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Ectomycorrhizal mediation of competition between coniferous tree species

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SUMMARY

To test the effect of ectomycorrhizal fungi (EMF) on interactions between host plants, *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* Dougl. ex. Laws., seedlings were grown in replacement series in pasteurized soil with (*a*) no EMF added, (*b*) two EMF species added – *Rhizopogon vinicolor* Smith (specific to Douglas-fir) and *R. ochraceorubens* Smith (specific to pine), and (*c*) four EMF species added – the two *Rhizopogon* species plus two host generalists, *Laccaria laccata* (Scop. ex Fr.) Bk. & Br. and *Hebeloma crustuliniforme* (Bull.) Quel. A replacement series in unpasteurized forest soil also was included. Seedlings without added EMF were colonized by the greenhouse contaminant, *Thelephora terrestris*. Without added EMF (but with *T. terrestris*), the tree species mutually inhibited one another, producing Relative Yield Totals significantly < 1; with EMF added, mutual inhibition disappeared. With four EMF species added, *Pseudotsuga menziesii* seedlings were significantly larger in mixture than in monoculture, with no corresponding decrease in the size of *Pinus ponderosa* seedlings; this was due solely to seedlings with *L. laccata*, which apparently enhanced nitrogen (N) and phosphorus (P) uptake by *Pseudotsuga menziesii* at the expense of luxury consumption by *Pinus ponderosa*. Graphical analysis suggested that better growth of *Pseudotosuga menziesii* in mixture with EMF added was related to improved P nitrogen. Both N and P nutrition of *Pinus ponderosa* was better in mixture with two than no EMF species added; there was no clear nutrient effect with four EMF species added. Results indicate that EMF can reduce competition between plant species and perhaps increase overall community P uptake. However, patterns were specific to both EMF and tree species and were quite different in unpasteurized soils. Hence generalizations about the effects of EMF on plant–plant interactions must be made cautiously.

Key words: Ectomycorrhizas, competition, *Pseudotsuga menziesii*, *Pinus ponderosa*.

INTRODUCTION

Few experiments have addressed the role played by mycorrhizal fungi in interactions between plant species. One of the most common mutualisms in nature, mediation of plant–plant interactions by mycorrhizal fungi may take several forms. The most obvious is in shifting site resources from one plant species to another, but with no effect on overall community productivity. In nutrient- or water-limited environments, for example, mycorrhizas may enable sparsely rooted plants such as trees or legumes to compete with densely rooted plants such as grasses (Hall, 1978; Bowen, 1980). On fertile sites, the effect may be quite different – the cost–benefit ratio of the symbiosis narrowing to a point that competitive advantage of the host is reduced (Fitter, 1977). The ability of at least some mycorrhizal fungi to detoxify allelochemicals is probably important in competitive relations between plants (St. John & Coleman, 1983; Perry & Choquette, 1987), and the general vigour mycorrhizas impart to hosts through factors such as improved nutrition and protection from pathogens is likely indirectly to influence how successfully plants compete. When plant species share species of mycorrhizal fungi, resources may be transferred from one plant to another through hyphal linkages (Read, Francis & Finlay, 1985; Francis, Finlay & Read, 1986).

Mycorrhizal fungi might also enhance overall community productivity rather than simply mediate transfer of resources from one plant species to another. Little research has addressed this possi-

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bility; however, Puga (1985) showed that vesicular–arbuscular (VA) mycorrhizas increased yield in mixtures of corn (*Zea mays* L.) and *Solanum ocraceo*, a common weedy shrub. Mycorrhizal fungi might increase overall community efficiency in various ways, one of which – mycorrhiza diversity – is particularly relevant to this paper.

Thousands of species of fungi form ectomycorrhizas (Malloch, Pirozynski & Raven, 1980). Of these, some are host generalists, some are host specific, and some exhibit host 'preference', i.e. although generalists, they tend to be more abundant on some plant species than on others. Species of mycorrhizal fungi differ both in their response to the environment and in the benefits they confer on hosts (Trappe, 1977; Bledsoe, 1986; Perry, Molina & Amaranthus, 1987). For example, fungus species vary widely in their response to allelochemics leaching from litter (Rose *et al.*, 1983), which may result in vertical stratification of fungi within the soil (Perry & Choquette, 1987); they also differ in temperature sensitivity (Parke, Linderman & Trappe, 1983*b*), hence seasonal activity. Fungi vary in ecophysiological factors such as water transport (Parke, Linderman & Black, 1983*a*), ability to decompose organic matter (D. Durall, R. Tood & J. Trappe, unpublished manuscript), production of phosphatases (Ho & Zak, 1979; Antibus *et al.*, 1981), nitrogen nutrition (Littke, Bledsoe & Edmonds, 1984), and respiration rates (Marshall & Perry, 1987). Mycorrhizae diversity probably enhances the ability of hosts to respond to spatial and temporal environmental variability, leading to more efficient utilization of resources (Perry, 1985; Bledsoe, 1986). Where different plant species host different mycorrhiza species, it is plausible that differing responses of mycorrhizas to the environment could also decrease competition between host plants.

Our objective in this study was to determine whether mycorrhiza diversity would influence interactions between two commonly associated conifers – *Pseudotsuga menziesii* (Mirb.) Franco and *Pinus ponderosa* Dougl. ex Laws. – growing in pots of reconstructed field-collected litter and forest soil. We hypothesized that competition between the tree species would be decreased by adding ectomycorrhizal fungi (EMF), and that more efficient resource utilization would result in a positive correlation between Relative Yield Total (Harper, 1977) and EMF diversity.

MATERIALS AND METHODS

Mycorrhizal inoculation

Pseudotsuga menziesii and *Pinus ponderosa* seed collected from trees in similar habitats in southwest Oregon were sown in plastic Ray Leach tubes 2·5 cm in diameter and 16 cm deep, filled with pasteurized potting mix (0·5 × 0·5, v/v) of vermiculite and commerical peat moss. Seedlings were either inoculated with one of four EMF species or left uninoculated. Inoculation procedures followed Molina & Trappe (1982). Inocula of *Laccaria laccata* (Scop. ex Fr.) Bk. & Br. and *Hebeloma crustuliniforme* (Bull.) Quel., both of which colonize the two tree species, were incorporated in the potting mix of the appropriate treatment before planting. Inocula of *Rhizopogon vinicolor* Smith (specific to *Pseudotsuga menziesii*) and *Rhizopogon ochraceorubens* Smith (specific to *Pinus ponderosa*) were added in a slurry when seedlings were 16 weeks old.

Replacement series

When 28 weeks old, seedlings verified as mycorrhizal were transplanted in a replacement series to plastic pots 20 cm in diameter and 20 cm deep. Each pot contained 12 randomly distributed seedlings in one of the following proportions: 12 *Pseudotsuga menziesii* (PM)/0 *Pinus ponderosa* (PP), 9PM/3PP, 6PM/6PP, 3PM/9PP, or 0PM/12PP. Four such replacement series were planted (Fig. 1*a*): one in unpasteurized forest soil with no EMF added, and three in pasteurized forest soil with (*a*) no EMF added, (*b*) *R. vinicolor* added to *Pseudotsuga menziesii* and *R. ochraceorubens* to *Pinus ponderosa* (2-EMF treatment), and (*c*) *R. vinicolor*, *L. laccata*, and *H. crustuliniforme* added to *Pseudotsuga menziesii* and *R. ochraceorubens*, *L. laccata*, and *H. crustuliniforme* to *Pinus ponderosa* (4-EMF treatment). In the 4-EMF treatment, individual seedlings within a pot were inoculated according to the scheme shown in Figure 1*b*. Each treatment combination (tree-species mix × EMF mix) was replicated five times (i.e. was represented by five pots).

Soils for the replacement series were collected from a mixed *Pseudotsuga menziesii*/*Pinus ponderosa* stand in the Siskiyou Mountains of southwestern Oregon, USA. Samples from the litter/humus layer (approx. 2 cm deep), A horizon, and B horizon were collected separately and reconstructed in pots such that one half of the soil depth in each pot was from each horizon, with the litter/humus layer added to the surface. Soils for the pasteurized treatments were steamed for 1·5 h at 70 °C.

Trees were grown for 12 months in a greenhouse. Natural light was supplemented by six Lumalux® 400 W bulbs and daylength during winter maintained at 16 h. Temperature generally varied from 25–30 °C, although it was higher for brief periods during summer. Pots were watered with tap water as necessary, generally every 2 weeks in winter and every other day in summer. Ammonium nitrate (50 mg l^{-1}) was added in irrigation water twice during the experiment.

We separated the different EMF treatments by at least 50 cm on the greenhouse bench to prevent cross contamination from irrigation splash and systemati-

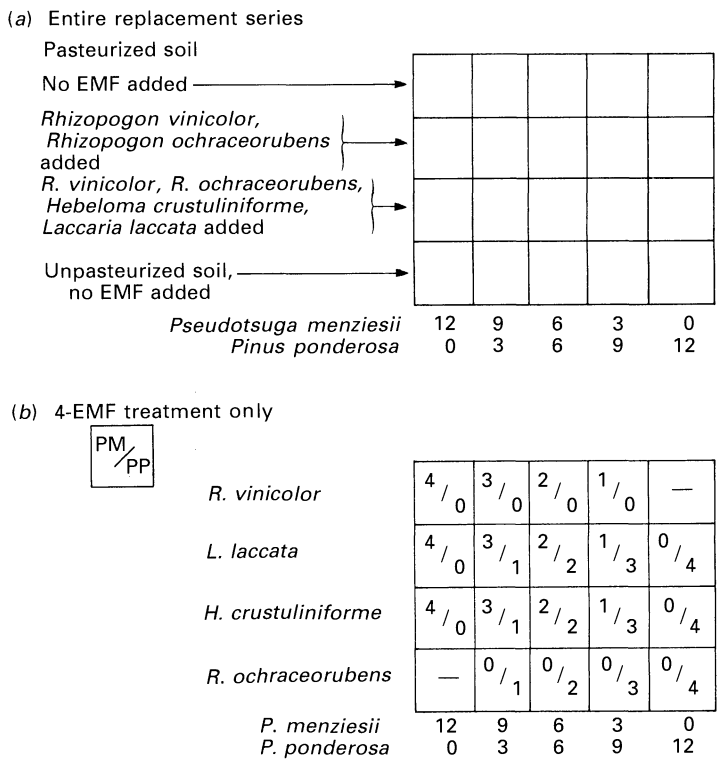

Figure 1. Schematic diagrams of the experimental design in which two conifer species – *Pseudotsuga menziesii* and *Pinus ponderosa* – were grown in mixture and monoculture in potted soil with and without the added ectomycorrhizal fungi (EMF) *Rhizopogon vinicolor*, *Rhizopogon ochraceorubens*, *Hebeloma crustulininforme*, and *Laccaria laccata*.

cally rotated pots to a different bench position at least once every 2 weeks to avoid positional effects. Pot surfaces were inspected at least once a week for fruiting bodies of the EMF *Thelephora terrestris* (Ehrh.) Fr., a common greenhouse contaminant, and were cleaned where necessary.

At lifting, trees were separated into roots, stems, and foliage, and oven dried for 48 h at 70 °C before weighing. All weights were on a per-tree basis (except for trees labelled with ^{32}P; see below). Before oven drying, mycorrhizal infection was estimated on roots of two individuals per tree species per pot. Root systems were placed under a dissecting microscope, and the percentage of root tips colonized by each mycorrhiza type was estimated by category (0–20, 21–40, 41–60, 61–80, > 80 %). For nutrient analyses, foliage samples were taken from a subset of trees in each pot in all treatments and digested by standard microKjeldahl technique. Nitrogen (N) and phosphorus (P) concentrations were then determined on an autoanalyzer.

^{32}P *labelling*

^{32}P labelling was used to assess whether EMF influenced spatial patterns (e.g. from A or B horizon) of nutrient uptake by the two tree species. Uptake of ^{32}P from two soil depths was determined for the 9PM/3PP and 3PM/9PP mixes from the pasteurized no-EMF and 2-EMF treatments. In October, after all trees had set bud, 30 holes slightly larger in diameter than a standard hypodermic needle were drilled into soil around the circumference of each of 16 pots; eight pots had holes 4 cm below the soil surface, eight 16 cm below. Each pot was injected with 4500 μCi (150 μCi per hole) of ^{32}P as orthophosphoric acid in a 0·001 mM HCl solution. Injected pots were kept in the greenhouse for 3 weeks, at which time tops were harvested, oven dried at 70 °C for 48 h and weighed individually. Roots were pooled for all individuals of a given tree species in a given pot (^{32}P counts in roots were too high for individual handling).

Approximately 0·9 g of foliage and 1·0 g of stems were placed in standard 25 ml glass liquid scintillation vials and ashed in a muffle furnace at 550 °C for 24 h. Then 1 ml of 6 N HCl was added to and evaporated from each vial twice, after which 4 ml of 0·1 N HCl were added. A 2 ml aliquot of the final mixture was transferred to a 1 dram counting vial, 2·5 ml of Aquasol-2 were added, and the vial was agitated on a vortex mixer. The 1 dram vial was placed inside a standard glass LSC vial and ^{32}P counted for 5 min on a Beckman LS-250 liquid scintillation counter.

Statistical analyses

We used Relative Yield Total (RYT) as a standard, comparing treatment yields for biomass and nutrient

uptake for significant departures from their respective RYT = 1 lines. For two species, I and J, growing in mixture (Harper, 1977),

$$\text{RYT} = \frac{\text{yield of I in mixture}}{\text{yield of I in pure stand}} + \frac{\text{yield of J in mixture}}{\text{yield of J in pure stand}}.$$

According to Harper (1977, p. 261), 'Values of RYT of *ca* 1·0 imply that the two species are making demands on the same limiting resources of the environment. Values of RYT > 1·0 suggest that the species make different demands on resources, avoid competition with each other or are showing some form of symbiotic relationship... Values of RYT < 1·0 imply a mutual antagonism.'

To test yields for significant differences from RYT = 1·0, we estimated two sets of standard errors, one for mean yields and one for each of the theoretical RYT = 1 points associated with each mean yield. We used residual mean squares from analysis of variance (ANOVA) of the entire data set (SAS Institute Inc., 1985) to estimate standard error of mean yields. Untransformed residuals were generally well distributed and not improved by log transformation, hence ANOVAs of untransformed data were used.

To calculate standard errors of the RYT = 1 points corresponding to a given tree-species mix (i.e. points lying along the theoretical RYT = 1 line) for each EMF treatment, we ran a simple linear regression using the five replicate values each for the pure *Pseudotsuga menziesii* and pure *Pinus ponderosa* pots. We then used the residual mean square from this regression to calculate the standard error associated with predicting a new Y value from regression (Snedecor & Cochran, 1980, p. 166). The significance of deviation of a given yield from RYT = 1 was then examined with a t test, a procedure used to check significance of the deviation of a point from a regression line (Snedecor & Cochran, 1980, p. 167) but here modified to account for uncertainty in the position of both the regression line and the points to be tested.

To compare treatment means with one another rather than with the RYT line, we calculated least significant differences (L.S.D.S) from the error mean square terms of the ANOVAs (Petersen, 1985).

RESULTS

Mycorrhizal colonization

When seedlings were transferred from synthesis tubes to pots, seedlings in the no-EMF treatment were mycorrhiza-free, but those inoculated with *L. laccata* and *H. crustuliniforme* had > 80 % of root tips colonized by one or the other fungus. Inoculation with the two *Rhizopogon* species was less successful, each colonizing 20–40 % of root tips. At the close of the experiment, > 80 % of tips of seedlings in the no-EMF treatment had been colonized by the common greenhouse contaminant *T. terrestris*, which had also colonized many tips in the 2-EMF treatment. In the 4-EMF treatment, there was little contamination with *T. terrestris*, but considerable transfer of mycorrhizas among seedlings. The fungus species initially inoculated on a given seedling generally predominated at final lifting; however, most seedlings had at least a few mycorrhizas of the other types.

Total pot biomass

In pasteurized soils with no EMF added, RYTs for tree species in mixture differed significantly from 1 (Fig. 2*a*). Mean RYT was 0·84 ($P < 0·001$) for 9PM/3PP, 0·93 ($P < 0·10$) for 6PM/6PP, and 0·87 ($P < 0·025$) for 3PM/9PP. When the species-specific *Rhizopogons* were added, antagonism between the two tree species disappeared (Fig. 2*b*). Mean RYTs were 0·97, 1·03, and 0·92 for 9PM/3PP, 6PM/6PP, and 3PM/9PP, respectively, and no value differed significantly from 1. When four EMF species were added, mean RYTs of trees in mixture reached their highest values (1·02, 1·05, and 0·96), but none differed significantly from 1 (Fig. 2*c*). Although no RYT value exceeded 1 at $P = 0·05$, within a given tree-species mix in the three pasteurized-soil treat-

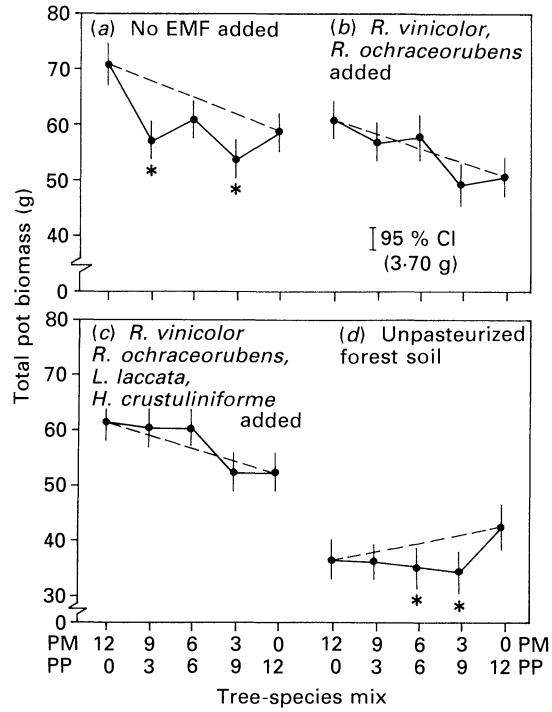

Figure 2. Influence, as indexed by Relative Yield Total (RYT), of ectomycorrhizal fungi (EMF) on total pot biomass (including roots) of various mixes of *Pseudotsuga menziesii* (PM) and *Pinus ponderosa* (PP) seedlings. Standard of comparison among treatments is RYT = 1 (dashed line); mean treatment RYTs significantly different from 1 are asterisked. CI = confidence interval.

ments, RYT consistently increased as numbers of EMF species increased.

Although seedlings grown in unpasteurized forest soil formed several types of mycorrhizas, mean RYTs were significantly ($P < 0{\cdot}05$) below 1 for 6PM/6PP and 3PM/9PP. In contrast to the results of the other treatments, biomass of *Pinus ponderosa* seedlings was, on average, greater than that of *Pseudotsuga menziesii* when the two tree species were grown in unpasteurized soil.

Individual seedling size

Pseudotsuga menziesii seedlings were taller than *Pinus ponderosa* (mean = 16 *vs.* 12 cm) in all pasteurized-soil treatments, but height of the two tree species did not differ (mean = 11 cm) in unpasteurized soil. In contrast to height, which was not affected by tree-species mix, seedling biomass varied significiantly with tree-species mix, particularly biomass of *Pseudotsuga menziesii* (Fig. 3*a*). With no EMF added, *P. menziesii* seedling biomass was significantly less in mixture than in monoculture, whereas *Pinus ponderosa* seedling biomass was reduced only in the 9PM/3PP mix (Fig. 3*b*). Adding EMF altered this pattern. When the two *Rhizopogon* species were added, *Pseudotsuga menziesii* seedling biomass was reduced only when it was the minor component in the mix (3PM/9PP), and *Pinus ponderosa* seedling biomass was unaffected by tree-species mix.

When four EMF species were added, *Pseudotsuga menziesii* seedlings were significantly larger in the 6PM/6PP mix than in monoculture, with no corresponding decrease in *Pinus ponderosa* seedling size (Fig. 3*a*, *b*). The response of individual seedlings in this mix differed according to the EMF species that had been synthesized on their roots (Fig. 4). Both tree species attained their largest size with *L. laccata* and their smallest size with *H. crustuliniforme*, and greater *Pseudotsuga menziesii* biomass in mixture than in monoculture was due primarily to seedlings with *L. laccata*. Curiously, in the 4-EMF treatment, *Pinus ponderosa* seedlings with *R. ochraceorubens* were significantly smaller in mixture than in monoculture. This was not true in the 2-EMF treatment, suggesting that growth in both mixture and monoculture was not so much a function of numbers of EMF species present as of the particular fungus species mix.

Biomass allocation

In uninoculated seedlings, biomass allocation to foliage, stems, and roots fluctuated widely depending on tree-species mix (see Fig. 5 on p. 507 below). In inoculated seedlings, biomass generally was allocated to leaves at the expense of stems and roots, and biomass allocation patterns were far more stable across the replacement series for inoculated than uninoculated seedlings.

The abrupt changes in allocation patterns of uninoculated pots were due primarily to *Pinus ponderosa*, and were consistent among all five replications. When uninoculated seedlings of *P. ponderosa* were grown in monoculture, 18% of their total biomass was foliage, 33% stem. When three seedlings of *Pseudotsuga menziesii* were added (3PM/9PP mix), this changed to 27% foliage, 18% stem. In the 6PM/6PP mix, allocation to foliage was 16% – similar to that in monoculture. In contrast, inoculated *Pinus pondersoa* seedlings consistently allocated a higher proportion of biomass to foliage than to stems. EMF produced a similar, but less dramatic, increase in the foliage proportion of *Pseudotsuga menziesii* seedlings, primarily at the expense of roots rather than stems.

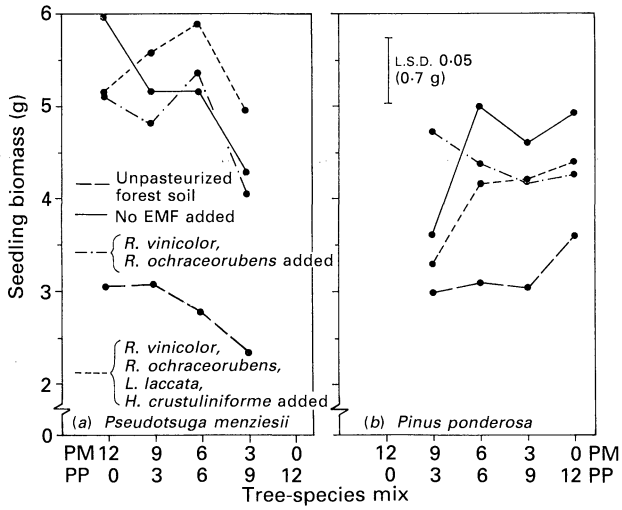

Figure 3. Individual seedling biomass, by tree species, of *Pseudotsuga menziesii* (PM) and *Pinus ponderosa* (PP) seedlings grown in mixture and monoculture in potted soil with and without added ectomycorrhizal fungi (EMF).

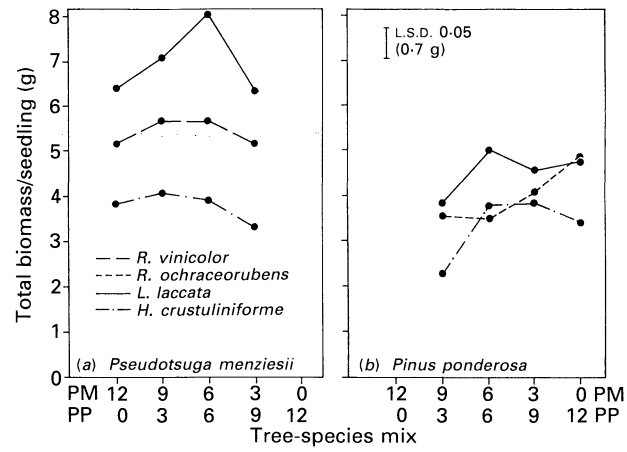

Figure 4. Mean total biomass, by ectomycorrhizal (EMF) species initially synthesized on seedlings, of *Pseudotsuga menziesii* (PM) and *Pinus ponderosa* (PP) seedlings grown in mixture and monoculture in pasteurized, potted soil to which four EMF species were added.

Table 1. *Mean foliar nutrient concentration (± SE) for* Pseudotsuga menziesii (*PM*) *and* Pinus ponderosa (*PP*) *grown in mixture and monoculture in potted soil with and without added ectomycorrhizal fungi (EMF)*

	Nutrient concentration (%), by tree-species mix*			
	Pseudotsuga menziesii		*Pinus ponderosa*	
Treatment†	12PM/0PP	6PM/6PP	6PM/6PP	0PM/12PP
		Nitrogen		
Pasteurized soil				
No EMF added‡	0·57 (0·02)a	0·62 (0·05)a	0·74 (0·05)b	0·94 (0·07)c
2 EMF added	0·70 (0·03)a	0·57 (0·04)b	0·81 (0·01)c	0·76 (0·04)a, c
4 EMF added	0·47 (0·01)a	0·57 (0·01)b	0·68 (0·12)c	0·93 (0·08)d
Unpasteurized soil	0·68 (0·02)a	0·71 (0·02)a	0·86 (0·05)b	0·77 (0·01)a, b
		Phosphorus		
Pasteurized soil				
No EMF added‡	0·12 (0·01)a, b	0·12 (0·002)a, b	0·11 (0·004)b	0·13 (0·007)c
2 EMF added	0·15 (0·009)a	0·16 (0·007)a	0·13 (0·02)b	0·10 (0·005)c
4 EMF added	0·14 (0·006)a, d	0·19 (0·004)b	0·11 (0·003)c	0·13 (0·005)d
Unpasteurized soil	0·35 (0·02)a	0·37 (0·01)a	0·16 (0·004)b	0·16 (0·009)b

* In a row; means followed by the same letter do not differ significantly at $P = 0·05$.

† 2 EMF = *Rhizopogon vinicolor*, *Rhizopogon ochraceorubens*; 4 EMF = *R. vinicolor*, *R. ochraceorubens*, *Laccaria laccata*, *Hebeloma crustuliniforme*.

‡ Colonized by the greenhouse contaminant, *Thelephora terrestris*.

Foliar nutrient concentration and total foliar nutrient content

Patterns of foliar N concentration (0·47–0·71 % in *Pseudotsuga menziesii*, 0·68–0·94 % in *Pinus ponderosa*) were inconsistent among treatments (Table 1). These values are quite low for conifer seedlings, although foliage did not show the chlorosis characteristic of N deficiency. In contrast, foliar P concentration (0·12–0·37 % in *Pseudotsuga menziesii*, 0·11–0·16 % in *Pinus ponderosa*) was average to high for conifers (Rodin & Bazilevich, 1967).

In most cases, foliar nutrient content was higher in the inoculated treatments than in the uninoculated (Fig. 6). For total foliar nutrients per pot, no RYT value differed significantly ($P = 0·05$) from 1·0; however, some consistent patterns were evident. The value of RYT for pot foliar N and P averaged 0·77 and 0·74, respectively, in the no-EMF treatment (Fig. 6*a*). Mean RYT for N remained < 1·0 in both the 2- and 4-EMF treatments. RYT for P averaged 1·15 in both the 2- and 4-EMF treatments (Fig. 6*b*, *c*) – 50 % greater than in the no-EMF treatment, but not significantly different from 1·0.

For uninoculated *Pseudotsuga menziesii*, both foliar N and P content was lower in mixture than in monoculture, but P content dropped the most, resulting in a wider N/P ratio in mixture. For uninoculated *Pinus ponderosa*, foliar nutrient concentrations were lower in mixture than in monoculture (Table 1), but there was no change in foliar biomass (Fig. 3), suggesting luxury consumption by the pine.

The two tree species responded quite differently to EMF with regard to average foliar nutrients per seedling (Fig. 6). In the 2-EMF treatment, N content and concentration in *Pseudotsuga menziesii* foliage dropped sharply in mixture relative to monoculture, whereas P content and concentration held stable (Table 1, Fig. 6*b*), suggesting that the beneficial effect of EMF on biomass of *Pseudotsuga menziesii* grown in mixture was due at least partly to improved P nutrition. Phosphorus content of *Pseudotosuga menziesii* apparently was not increased at the expense of the *Pinus ponderosa*, whose foliar P concentration also increased in mixture relative to monoculture.

The 4-EMF treatment conferred a particular advantage to the *Pseudotsuga menziesii* growing in mixture. Relative to monoculture, *P. menziesii* in mixed pots had significantly higher foliar N and P concentration and content (Table 1, Fig. 6*c*). Relative to monoculture, *Pinus ponderosa* in mixed pots had sharply lower foliar N concentration and content; foliar P concentration also was reduced, but not foliar P content. As was the case for the no-EMF treatments, reduced N uptake by *P. ponderosa* in mixture was not accompanied by reduced seedling biomass; the pine in monoculture apparently sequestered N and perhaps P in excess of growth requirements.

In unpasteurized soil, neither tree species grown either in mixture or in monoculture differed in nutrient uptake (Fig. 6*d*). Seedlings of *Pinus ponderosa* contained the most N, *Pseudotsuga menziesii* the most P, the latter sequestering very large amounts of P in foliage (N/P ratios < 2). Comparing foliar nutrient concentrations in this treatment with those in the other treatments (Table 1) suggests that the relatively poor growth of both tree species in uninoculated soils was not due to N or P deficiency.

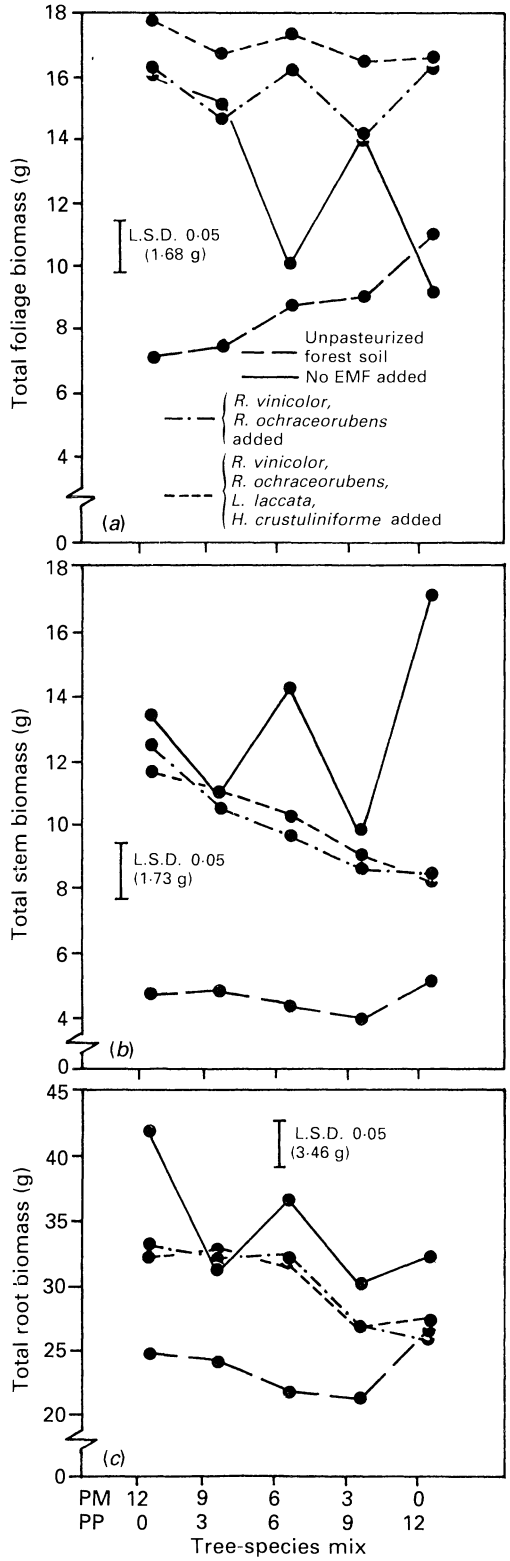

Figure 5. Total biomass allocation to foliage, stems, and roots of *Pseudotsuga menziesii* (PM) and *Pinus ponderosa* (PP) seedlings grown in mixture and monoculture with and without added ectomycorrhizal fungi (EMF).

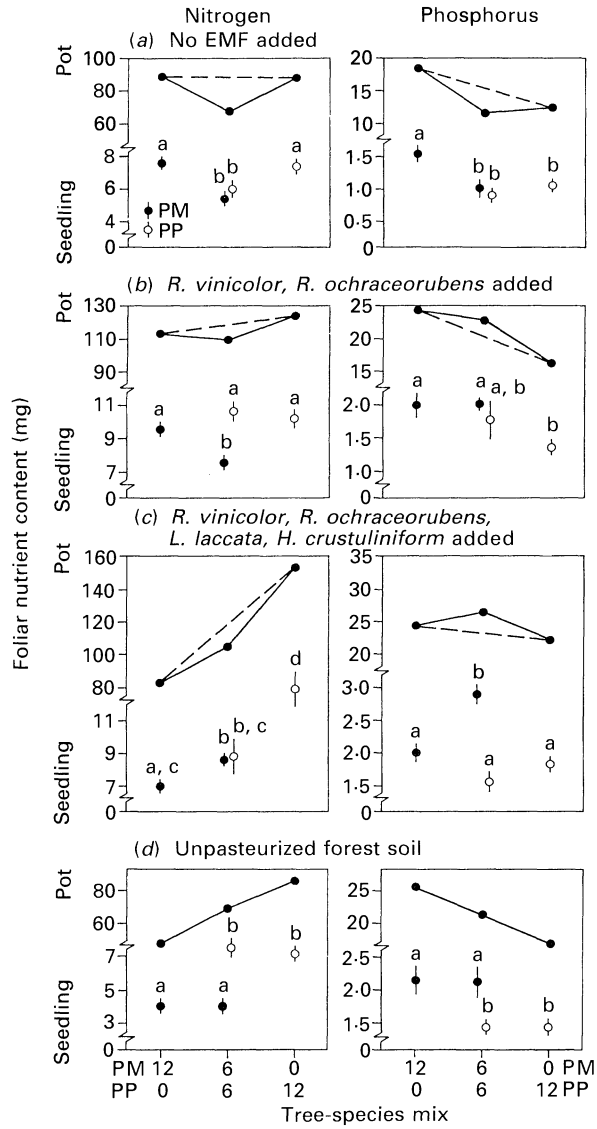

Figure 6. Mean foliar nutrient content per seedling and total foliar nutrient content per pot, as indexed by Relative Yield Total (RYT), for *Pseudotsuga menziesii* (PM) and *Pinus ponderosa* (PP) seedlings grown in mixture and monoculture with and without added ectomycorrhizal fungi (EMF). Standard of comparison among treatments is RYT = 1 (dashed line); no mean treatment RYTs significantly differed from 0 ($P = 0{\cdot}05$). Means denoted by the same letter do not differ significantly ($P = 0{\cdot}05$).

^{32}P *uptake*

With no added EMF, *Pinus ponderosa* in pots with ^{32}P injected at the 4 cm soil depth had 7 times more ^{32}P per unit stem tissue than did *Pseudotsuga menziesii* seedlings (Fig. 7*a*). With the two *Rhizopogon* species, however, this pattern was reversed. The ^{32}P counts in foliage showed the same pattern as in stems, but variability was greater (Fig. 7*b*). Uptake of ^{32}P injected at the 16 cm depth was highly variable and showed no consistent pattern. Root systems at this depth clearly were influenced by the bottom of the pots. Taking into account the different sizes of seedlings by expressing ^{32}P uptake on a whole-tissue basis (i.e. total counts per minute in foliage or stems) did not change overall patterns.

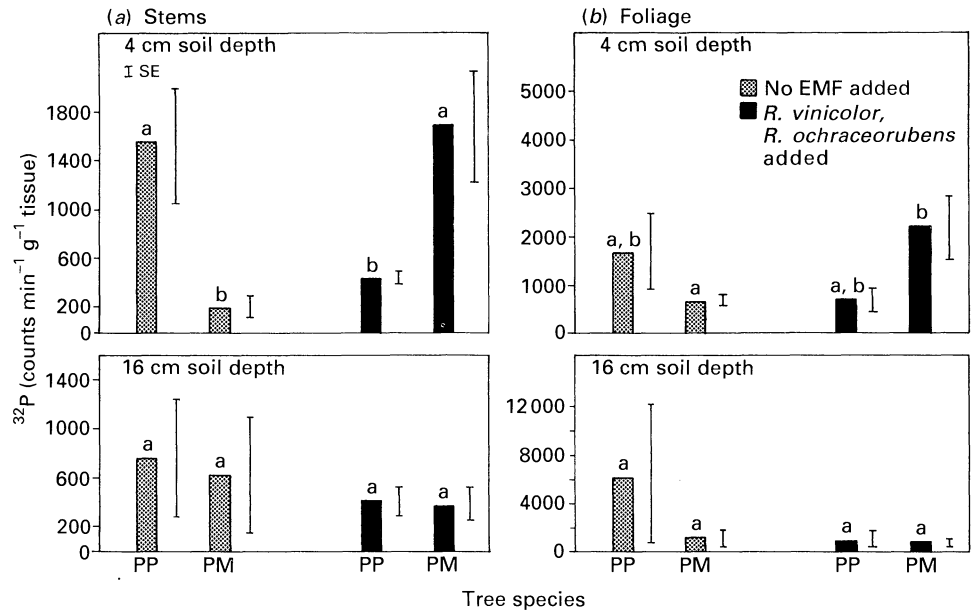

Figure 7. ^{32}P counts per minute per gram tissue for *Pseudotsuga menziesii* (PM) and *Pinus ponderosa* (PP) seedlings grown in mixture (9PM/3PP, 3PM/9PP) with and without added ectomycorrhizal fungi (EMF). Soil depths refer to point of ^{32}P injection into pots. Means denoted by the same letter do not differ significantly ($P = 0{\cdot}05$).

DISCUSSION

Pseudotsuga menziesii and *Pinus ponderosa* growing in uninoculated mixtures (but contaminated with *T. terrestris*) had RYTs significantly < 1, indicating that the two tree species were mutually antagonistic (Harper, 1977). Inoculation with EMF eliminated the mutual antagonism, neutralizing it when two EMF species were added and perhaps fostering a positive tree-species interaction when four EMF species were added. Although no RYT value significantly exceeded 1, when changes in mean seedling size were compared rather than RYT values, *Pseudotsuga menziesii* seedlings were clearly larger in mixture than in monoculture in the 4-EMF treatment, without a corresponding decline in the size of *Pinus ponderosa* seedlings. This outcome was not due, however, to EMF diversity *per se*, but to the positive effects of a single EMF species – *L. laccata* – on *Pseudotsuga menziesii*.

Although growth of uninoculated *P. ponderosa* seedlings was reduced in mixture only when that tree species was in the minority, relative proportions of stem and foliage fluctuated significantly when *P. ponderosa* seedlings were in the majority. Increased biomass allocation to foliage by *P. ponderosa* seedlings may have been triggered by shading by the taller *Pseudotsuga menziesii*; however, the fact that this did not occur in the 6/6 mixture suggests some other mechanism. Both tree species responded to inoculation by increasing allocation of biomass to foliage – in *Pseudotsuga menziesii* at the expense of roots, in *Pinus ponderosa* at the expense of both roots and stems – further suggesting influence of factors other than shading. Ectomycorrhizal plants commonly have lower root/shoot ratios than do non-ectomycorrhizal plants (Harley & Smith, 1983).

The effect of inoculation on seedling biomass and foliar nutrients was highly specific to both EMF species and tree-species mix. However, both the 2- and 4-EMF treatments had a greater relative effect on P than N uptake when seedlings were grown in mixture. This is consistent with the well-known tendency of mycorrhizas to enhance uptake of poorly mobile nutrients, such as P, more than that of more mobile nutrients, such as mineral N (Bowen, 1980; Harley & Smith, 1983). Without the N limitations, we may have seen an increase in total biomass in the EMF treatments, but this is only speculation.

When four EMF species were added, the two tree species clearly differed in N nutrition when growing in monoculture but not in mixture – a pattern evident both in foliar N content and concentration. The fact that enhanced *Pseudotsuga menziesii* growth in mixture was due solely to *L. laccata* – which formed mycorrhizas on both tree species – raises the possibility of nutrient transfers between plants through shared mycorrhizas. Various studies have provided strong evidence for such transfers (Reid & Woods, 1969; Read *et al.*, 1985; Finlay & Read, 1986*a*, *b*; Francis *et al.*, 1986), and we did see cross inoculation – i.e. trees inoculated only with *Rhizopogon* formed mycorrhizas with *Laccaria* and *Hebeloma*. It is of course also possible that *L. laccata* is simply a more effective symbiont of *Pseudotsuga menziesii* than of *Pinus ponderosa*; however, if this were the case, *Pseudotsuga menziesii* growth and

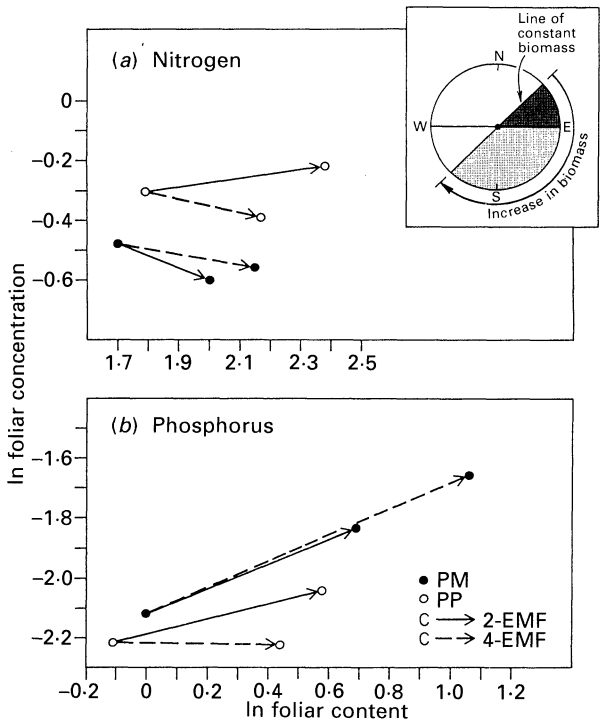

Figure 8. Relationship between foliar nutrient content and concentration in *Pseudotsuga menziesii* (PM) and *Pinus ponderosa* (PP) seedlings grown in 6/6 mixtures with and without added ectomycorrhizal fungi (EMF). Direction of the arrows drawn from the data points for the uninoculated seedlings (controls, C) to those for the seedlings inoculated with either two or four EMF species (2- or 4-EMF treatment) provides information concerning treatment effects on seedling (*a*) nitrogen and (*b*) phosphorous nutrition. (See the text for explanation of this graphical analysis.)

nutrient uptake should have been enhanced in monoculture as well as mixture, and it was not. *H. crustuliniforme*, also forming mycorrhizas on both tree species, conferred no growth advantage on either.

We used a slightly modified version of the graphical technique of Weetman & Fournier (1982) and Timmer & Morrow (1984) to help identify possible contributions of N and P to the positive effect of mycorrhizal inoculation on productivity of seedling mixtures (Fig. 8). In this approach, foliar nutrient contents of treatments (in our case, 2- and 4-EMF) and controls (no EMF) are plotted against their foliar nutrient concentrations, and radii drawn from the control to each treatment. To see this, envisage the point corresponding to the control as the centre of a circle (see inset, Fig. 8). When a log–log transformation is used and scaled the same on the x and y axes (our modification), a diagonal drawn through the centre (the control) from SW to NE is a line of constant foliar biomass. Because foliar biomass is the quotient of content divided by concentration, the direction and magnitude of these radii contain information about all three.

Consider some treatment designated FERT. If the radius drawn from the centre (control) to FERT (treatment) falls within the region from (moving clockwise) NE to SW then FERT has increased foliar biomass. If the radius to FERT falls within the region from NE to E (heavily shaded in the inset), foliar concentration (of whatever nutrient has been plotted) has either increased or remained constant in FERT relative to the control, suggesting that the nutrient was limiting in the control. If the radius to FERT falls within the region from E to SW (lightly shaded in the inset), foliar concentration of the nutrient has declined and is considered non-limiting.

In Figure 8, which includes data for the 6/6 seedling mixtures only, the direction of the radii suggests that the positive effects of both treatments on *Pseudotsuga menziesii* were related to improved P rather than N nutrition. This is consistent with the pattern of nutrient change in the controls, where lower *P. menziesii* foliar biomass in mixture compared to monoculture was accompanied by a drop in P content relative to N content. Yet all N/P ratios in this study were below the value considered to indicate P deficiency in conifers (Binkley, 1983; Mohren, Van Den Burg & Burger, 1986). Perhaps uptake of P correlated with that of some other, more limiting nutrient; uptake of P and divalent cations are closely associated (e.g. Strullu *et al.*, 1986). Or it may be that we do not understand P nutrition of conifers very well, or that the graphical technique does not accurately reflect changes in plant nutrition.

The graphical analysis suggests that both N and P nutrition of *Pinus ponderosa* was improved in mixture for seedlings in the 2-EMF treatment relative to the controls, whereas neither nutrient appeared to play a role in the 4-EMF treatment. In general, luxury consumption seemed to have a larger impact on nutrition of *Pinus ponderosa* than of *Pseudotsuga menziesii*.

The results of the ^{32}P labelling were equivocal. Showing that the 2-EMF treatment significantly affects *Pseudotsuga menziesii* P uptake, they support the outcome of the graphical analysis for *Pseudotsuga menziesii* but not for *Pinus ponderosa*. This inconsistency may reflect differing patterns of P uptake between the period of shoot growth – when most foliar P was presumably taken up – and the period following budset – when ^{32}P was applied. Interpretation of ^{32}P-uptake patterns is further confounded because uptake of applied P can reflect either ability to compete for soil P or, quite the opposite, P deficiency (Harrison & Helliwell, 1979).

Various *caveats* must be applied to our results. Because uninoculated seedlings formed mycorrhizas from the greenhouse contaminant *T. terrestris*, differences between treatments with and without added EMF cannot be conclusively attributed to presence or absence of mycorrhizas. Weekly inspection and removal of *T. terrestris* fruiting bodies may have delayed spread of the contaminant, and it

seems likely that at least during the first few months, growth patterns were influenced more by mycorrhizas in those seedlings that were deliberately inoculated than in those accidentally contaminated. However, we have no way of verifiying that. With regard to the replacement series as an experimental technique, Harper (1977) pointed out that the theoretical RYT = 1 may be unrealistic, particularly when the resource demand per individual varies between the two species in the series. In our study, the RYT = 1 line serves primarily as a convenient point of reference for comparing among the four different replacement series. Inferences concerning the role of species diversity in 'underyielding' or 'overyielding' within any given series must take into account that RYT = 1 does not necessarily represent a real biological expectation.

Two experimental conditions in particular are likely to have influenced the outcome of our study. One is the N deficiency suggested by foliar N concentrations of both species (although, as we have discussed, it is not clear whether N or P was more limiting, at least for *Pseudotsuga menziesii*). Results might have been quite different had we fertilized more heavily. The second is, as with all 'pot' experiments, the relatively shallow rooting depth available in pots. In nature, rooting competition between deep-rooted *Pinus ponderosa* and (relatively) shallow-rooted *Pseudotsuga menziesii* is likely to be considerably less than occurred in this experiment. On the other hand, we intended to force competition between the two tree species, and the pots accomplished this purpose.

Specificity of our findings to the experimental context is underscored by the results in unpasteurized soil. There, growth of both tree species – particularly *Pseudotsuga menziesii* – was lower than in pasteurized soils and, despite good EMF formation, the two tree species were mutually inhibitory. Phosphorus clearly was not limiting in unpasteurized soil, but N limitation may have been aggravated by competition between mycorrhizas and soil microbes. The soil holds a rich variety of organisms, many of which influence plant grown (Bowen, 1980; Coleman, 1985; Linderman, 1985; Newman, 1985; Foster, 1986; Perry *et al.*, 1987). Not only biotic but abiotic factors in the field are likely to produce interactions quite different from those in the greenhouse. For example, because *Pseudotsuga menziesii* and *Pinus ponderosa* differ in rooting characteristics, the latter tending to root much deeper, interaction between the two species is likely to vary with soil depth and growth. Variable field environments – both biotic and abiotic – create an array of niches for EMF that are not present in the greenhouse, and almost certainly increase the complexity of interaction among EMF species and plant species.

Despite these *caveats*, our study demonstrates that ectomycorrhizal fungi can significantly influence plant–plant interactions. The similarity of our results with those of Puga (1985), who worked with a VA mycorrhizal crop plant and a VA mycorrhizal shrub, suggests that, depending on fungus species, mycorrhizal fungi may commonly reduce interference between plant species in nature. As Puga (1985) points out, this has considerable implication for agronomic polycultures and agroforestry.

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