is evident that mycorrhizae reduced the number of damaged leaves in −E plants (custom hypothesis test, $P < 0.001$) but not in +E plants ($P = 0.801$). The foliar endophyte reduced the number of damaged leaves in both −M and +M plants ($P < 0.001$ for both; Fig. 3).

DISCUSSION

Barker (1987) found that the antixenotic effect of *Neotyphodium lolii* on an insect herbivore was reduced when the arbuscular mycorrhizal (AM) fungus *Glomus fasciculatum* was also present in the host. Our findings confirm and extend those of Barker; the presence of another AM fungus, *G. mosseae*, reduced the difference between +E (endophyte present) and −E (endophyte absent) plants in terms of the amount of damage suffered from herbivory (Figs. 2 and 3). Our findings differ from Barker's, however, in two major respects: (1) mycorrhizae (M) themselves reduced feeding damage by larvae; and (2) the M × E interaction described above was caused by an apparent disappearance of the mycorrhiza effect in the presence of the foliar endophyte, rather than vice versa (Fig. 3). We have also gone fur-

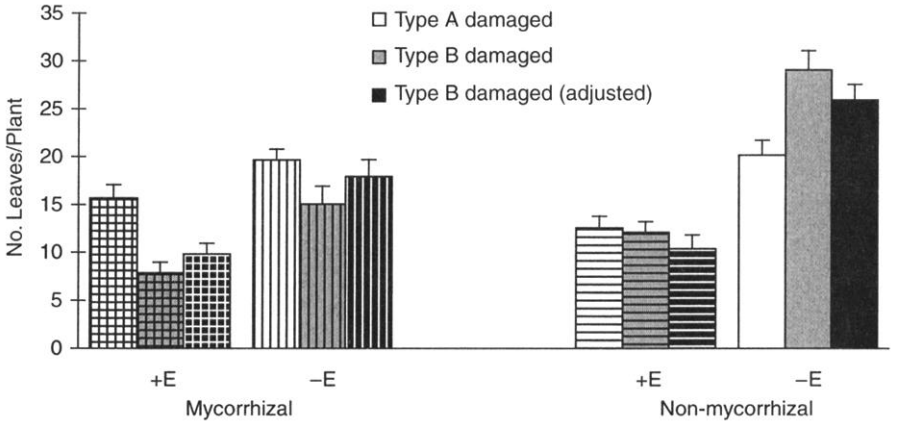

FIG. 3. Number of leaves of *Lolium perenne* showing type-A (minor) or type-B (moderate to severe) feeding scars; number of type-B scarred leaves adjusted for differences in plant size (i.e., total number of leaves per plant) is also shown. +E = foliar endophyte-infected; −E = foliar endophyte-free. Data are means and 1 SE. N = 18 plants for all treatments. Vertical hatching = +M treatments; horizontal hatching = +E treatments.

ther than Barker in investigating the antibiotic effects of foliar endophytes and mycorrhizae on the insect, some of which were additive (Fig. 1), while others were not (Table 2).

Effect of the two fungi on insect performance in the absence of host P limitation

The occurrence of interactions between different plant-symbiotic fungi with respect to their effects on herbivore performance appears to be a widespread phenomenon. Gange (2001) reported that two AM fungi, *G. mosseae* and *G. fasciculatum*, had detrimental effects on an insect herbivore when present individually in the host *Fragaria* × *ananassa*. When both fungi were present together, however, there was no statistically significant effect of AM infection on herbivore performance; each fungus appeared to cancel out the effect of the other. Most plants in their natural settings are likely to be infected by multiple species of both mycorrhizal (Clapp et al. 1995) and foliar endophytic (Bills 1996) fungi. Thus, the findings of Barker (1987) and Gange (2001) suggest that the effect of any individual plant-symbiotic fungus on herbivores in the "real world" may be difficult to assess accurately without controlling for the effect of other fungi that would normally be present.

Our findings concur with those of Barker (1987) and Gange (2001) in that the leaf endophyte and AM fungus did not have additive effects on the growth and consumption of final-instar *Phlogophora meticulosa* larvae (experiment 2, Table 2). However, in those two studies, plants infected by multiple fungi were better quality and/or more preferred food for the herbivore than plants infected by only one fungus capable of causing detrimental effects on the herbivore. Except where plants were P limited (see *Effect of host P limitation. . .* , below), this was not the case in our study. The two fungi had additive detrimental effects on the overall survivorship of larvae between their second and fifth instar (Fig. 1); mycorrhizae also slightly reduced development rate and (possibly) fresh mass of larvae at the end of their fifth instar, independently of the foliar endophyte. Final-instar larvae feeding on +M+E plants were similar in mass to those feeding on plants with only one fungus (Table 2). Collectively, these results suggest that when P is not limiting to plant growth, the fitness of *P. meticulosa* should be greatest when feeding on −M−E plants, lower on plants infected by only one of the two fungi, and lower still on +M+E plants. The presence of both fungi together yielded the poorest overall quality food for the herbivore, contrary to the findings of Barker (1987) and Gange (2001). Thus it appears that the only generalization that can be made (based on the limited number of available studies) regarding the effects on herbivores of multiple infection of plants by fungal symbionts is that the effects of different fungi are unlikely to be completely additive.

The detrimental effects of *Neotyphodium* on some herbivores are believed to be primarily due to the production of one or more of a variety of secondary metabolites by the fungus (Bush et al. 1997), some of which may as yet remain undiscovered (Brem and Leuchtmann 2001). The mechanisms by which mycorrhizae affect herbivores are less well understood; several foliar-feeding Lepidoptera (Rabin and Pacovsky 1985, Gange and West 1994), a gall fly (Gange and Nice 1997), a root-feeding larval weevil and its conspecific leaf-feeding adult (Gange 2001) have all been shown to be negatively affected by AMs. It is often the case that the induction of resistance to herbivores in plants may affect the induction of resistance to pathogens, and vice versa (Felton and Korth 2000, Paul et al. 2000) but it remains to be determined if this is the case for mycorrhiza-mediated resistance.

Mycorrhizae are sometimes associated with reductions in foliar N concentration that are correlated with

detrimental effects on herbivore performance (Gange and West 1994, Gange and Nice 1997), but this is not always the case (Rabin and Pacovsky 1985). In our study, mycorrhizae had no effect on Kjeldahl N, supporting Rabin and Pacovsky's finding that negative effects on herbivores need not be correlated with changes in plant N concentration. The mechanisms by which mycorrhizae affect insect performance are clearly different from those associated with foliar endophytes, as indicated by differences in the timing of larval mortality (Fig. 1 and the Appendix) and in the ability of insects to discriminate between infected and uninfected plants (Table 3). Thus, +M+E plants may gain resistance to a wider range of herbivores than singly infected plants, even if the effects of the two fungi are not additive with respect to individual herbivore species. Further work is needed to determine the mechanism(s) by which mycorrhizae affect herbivore performance.

As noted above, the effects of the AM fungus and the foliar endophyte on the dry mass attained by sixth-instar larvae were not additive (experiment 2, Table 2). The mechanisms underlying the nonadditivity of these effects could not be elucidated from the calculated nutritional indices, since none of them showed significant first-order interactions. Additional trials to increase the sample size of larvae might circumvent this problem. The foliar endophyte itself increased the relative consumption rate (RCR) and the relative egestion rate (RER) without affecting the relative growth rate (RGR); it also reduced the efficiency of conversion of ingested food (ECI). Thus it is clear that the foliar endophyte had post-ingestive effects on *P. meticulosa* in addition to the antixenotic effects observed in experiments 3 and 4. Similar effects of systemic fungi on RCR and ECI were observed in two other lepidopteran herbivores feeding on dicot hosts (Kingsley et al. 1983, Raps and Vidal 1998). However, a low-alkaloid *Neotyphodium* endophyte actually increased RGR in the grasshopper *Xanthippus corallipes* (Saikkonen et al. 1999). ECI is influenced by the water and N content of host-plant tissue (Scriber and Slansky 1981), but we did not detect any effect of the foliar endophyte on either of these variables. It is likely that alkaloid production influences insect nutritional indices, although other mechanisms (e.g., the competitive consumption of foliar nutrients by the endophyte; Raps and Vidal 1998) cannot be ruled out.

In experiment 1 there were significant differences between trials in overall larval survival, development rate, and fresh mass following the fifth molt (Table 1). Trial 3 stands out as one with exceptionally high overall larval mortality and slow larval development. Viral infections, which are common in Lepidoptera and a frequent problem in artificial colonies (Rivers 1976), often produce such symptoms (Rothman and Myers 1996). Genetic differences between larvae used in different trials could also be responsible for some of the trial effects (Goverde et al. 2000). Elimination of the most "extreme" trials from the analyses did not significantly alter the conclusions.

Effect of the two fungi on plant resistance

Plant fitness, in a competitive environment, should be improved most effectively by fungal symbionts if the latter cause herbivores to inflict more damage on competing genotypes and/or species that are uninfected. In experiment 4 we found that mycorrhizae reduced, but did not eliminate, this effect of the foliar endophyte. Plant biomass in the −M treatments was significantly reduced by herbivory in −E but not in +E plants (Fig. 2). In the +M treatments herbivory had no significant effect on the biomass of −E or of +E plants. However, the type-B feeding-scar counts (Fig. 3) show that the endophyte effect in the +M treatments was not completely eliminated; +E plants had 55% as many feeding scars as −E plants (compared with 40% as many in the −M treatments). These percentages are similar to those calculated from Barker's (1987) data. However, mycorrhizae apparently did not increase the palatability of +E genotypes to the herbivore, as was the case in Barker's study. Three pieces of evidence support this conclusion: (1) the foliar endophyte–mycorrhiza interaction with type-B feeding-scar number appears to arise as a result of the mycorrhizal effect being eliminated in the presence of the foliar endophyte, rather than the other way around (Fig. 3); (2) the tendency of larvae to taste +E plants and move to another plant was unaffected by mycorrhizae (type-A damage, Fig. 3); and (3) in experiment 3, mycorrhizae did not affect insect preference for −E over +E leaf blades (Table 3a). The discrepancies between our findings and those of Barker (1987) could be due to the fact that different species of AM fungi, and/or genotypes of *N. lolii* (which vary in the types and quantities of alkaloids they produce; e.g., Christensen et al. 1993), were used. The ecological and evolutionary role of variation between and within different endophyte/mycorrhiza/host-plant species combinations, including its implications for the herbivore defense hypothesis, is an important area for future exploration.

It has been suggested that the role of mycorrhizae in plant nutrition may, at least in some hosts, be secondary to their importance in pathogen (Newsham et al. 1995) and/or herbivore (Gange and Bower 1997) resistance. Although we did not detect any antixenotic effect of mycorrhizae on *P. meticulosa* (Table 3b), we did observe reductions in larval growth and survival that could improve plant fitness. Our findings support those of Rabin and Pacovsky (1985), Gange and West (1994), Gange and Nice (1997), and Gange (2001) who have reported a detrimental effect of mycorrhizae on insect herbivores. However, mycorrhizal effects on herbivores are highly variable. Mycorrhizal colonization of sorghum had no effect on an aphid (Rabin and Pacovsky 1985), and other studies have shown that my-

corrhizae can actually improve insect performance (Borowicz 1997, Gange et al. 1999, Goverde et al. 2000). Our findings show that foliar endophytes can alter the effect of mycorrhizae on insect herbivores. In addition to the above-mentioned interaction with type-B feeding-scar number (experiment 4, Fig. 3), there was a tendency for the foliar endophyte to eliminate the depressive effect of mycorrhizae on total larval consumption in a no-choice feeding situation (experiment 2, Table 2). Clearly, the presence or absence of foliar endophytes should be taken into account when assessing the effect of AM fungi on plant resistance, and not merely vice versa.

There were costs—in the form of reduced shoot biomass—to *L. perenne* of infection by each of the two fungi in experiment 4 (Fig. 2). While some foliar endophytes can improve host vigor even in the absence of herbivory (e.g., Marks et al. 1991), there is considerable variation between and within host species (Cheplick 1997, Johnson-Cicalese et al. 2000). Mycorrhizae often have detrimental effects on the shoot biomass of C_3 grasses when phosphorus is not limiting to growth (e.g., Buwalda and Goh 1982). Thus, in order for either fungus to improve host fitness because of reduced herbivory, the intensity of herbivory would have to be great enough to outweigh the costs of infection.

Effect of host P limitation on foliar endophyte–mycorrhiza interactions

Due to the well-known importance of arbuscular mycorrhizae in plant P nutrition (Smith and Read 1997), in experiment 1 we included P nutrition as a factor in our investigation of the performance of second- to fifth-instar larvae. Mycorrhizae did not markedly improve insect performance when plants were P limited, as they have been reported to do in some studies (Borowicz 1997, Gange et al. 1999). There was an M × P interaction with larval development rate, but the beneficial effect of mycorrhizae at low P was small. Mycorrhizae reduced overall larval survivorship at both low and high P, independently of the foliar endophyte; they did not mitigate larval mortality associated with low P. This is consistent with the fact that plants with fine roots, including C_3 grasses, tend not to be dependent on mycorrhizae for P uptake (Hetrick et al. 1991). The foliar endophyte, in contrast, dramatically improved the survivorship of fifth-instar larvae in the low-P treatments, compensating for endophyte-associated mortality in earlier instars (Fig. 1a). Thus, when P is limiting to plant growth, the fitness of *P. meticulosa* may be lowest on +M−E plants, rather than +M+E plants (although we did not consider final-instar larvae in this part of our study). It is important to emphasize that this was due to a beneficial effect of the foliar endophyte, independent of mycorrhizae, on survivorship at low P; it was not due to a "cancellation" of a detrimental effect of the foliar endophyte by the mycorrhizal fungus. Recent studies (Malinowski et al. 1998, Malinowski and Belesky 1999) have indicated that the endophyte *Neotyphodium coenophialum* can improve P uptake in its host *Festuca arundinacea*. Our findings suggest that other *Neotyphodium* spp. may also do this.

We did not investigate the effects of P limitation on fungal-mediated antixenosis. However, the foliar endophyte reduced the survivorship of second-instar larvae independently of host P nutrition and mycorrhizae (Fig. 1a and the Appendix), suggesting that P-limited plants did contain alkaloids. In Barker's (1987) study, P limitation of *L. perenne* had no effect on *N. lolii*-mediated antixenosis when insects were offered a choice between +E and −E plants. Thus, even under P limitation (and in the presence of mycorrhizae), it is possible that the host plant could benefit from foliar endophyte infection via reduced palatability to insect herbivores. The influence of host P nutrition on foliar endophyte–herbivore interactions merits further investigation.

Conclusions

What are the implications of our findings for the herbivore defense hypothesis? Our results concur with those of Barker (1987) in that the host plant gained less benefit, in terms of protection from herbivory, from its foliar endophyte in the presence of mycorrhizae. Furthermore, the foliar endophyte's detrimental impact on the growth of final-instar larvae was eliminated (or masked) in the presence of mycorrhizae. However, there were no interactions between mycorrhizae and foliar endophytes with respect to their effects on the overall survivorship of second- to fifth-instar larvae, and there was no evidence that mycorrhizae reduced the antixenotic properties of the foliar endophyte. Thus, it would be premature to conclude that mycorrhizae render foliar endophytes ineffective as a herbivore defense, although they do appear to reduce their impact to a degree. The differences between our findings and those of Barker (1987) suggest that not all species or strains of AM fungi interact with foliar endophytes in the same way. Further work is needed to characterize the foliar endophyte–mycorrhiza associations found in the field, and to determine their impact on plant and herbivore dynamics.

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APPENDIX

A table presenting the results of logistic regression analyses of the effects of the foliar endophyte, mycorrhizae, and host P nutrition on the survivorship of specific instars (second, third, fourth, and fifth) of *Phlogophora meticulosa* larvae is available in the ESA's Electronic Data Archive: *Ecological Archives* E083-049-A1.