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Plant–plant interactions vary with different mycorrhizal fungus species

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Because different species of mycorrhizal fungi have different effects on the growth of particular plant species, variation in mycorrhizal fungus species composition could cause changes in the strength of plant–plant interactions. Results are presented from a growth chamber experiment that compared the strength of interactions among seedlings of ponderosa pine (*Pinus ponderosa*) when the pines were colonized by two different groups of ectomycorrhizal fungi in the genus *Rhizopogon*. Plant density effects differed between the two groups of mycorrhizal fungi: plant growth was low regardless of density when plants were colonized with pine-specific *Rhizopogon* species, while plant growth declined with plant density when plants were colonized by *Rhizopogon* species having a broader host range. This result parallels results from previous studies showing that plant interactions are more antagonistic with mycorrhizal fungi than without, implying that plant responsiveness to beneficial mycorrhizal fungi declines with increasing plant density. If such effects are prevalent in plant communities, then variation in mycorrhizal fungus community composition is predicted to have a density-dependent effect on plants.

Keywords: density-dependence; mycorrhizal fungi; *Pinus ponderosa*; plant competition; *Rhizopogon*

1. INTRODUCTION

A pattern that has been repeatedly observed in interactions between arbuscular mycorrhizal (AM) fungi and plants is that plant competition is stronger with AM fungi than without (reviewed by Koide & Dickie 2002). However, plants and the mycorrhizal fungi that colonize their roots usually occur as diverse communities that vary in species composition at multiple spatial and temporal scales (Horton & Bruns 2001; Clapp *et al.* 2002). Because mycorrhizal fungus species often vary in their growth effects on particular plant species (e.g. Chu-Chou & Grace 1985; Hetrick *et al.* 1986; van der Heijden *et al.* 1998), the potential for plant–plant interactions to be affected by changes in the mycorrhizal fungus community, rather than simply the presence versus absence of it, warrants consideration (van der Heijden *et al.* 1998; Bever & Schultz in press).

Because previous studies have shown that ectomycorrhizal fungi differing in host specificity also frequently differ in their abilities to acquire nutrients for and promote the growth of particular host plant species (e.g. Chu-Chou & Grace 1985), I hypothesized that the strength of intraspecific plant interactions would change when plants were colonized by mycorrhizal fungi differing in host specificity. Here, I describe the results of a simple experiment designed to test whether ectomycorrhizal fungi differing in host specificity cause differences in the strength of interactions among seedlings of ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.).

2. MATERIAL AND METHODS

(a) *Experimental organisms*

Ponderosa pine (*P. ponderosa*) is a common constituent of coniferous forests throughout western North America. Like other conifers, ponderosa pine harbours a diverse ectomycorrhizal fungus community, of which *Rhizopogon* Fries (Basidiomycota, Rhizopogonaceae) species are among the more commonly found taxa. Four different *Rhizopogon* species were used for this study: one each from the pine-specialist subgenera *Rhizopogon* (*R. truncatus*) and *Roseoli* (*R. vulgaris*) and two from the subgenus *Amylopogon* (*R. ellenae* and *R. arctostaphyli*), the members of which tend to have some ability to colonize a broader range of coniferous host plant species, including Douglas fir (e.g. Massicotte *et al.* 1994). *Rhizopogon* sporocarps (provided by Mycorrhizal Applications Inc., Grants Pass, OR) and a mixed genetic stock of ponderosa pine seeds (provided by the J. Herbert Stone Nursery, Central Point, OR) were collected at various locations in southwestern Oregon between 400 and 1200 m in elevation. The pine-specificity of *R. truncatus* and *R. vulgaris* and the broader host-range (ability to colonize Douglas fir) of the two *Amylopogon* species were confirmed in a separate simultaneous experiment (Hoeksema 2002). Voucher specimens of dried mycorrhizal root tips of each of the four fungal species were deposited in the Museum of Natural History Collections at the University of California, Santa Cruz (Collection nos JDH 147, 148, 129 and 130, respectively).

(b) *Experimental design*

Pine seedlings were grown at a range of plant densities and with one of the four *Rhizopogon* species, in order to estimate the impact of differing mycorrhizal fungus species on the magnitude of intraspecific plant–plant interactions. Specifically, each pot (6.6 cm diameter, 12 cm deep) contained a single randomly-chosen 'target' pine seedling and 0–4 'neighbour' seedlings (all grown from seed). Higher numbers of replicates were concentrated at lower neighbour densities to facilitate the detection of curvilinear changes in plant performance with density (Goldberg & Scheiner 2001).

Soil used for the experiment was the upper 8 cm from a xeric palehumult soil in a mixed conifer forest (of which *Pinus ponderosa* is a common constituent) in the Sierra Nevada mountains of northern California, USA (El Dorado County, 39° 15′latitude, 120° 32′ 45″ longitude). The field-collected soil was thoroughly mixed, passed through a 2 mm sieve and autoclaved at 121 °C for 3 hours.

After planting, the pots were placed in a growth chamber (14 hour day, ~30,000 lumens m^{-2} of light, 20 °C daytime, 7 °C nighttime, except weeks 13–17 during which temperature was 24 °C daytime and 10 °C nighttime) and completely randomized with respect to treatment. During the period of seed germination (weeks 1–8) and after mycorrhizal inoculation (week 13), each pot was watered from the surface with 50 mL of de-ionized water every 3–5 days. During weeks 8–12 of the experiment, every other watering was with a dilute nutrient solution (61 ppm nitrogen, 20 ppm soluble potash, 56 ppm phosphoric acid, 31 ppm magnesium, 40 ppm sulphur and 59 ppm calcium).

After 13 weeks, mycorrhizal fungus spores were added to all pots by pipetting onto the soil surface ~10^7 spores of a single *Rhizopogon* species in 10 mL of aqueous slurry (homogenized fresh sporocarp material in de-ionized water). Four mycorrhizal fungus species treatments were created—Generalist 1 (*R. ellenae*), Generalist 2 (*R. arctostaphyli*), Specialist 1 (*R. truncatus*) and Specialist 2 (*R. vulgaris*)—nested within two mycorrhizal specificity treatments, Generalist and Specialist. Sterilized coarse quartz sand was placed in a 5 mm layer over the surface of the soil in each pot, to minimize potential splash of spores among pots during watering.

(c) *Assessment of plant performance*

Thirty-three weeks after mycorrhizal fungus inoculation, all pots were removed from the growth chamber and the plants were harvested over the next 54 hours. Soil was carefully washed from

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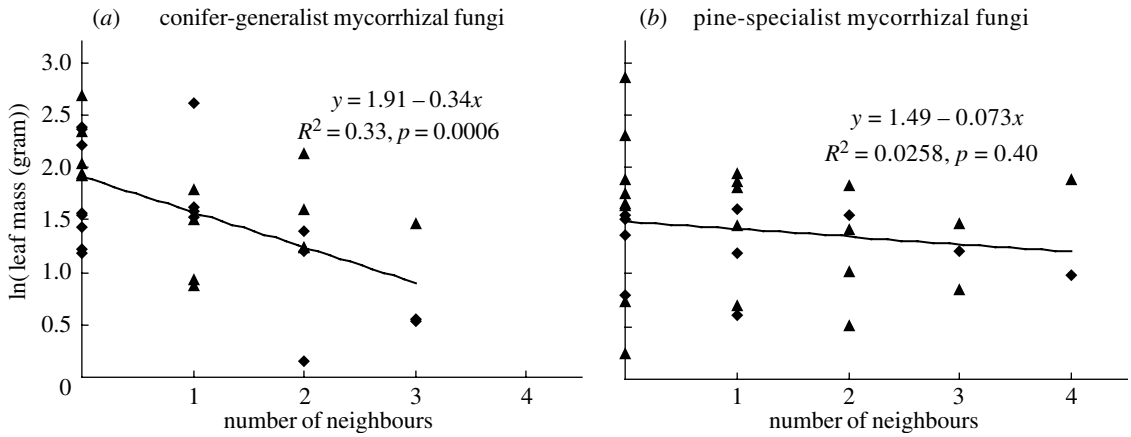

Figure 1. Target plant aboveground performance (natural log of final target dry leaf mass (gram) +1.5) as a function of Neighbour Number and Fungus Specificity. Shown are separate simple linear regressions for (*a*) Generalist and (*b*) Specialist mycorrhizal fungi: plant–plant interactions were negative with Generalist mycorrhizal fungi and lacking with Specialist mycorrhizal fungi. Values on the *y*-axis are residuals from a separate regression of plant performance on the covariate, Initial Plant Height. Triangles represent (*a*) *R. arctostaphyli* and (*b*) *R. vulgaris* and diamonds represent (*a*) *R. ellenae* and (*b*) *R. truncatus*.

roots on a 2 mm sieve and roots were examined under a dissecting microscope to obtain a qualitative estimate of the extent of mycorrhizal infection (0, no colonization; 1, <5% of root tips colonized; 2, 6–20% of root tips colonized; 3, 21–50% of root tips colonized; 4, >50% of root tips colonized). Seedlings were found to be colonized by mycorrhizal fungi to an approximately equal extent in all treatments—96% of seedlings were given a score of 3 or 4 on the qualitative scale (data not presented here; see Hoeksema 2002). No contamination with foreign mycorrhizal fungi was observed. Also, the two pine-specialist *Rhizopogon* species are readily distinguished from each other and from the other two species under a dissecting microscope and no instances of cross-contamination among pots within the experiment were observed. Shoots and roots were separated and air-dried at approximately 30 °C over 7 days (air-drying, rather than oven-drying, was used in order to facilitate more reliable stable isotope analysis of plant tissues for a separate study). After drying, roots, stems and leaves of all target and neighbour plants were separated and weighed.

(d) *Statistical analysis*

To analyse the effects of plant density and mycorrhizal fungus specificity on plant performance, a mixed, cross-nested linear model (Goldberg & Scheiner 2001) was fit to the data using restricted maximum likelihood estimation of parameters in the Mixed procedure in SAS (v. 8.02, SAS Institute, Inc.). Target plant final leaf mass and final root mass were used as response variables and both were log-transformed to achieve normality. Neighbour Number, Fungus Specificity (Generalist versus Specialist) and the interaction between Neighbour Number and Fungus Specificity were considered fixed factors of interest, while Fungus Species (Generalist 1, Generalist 2, Specialist 1 and Specialist 2) and the interaction between Fungus Species and Neighbour Number were treated as random factors nested within Fungus Specificity. Due to mortality of a small number of neighbour plants during the first few weeks of the experiment, the distribution of replicates among neighbour densities varied somewhat among the four mycorrhizal fungus treatments; thus, the correct *F*-tests were obtained in SAS by requesting Satterthwaite corrections of the degrees of freedom. To account for variance in target plant final mass due to initial differences in plant growth rate (prior to mycorrhizal inoculation), initial target height (4 days after mycorrhizal inoculation) was used as a covariate in the analyses. When a significant interaction was found between Neighbour Number and Fungus Specificity, separate simple linear regressions of plant performance (after removing the effect of the covariate) on neighbour number were also performed for the Generalist and Specialist fungus species groups, to explicitly characterize interaction effects. In these regressions, a significant negative slope indicates antagonistic effects of neighbour plants on target plants.

3. RESULTS

The measure of aboveground seedling performance (natural log of final target leaf mass) was significantly affected by an interaction between neighbour density and mycorrhizal fungus specificity (Neighbour Number: $F_{1,55}=11.53$, $p=0.0013$; Fungus Specificity $F_{1,4.4}=4.23$, $p=0.10$; Neighbour Number×Fungus Specificity $F_{1,55}=5.03$, $p=0.029$; Initial Target Height $F_{1,55.6}=6.61$, $p=0.013$). This result suggests that for this measure of plant performance, the strength of plant–plant interactions differed between mycorrhizal fungus treatments. Stated another way, this result implies that plant growth differences due to fungus specificity change with neighbour density. Separate simple linear regressions (using residuals from a regression on the covariate) for the two species groups of mycorrhizal fungi (Specialist and Generalist) revealed that target plant performance declined with neighbour density when colonized by generalist *Rhizopogon* species, but not when colonized by pine-specialist *Rhizopogon* species (figure 1). Though such regression slopes quantify interaction effects in an absolute sense, the fact that antagonistic effects were completely lacking in the Specialist treatment suggests that relative interaction intensity, i.e. the proportional reduction in target plant performance with neighbour density, also differed between the Specialist and Generalist treatments.

In contrast to aboveground seedling performance, belowground performance (natural log of final root biomass) was negatively affected by neighbour number but not by mycorrhizal fungi, i.e. there was a simple antagonistic effect of neighbours and no effect of mycorrhizal fungus treatments on plant performance or on the strength of interactions (Neighbour Number: $F_{1,54.8}=14.4$, $p=0.0004$; Fungus Specificity $F_{1,4.1}=0.01$, $p=0.94$; Neighbour Number× Fungus Specificity $F_{1,54.8}=1.03$, $p=0.316$; Initial Target Height $F_{1,55.4}=1.06$, $p=0.31$).

4. DISCUSSION

The experimental results presented here demonstrate how functional differences among mycorrhizal fungus species can translate into changes in the strength of

interactions among plants: when pines were colonized by ectomycorrhizal fungi that caused higher average plant growth rates at low plant density (see intercepts of the regressions in figure 1), antagonistic effects among ponderosa pine seedlings were stronger (see slopes of the regressions in figure 1). These results are not dissimilar to the pattern often observed in studies of the effect on plant interactions of the presence versus absence of mycorrhizal fungi, in which plant interactions are more negative with mycorrhizal fungi than without (reviewed by Koide & Dickie 2002). If we consider that ineffective mycorrhizal partners may be functionally equivalent to a lack of mycorrhizal partners altogether and may be analogous to low-productivity habitats for plants, then all such results may provide support for the Ruderal-Competitor-Stress Tolerator model of plant community organization (Grime 1979), which predicts that plant competition intensity should decrease with decreasing habitat productivity (Grace 1995).

Though data were not collected to determine the mechanism of the antagonistic plant–plant interactions observed in this study, and indeed it is beyond the scope of most studies to definitively distinguish between allelopathy and resource competition and to determine the specific resource(s) involved in such interactions (Fuerst & Putnam 1983), the most likely mechanism for the antagonistic interactions seen here is competition for soil macronutrients such as N, Ca, Mg and K. All of these soil nutrients are found in very low plant-available concentrations in the acidic, highly leached humult ultisol soil used in this experiment (Buol *et al*. 2003), very few nutrients were added as fertilizer and water and light were provided in such quantities as to not be limiting to seedling growth. Thus, at low plant densities, the broader host range mycorrhizal fungi may have allowed more efficient acquisition of one or more soil macronutrients, alleviating such limitations on plant growth; at higher plant densities, soil resource concentrations per plant may have been too low to allow for functional differences between mycorrhizal fungi to translate into differences in plant growth. Finally, the specific pattern of change in plant performance with density observed here is more consistent with the pattern predicted for resource competition than for allelopathy (e.g. Thijs *et al*. 1994).

A correlate of data showing a lack of plant–plant interactions in the absence of mycorrhizal fungi is that plant responsiveness to the presence of mycorrhizal fungi may be density-dependent, with less impact of the presence of mycorrhizal fungi at high plant densities (Koide & Dickie 2002). I suggest that plant responses to variation in benefits received from *different* mycorrhizal fungi may also be density-dependent. It has been suggested that density-dependent responses of plants to the presence of mycorrhizal fungi could act to stabilize the mycorrhizal mutualism (Bever & Schultz in press); density-dependent responses of plants to variation among different mycorrhizal fungi, as suggested by the results presented here, could seemingly provide a similar stabilizing influence on the dynamics of the interaction.

Because only two pine-specialist and two broader host-range *Rhizopogon* species were used in this experiment, these results should not be extrapolated to all ectomycorrhizal fungi differing in host specificity. However, future studies of the impact of the impact of mycorrhizal fungus community structure on plant community dynamics should consider fungal host-specificity as a potentially informative variable. It will be interesting to see if such studies begin to reveal that the evolution of host-specificity in mycorrhizal fungi has potential consequences for current plant population dynamics.

Based on what we now know about the diversity in function of mycorrhizal fungi and the prevalence of strong plant–plant interactions in plant communities, it seems clear that in order to understand the role of mycorrhizal diversity in affecting plant populations and communities, we need a better understanding of the impact of mycorrhizal fungus community structure on plant–plant interactions. The experimental results presented here represent one step towards such an understanding.

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