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Pisolithus arhizus ectomycorrhiza affects plant competition for phosphorus between *Pinus elliottii* and *Panicum chamaelonche*

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Abstract Our objective was to evaluate the ability of an ectomycorrhizal fungus to alter the competitive interaction of pine seedlings growing with grass, and to determine whether the interaction was modified by soil-phosphorus (P) concentration. Slash pine (*Pinus elliottii*), inoculated with the ectomycorrhizal fungus *Pisolithus arhizus* or fortuitously colonized by *Thelephora terrestris*, and a native grass (*Panicum chamaelonche*) were grown in a greenhouse at three P levels (0.32, 3.22, 32.26 μM H_3PO_4). Pine inoculated with *P. arhizus* took up more P when competing with the nonmycorrhizal grass than when competing with another pine (irrespective of pine mycorrhizal status). Phosphorus uptake kinetics (C_{min}, the minimum concentration at which P can be absorbed from a solution; I_{max}, the maximum uptake rate) for pine and grass were also determined under hydroponic conditions. Pine had a higher I_{max} than grass but grass had a lower C_{min}, suggesting that pine is more competitive at higher nutrient concentrations while grass is more competitive at lower nutrient concentrations. The controlled conditions used in these experiments allowed us to evaluate specific parameters (P uptake and absorbing surface area) affecting plant competition.

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Introduction

Soil fertility is a factor determining the plant biomass an environment can support (Donald 1951). In environments with low nutrient concentrations, plants are stressed directly by the lack of adequate nutrients and they survive primarily by stress-tolerance mechanisms (Grime 1979). An environment with more nutrients has the potential to produce more plant biomass and, as plants grow, nutrient depletion zones around roots may overlap. As a consequence, plant competition for nutrients becomes a factor governing survival and plant growth. Thus, environmentally induced stress on a plant can be considered a gradient extending from direct physical stress on an individual plant to stress produced biologically by plant interactions (Berkowitz et al. 1995; Grime 1979).

Autecological studies have shown that mycorrhiza can increase plant tolerance to environmental stresses and contribute to a plant's survival and growth (Sylvia and Williams 1992). This enhancement of individual plant health benefits a plant in competion with neighboring plants. Much less research has addressed quantitatively the influence of mycorrhiza on plant synecology (Pedersen and Sylvia 1996). Several researchers have demonstrated that mycorrhiza can enhance plant competitive ability (Allen and Allen 1984, 1986; Fitter 1977; Hall 1978; Hartnett et al. 1993; Hetrick et al. 1989; West 1996). The majority of these studies relate to arbuscular-mycorrhizal (AM) plants. To our knowledge, only one study has addressed ectomycorrhizal (EM) effects on plant competition (Perry et al. 1989).

The goal of our research was to assess the effect of the ectomycorrhizal fungus *Pisolithus arhizus* (Scop. Per Pers.) [Syn.: *P. tinctorius* (Pers.) Coker & Couch], on the competitive ability of slash pine (*Pinus elliottii*

Engelm. var. *elliottii*), which is grown for pulpwood in the southeastern United States. Grasses, among other plants, compete extensively in new slash pine plantations because weed control is practiced infrequently. Our specific objectives were to determine (i) whether the ectomycorrhizal fungus alters the competitive ability of pine when growing with grass and (ii) how competition is modified by soil-phosphorus (P) concentration.

Materials and methods

Competition study

Experiments were conducted in sand acid-washed by treatment with 25% HCl for 24 h, then draining the acid and rinsing until the pH increased to that of the deionized water being used. Eighty percent of the sand was in the particle size range of 0.160–1 mm with the rest larger than 1 mm.

Slash pine seeds were surface disinfected for 2 min in a 5.25% sodium hypochlorite solution with 0.2 ml Liqui-Nox surfactant (Alconox, Inc., New York) and then rinsed thoroughly with tap water. Seedlings were raised from seed for 2 weeks in a growth chamber (29/23 °C day/night, with a 15-h light period and irradiance of 1000 μmol m^{-2} s^{-1}) in sand and then transplanted to 50-ml pots and grown in sand in the greenhouse for 8 weeks, where they received water only. To inoculate pine, washed roots were dipped in a slurry of rinsed and chopped mycelium of *P. arhizus*, isolate S106 (see Farmer and Sylvia 1998), previously grown in a liquid suspension culture containing modified Melin-Norkrans liquid medium (Marx 1969) using glucose instead of sucrose (these plants were designated as $pine^{Pa}$). Roots of control plants were dipped in tap water. After a further 6 weeks of growth in 500 ml of sand in Deepots (McConkey, Sumner, Wash.), root systems were gently rinsed free of adhering sand particles and seedlings were placed in the appropriate competition treatments.

Plants of a native competing grass species (*Panicum chamaelonche* Trin.) were started from field-collected seed and maintained in sand in the greenhouse. Just prior to the experiment, the roots were washed free of sand and plants were placed in the appropriate competition treatments. Our original intent was to include mycorrhizal grass plants in the study; however, repeated efforts with exotic and indigenous isolates failed to achieve AM colonization under the experimental conditions.

At the beginning of the competition experiment, noninoculated pine had no visual indication of colonization, whereas inoculated pine was heavily colonized by *P. arhizus*. There were no significant differences in dry mass between inoculated and noninoculated pine. Intraspecific and interspecific paired combinations of pine (inoculated and noninoculated) and grass were made by planting appropriate plants together in 500 ml of sand with six replications per combination.

Plants were grown in the greenhouse at mean temperatures of 21/34 °C (min./max.) and a mean photosynthetic photon flux density of 1240 μmol m^{-2} s^{-1} from January to June 1994. The plants were fertilized twice a week with a solution containing 660 μM NH_4NO_3, 660 μM $(NH_4)_2SO_4$, 616 μM KCl, 80 μM $MgSO_4$, 54 μM NaFeEDTA, 600 μM $CaCl_2$, 0.25 μM $CuSO_4$, 14 μM H_3BO_3, 40 μM $NaMoO_4$, 2.75 μM MnCl, and 1.25 μM $ZnSO_4$. P was supplied as 0.32, 3.23 or 32.26 μM H_3PO_4. These P levels were chosen because they were shown to enhance growth of *P. chamaelonche* (Pedersen 1995). Soil solution pH in several pots was measured during the experiment by thoroughly watering the selected pots with deionized water and collecting the leachate. The resulting mean pH was 4.0.

Plants were removed from the pots after 18 weeks, and the roots of individual plants were carefully separated. Roots of the intraspecific grass combination, however, were treated as one unit and half the value allotted to each plant. Because the ubiquitous EM fungus *Thelephora terrestris* (Ehrh.) Fr. was observed sporadically in the noninoculated pine treatments but not in the inoculated pine treatments, the control treatment was designated $pine^{N/Tt}$. The grass plants were not colonized by AM fungi.

Root and shoot wet and dry masses were determined. An estimate of root length was obtained using calculations of specific root length (cm root g^{-1} root fresh mass) for pine and grass from a previous experiment (unpublished data) and expressed here as root-length density (cm root ml^{-1} soil). Root ergosterol concentration was used as an estimate of EM fungal biomass as described previously (Pedersen and Sylvia 1997). For P analysis, the shoots were ground and ashed at 500 °C for a minimum of 4 h and analysis performed using the method of Murphy and Riley (1962).

To compare the competitive abilities of the two plant species, the relative crowding coefficient (RCC, Harper 1977) was calculated using the means of total dry mass as follows:

$$\mathrm{RCC} = \frac{\dfrac{\text{grass (interspecific)}}{\text{grass (intraspecific)}}}{\dfrac{\text{pine}^{\text{Pa}}\text{ (interspecific)}}{\text{pine}^{\text{Pa}}\text{ (intraspecific)}}}$$

Data for each plant species were subjected to analysis of variance and statistically planned contrasts, if appropriate, using the General Linear Model (SAS Institute 1989). The least-squares means statement within SAS was used to compare means. Statistical significance was at $P < 0.05$ unless otherwise stated.

Phosphorus uptake kinetics

Pine seedlings were inoculated with *P. arhizus* or left noninoculated (but were also fortuitously colonized by *T. terrestris*) and were grown in Deepots for 24 weeks prior to the uptake study. Grasses were obtained from greenhouse stock cultures.

Roots of selected plants were gently rinsed free of adhering sand and a single plant placed in a 1-l Erlenmeyer flask sealed with aluminum foil. Plants were grown in a growth chamber (29/23 °C day/night, with a 15-h light period and irradiance of 1000 μmol $m^{-2}s^{-1}$) in a continuously aerated nutrient solution containing 660 μM NH_4NO_3, 616 μM KCl, 800 μM $MgSO_4$, 54 μM NaFeEDTA, 600 μM $Ca(NO_3)_2 \cdot 4\,H_2O$, 0.75 μM $CuSO_4$, 52 μM H_3BO_3, 120 μM $NaMoO_4$, 8.25 μM MnCl, and 3.75 μM $ZnSO_4$. P was supplied as 3.23 μM H_3PO_4. The solution was changed twice a week. A minimum 4-week acclimatization period was provided to allow external hyphae to grow from the colonized roots.

To quantify uptake kinetics, root systems were rinsed with deionized water and placed in additional deionized water for 1 h. One liter of fresh nutrient solution, identical to that used previously, was added to 1-l, acid-washed Erlenmeyer flasks. At the start of the experiment, plant roots were gently dried with paper towels, placed in the nutrient solution and weighed. Approximately every 15 min through the first 2 h and then hourly through 7 h, 23 ml of solution was removed for P analysis and immediately filtered through 0.45-μm syringe filters. The solution was replaced with sufficient deionized water to bring the system back to its original starting weight. Aliquots (20 ml) of the sample removed for P analysis were evaporated to dryness. Concentrated HCl (20 ml) was added to the sample and also evaporated to dryness. Phosphorus was determined colorimetrically (Murphy and Riley 1962). The absorbing surface areas of roots and hyphae were estimated using image analysis software (Mocha, Jandell Scientific, San Rafael, Calif.) and a gridline-intersect method as previously described (Rousseau et al. 1994). An estimate of the maximum uptake rate (I_{max}) was made from the absorbing surface areas and the quantity of P absorbed from the nutrient solution during the first 45 min. The minimum solution concentration at which P could be absorbed (C_{min}) was considered to be the

asymptotic value where the solution-P concentration no longer decreased based on a P-depletion curve fitted to the raw data and obtained using a curve-fitting procedure (SigmaPlot, Jandell Scientific, San Rafael, Calif.).

Results

Competition study

Pine shoot-P concentration and content increased in all treatments with increasing level of applied P (Fig. 1A, B). When data for all treatments were combined and evaluated together using planned contrasts, a higher shoot-P concentration was observed in pine^{Pa} than in pine$^{N/Tt}$ plants ($P \leq 0.001$). In the interspecific competition treatments where pine was grown with grass, pine^{Pa} had a significantly higher shoot-P content than pine$^{N/Tt}$ and the difference became more apparent with increasing P level. Total dry mass of pine was not affected by the level of applied P; however, pine^{Pa} had a higher total dry mass than pine$^{N/Tt}$ ($P = 0.07$), more so when grown with grass (Fig. 1C). Pines growing intraspecifically had significantly less dry mass than when grown with grass. Ectomycorrizal colonization, as indicated by ergosterol concentration, was significantly higher in the pine^{Pa} than in the pine$^{N/Tt}$ treatments (Table 1).

Table 1 Ergosterol concentration ($\mu g\ g^{-1}$) of *Pinus elliottii* roots inoculated with *Pisolithus arhizus* (pine^{Pa}) or fortuitously colonized by *Thelephora terrestris* (pine$^{N/Tt}$), and grown in combination with another pine or a grass (*Panicum chamaelonche*) at 0.32, 3.23 or 32.26 μM P for 18 weeks. Each value represents the mean of six replicates ± SE

Competition treatment	Applied P (μM)		
	0.32	3.23	32.26
Pine^{Pa} × pine^{Pa}	181 ± 20	282 ± 34	297 ± 33
Pine$^{N/Tt}$ × pine$^{N/Tt}$	129 ± 13	104 ± 11	150 ± 11
Pine^{Pa} × grass	192 ± 8	297 ± 46	260 ± 26
Pine$^{N/Tt}$ × grass	137 ± 21	139 ± 12	140 ± 17

There was a significant interaction between applied P and competition treatment for grass shoot-P concentration and content. There was no affect of competition

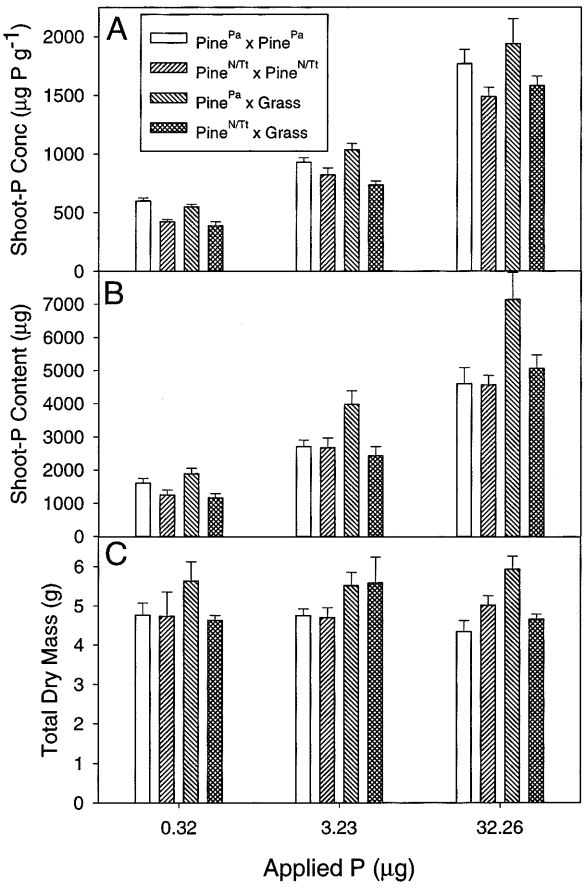

Fig. 1 *Pinus elliottii* (**A**) shoot-phosphorus (P) concentration, (**B**) shoot-P content, and (**C**) total dry mass in response to different competition treatments and grown at either 0.32, 3.23 or 32.26 μM P for 18 weeks; *bars* represent the mean of six replicates ± SE

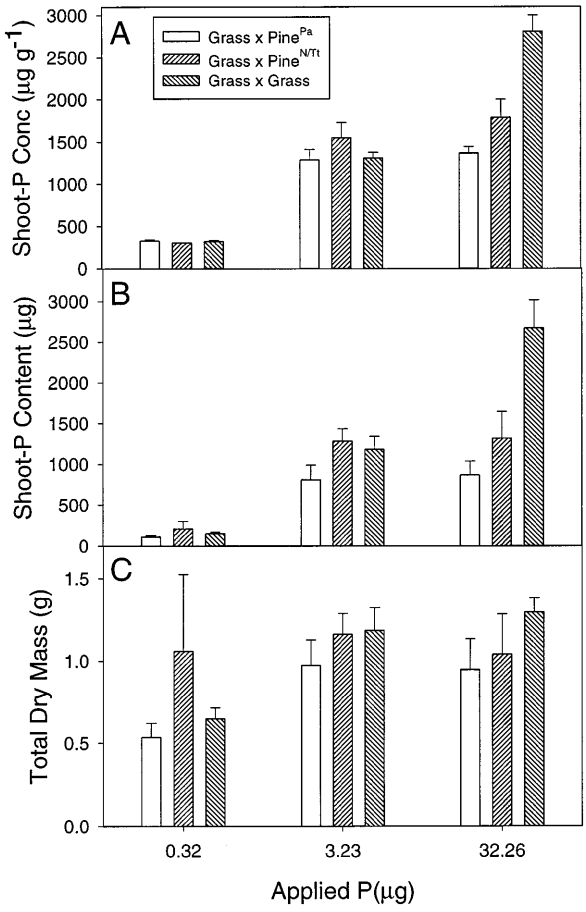

Fig. 2 *Panicum chamaelonche* (**A**) shoot-P concentration, (**B**) shoot-P content, and (**C**) total dry mass in response to different competition treatments and grown at either 0.32, 3.23 or 32.26 μM P for 18 weeks; *bars* represent the mean of six replicates ± SE

treatment at the two lower levels of applied P. At the highest P level, grass grown in intraspecific combination had higher shoot P than grass grown in interspecific combination with pine^{Pa} or pine$^{N/Tt}$ (Fig. 2A, B). Furthermore, the shoot-P of grass when grown with pine^{Pa} was significantly lower than when grown with pine$^{N/Tt}$. The total dry mass of grass was not significantly different between competition treatments (Fig. 2C).

Pine^{Pa} had higher root-length density than pine$^{N/Tt}$ for all treatments ($P \leq 0.001$, Fig. 3A). When grass was grown intraspecifically, there was an increase in grass root length density with increasing P level (Fig. 3B) which paralleled the increase in shoot-P content (Fig. 2A). At the highest applied P level, grass growing with grass had a higher root-length density than grass in the interspecific treatments ($P \leq 0.0001$).

Pine^{Pa} had a higher RCC than grass (Fig. 4). In contrast, the RCC of pine$^{N/Tt}$ was similar to that of the grass.

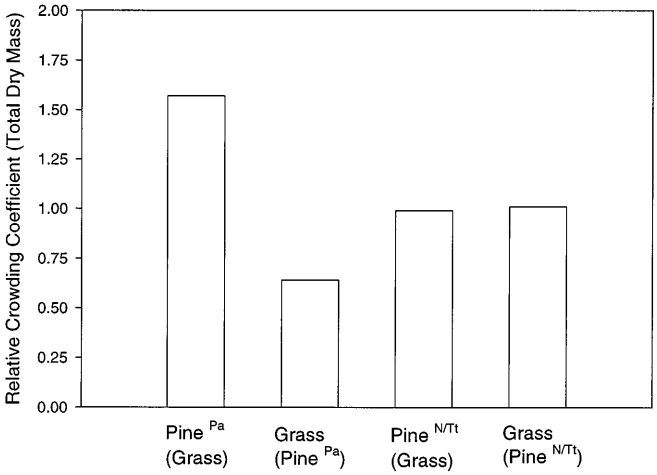

Fig. 4 Relative crowding coefficients of *P. elliottii* seedlings (*pine*) grown in combination with *P. chamaelonche* (*grass*) across three P levels. Pine^{Pa} was inoculated with *Pisolithus arhizus* and pine$^{N/Tt}$ was fortuitously colonized by *Thelephora terrestris*

Phosphorus uptake kinetics

On a root surface area basis (pine^{Pa} 428 cm^2, pine$^{N/Tt}$ 511 cm^2), I_{max} was not different between pine^{Pa} and pine$^{N/Tt}$. On the basis of total absorptive surface area (pine^{Pa} 3725 cm^2, pine$^{N/Tt}$ 523 cm^2), which included mycorrhizal hyphae, I_{max} was much lower in pine^{Pa} than pine$^{N/Tt}$. This suggests that depletion zones around the hyphae were overlapping, possibly due to inadequate mixing of the solution around a dense hyphal mass. Consequently, only the values for pine$^{N/Tt}$ and grass are reported (Table 2). Both I_{max} and C_{min} were higher for pine than for grass.

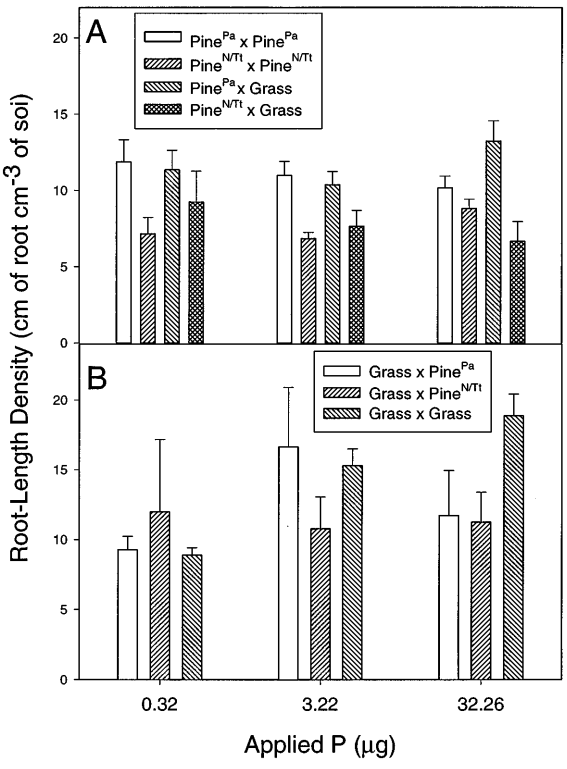

Fig. 3 Root-length density of (**A**) *P. elliottii* and (**B**) *P. chamaelonche* in different competition treatments and grown at 0.32, 3.23 or 32.26 µM P for 18 weeks; *bars* represent the mean of six replicates ± SE

Discussion

Abundant colonization of slash pine seedlings with *P. arhizus* enhanced their P acquisition when grown with nonmycorrhizal grass. In these experiments using acid-washed sand, soluble inorganic nutrients were the only source of nutrients available to both the roots and mycorrhizal fungi. The differences in P acquisition were, therefore, primarily affected by absorbing surface area and uptake rate. Plant density also may affect plant competition (Hartnett et al. 1993; Taylor and Aarssen 1989) but we did not specifically test this parameter.

Previous researchers have observed higher P-uptake rates for mycorrhizal plants than nonmycorrhizal plants (Cress et al. 1979; Karunaratne et al. 1986; Pacheco and Cambraia 1992), but these estimates generally are reported only on a root weight or root length basis. If

Table 2 Estimated maximum uptake rate, I_{max} (pmol P cm^{-2} s^{-1}), and the minimum concentration at which P can be absorbed, C_{min} (µM P), for *P. elliottii* (pine$^{N/Tt}$ treatment) and *P. chamaelonche* grown in a hydroponic solution containing 0.32 µM P. Each value represents the mean of three replicates ± SE

Plant species	I_{max}	C_{min}
Pinus elliottii	0.116 ± 0.027	0.080 ± 0.018
Panicum chamaelonche	0.075 ± 0.016	0.028 ± 0.014

such estimates included hyphal surface area, the specific uptake rates would be greatly reduced for mycorrhizal plants. In our study, pine^{Pa} and pine$^{N/Tt}$ had similar uptake rates based on root surface area estimates. However, the lower uptake rate of pine^{Pa} than pine$^{N/Tt}$, based on total absorptive surface area, indicates that hyphal nutrient depletion zones were overlapping and that the rate-limiting step for uptake was the replenishment of P in the dense fungal mass.

The P-uptake kinetics of pine and grass were different and this may contribute to the competition between these plants. The higher I_{max} of pine may permit pine to sequester more P than grass where nutrient pulses occur, such as when a rain follows a dry period and releases nutrients stored in an organic form, or during regular fertilization. The lower C_{min} of grass may be advantageous where low nutrient concentrations persist over longer time periods, such as in a nonfertilized field, but not in our greenhouse study, where the fertilization interval was relatively brief.

The relationship between resource abundance and intensity of competition depends on environmental conditions and the plant species involved (Di Tommaso and Aarssen 1991; Grace 1995). Tilman (1982) stated that competition increases with decreasing resource availability. An alternate view, espoused by Grime (1979), maintains that competition intensity increases with increasing habitat fertility. Competition is often manifested as reduced plant biomass, survival or reproduction. In our study, pine biomass generally did not respond to P application, suggesting that P was not the nutrient limiting growth. Nonetheless, P uptake was affected by P and competition treatments. Because P uptake often leads to increased plant biomass in the field, this mechanism could influence plant competition in pine plantations.

The P captured by pine reduced the amount of P taken up by the nonmycorrhizal grass. Pine^{Pa}, based on its greater RCC, was more competitive than nonmycorrhizal grass when the two were grown together. It must be noted, however, that competitive interactions in the field could be quite different. Field-collected *P. chamaelonche* has abundant AM fungal root colonization (Pedersen and Sylvia 1997) and this would alter the dynamics of nutrient competition between pine and grass. Nonetheless, our experiment serves to underscore the importance of pine mycorrhiza to interplant competition.

Intraspecific pine competition was not influenced by inoculation. However, because the noninoculated pine plants were not free of mycorrhiza, we were not able to determine whether ectomycorrhiza altered the intensity of intraspecific competition. In an EM competition study by Perry et al. (1989), the competition intensity varied with the fungus species.

We conclude that *P. arhizus* can increase P acquisition by pine when grown with grass, which may subsequently lead to an increase in the competitive ability of pine. The controlled conditions used in these experiments allowed us to determine specific parameters (P uptake and absorbing surface area) affecting plant competitive ability. However, the actual contribution of the mycorrhizal component to competitive ability can only be determined under field conditions, where soil chemical, physical, and biological parameters modify plant interactions and the mycorrhizal response. Our ability to determine individual components of plant competitive ability and the relative importance of each component is limited precisely by the interwoven nature of the plant/soil complex.

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