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Root age and phosphorus effects on colonization of *Andropogon gerardii* by mycorrhizal fungi

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Abstract

We examined the effects of plant age and root age on mycorrhizal colonization in the obligate mycotrophic grass *Andropogon gerardii* grown at high and low rates of soil phosphorus availability. There were significant interactions between soil P availability, plant age, root age and mycorrhizal colonization. Mycorrhizal colonization of 1-week-old roots increased with plant age, but was not significantly affected by soil P availability. Colonization of roots increased significantly with increasing root age only in the low-P treatment. Although the high-P treatment increased rates of root extension for some root age classes, there were not consistently lower rates of colonization in these roots. Studies of P acquisition by *A. gerardii* and other mycotrophic plants should take these interacting factors into account, especially when examining exploitation of nutrient-enriched patches.

Keywords: Phosphorus; Root age; Root growth; Vesicular-arbuscular mycorrhizal fungi; *Andropogon gerardii*

1. Introduction

We examined the effects of plant age and root age on mycorrhizal colonization in the obligate mycotrophic grass *Andropogon gerardii* grown at high and low rates of soil phosphorus availability. *A. gerardii* is a C-4, warm-season, native North American prairie grass known to be strongly dependent upon mycorrhizal colonization for P acquisition from low-P soils (Miller et al., 1997). There was a significant interaction between effects of root age and P availability on mycorrhizal colonization of roots; colonization of older root segments was lower in the high-P treatment.

Models and observations of mycorrhizal colonization dynamics indicate that root age, plant age, and phosphorus availability can interact to affect rates of root colonization (Buwalda et al., 1984; Amijee et al., 1993; Bruce et al., 1994). Older portions of roots are typically more resistant to primary infection by propagules in soil and secondary infection (hyphal growth along the root from primary lesions) than young portions of roots (Afek et al., 1990; Amijee et al., 1993; Bruce et al., 1994). Phosphorus additions to the soil may reduce percent root colonization by increasing root growth (Smith and Walker, 1981), and also by reducing primary and secondary infection rates (Amijee et al., 1993; Bruce et al., 1994). Infection rates are more strongly reduced by higher phosphorus concentrations in older roots than in younger roots (Amijee et al., 1993; Bruce et al., 1994).

To our knowledge, studies on the interactions between P additions and root age have only been carried out with cucumber (Bruce et al., 1994), leek, and clover (Amijee et al., 1993), none of which are obligately mycotrophic. Interactions between soil P and root age on mycorrhizal colonization may differ in obligately mycotrophic species, which typically have very high amounts of mycorrhizal colonization (Hetrick et al., 1994). Information on root age effects

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on colonization of obligate mycotrophs would be especially useful in interpreting studies of plant exploitation of enriched patches of P (Duke et al., 1994), where the dynamics of root and mycorrhizal proliferation may affect plant ability to compete for P within the enriched patch.

2. Materials and methods

A. gerardii seeds were germinated and grown in a soil mixture for 2 months. The soil mixture contained a 6:1:1 ratio by weight of peat moss, commercial potting soil (Sunshine Mix No. 1, Sungro Horticulture, Bellevue, WA), and field soil. Seedlings were then transplanted into an unsterilized mixture of field soil and washed quartz sand (1:3 w/w field soil:sand, 620 cm^3 soil mix per 750 cm^3 square pot). The field soil (Spinks sand, mesic Pasammentic Hapludalfs) in both mixtures was collected from a prairie remnant near Augusta, MI, which contained a large population of *A. gerardii*. All soil was collected within 30 cm of *A. gerardii* plants to ensure that roots of *A. gerardii* and associated VAM fungi were included. To ensure that inoculum potential would not be limiting to mycorrhizal colonization of the transplanted seedlings, 0.2 g of air-dried, crushed *A. gerardii* roots collected from the field were mixed into the soil in each pot. Two seedlings were planted on opposite sides of each pot, each plant 1 cm from a 5-cm-square Mylar root observation window. The observation windows were kept dark by nesting the windowed pots inside intact pots.

Plants were grown for 2 weeks after transplanting before the experimental P treatments were started. The mixture of field soil and sterilized quartz sand in the low-P treatment contained 12.6 mg P kg^{-1} dry soil, well below the level which would decrease mycorrhizal colonization in *A. gerardii* (Hetrick et al., 1990). Plants in the high-P treatment received 35 ml of 400 μM potassium phosphate (pH 7.0) every week to increase soil P levels to about 63 mg P kg^{-1} soil, a level previously shown to decrease colonization (Hetrick et al., 1990). To ensure that no other nutrients were limiting, both treatments received 150 ml per pot of a modified P-free Johnson solution (Epstein, 1972) twice weekly. All soil P analyses were done using the Bray P1 extraction and an ammonium molybdate colorimetric assay (Council on Soil Testing and Plant Analysis, 1980).

The experiment was set up as a randomized block design, with eight replicate pots (10.5 cm square, 9.5 cm deep) of each treatment placed in each of seven trays (blocks: 33×52.5 cm). Tray positions in the greenhouse were changed weekly to reduce environmental effects on plant growth. After P treatments were started, root growth on the windows was monitored on a regular basis (usually every week) by tracing new root length produced since the last tracing date; a different color pen was used at each tracing date. Root segments were harvested for analysis of mycorrhizal colonization by age class 3, 4, 5, 6 and 8 weeks after transplanting. The root tissue produced during each tracing interval was cut out with scissors, using the colored tracings on the Mylar windows as a guide. At each harvest date, we collected an average of 11 (range 6–21) root segments of 15.0 mm average length for each age class in the high-P treatment, and an average of 9 (range 5–15) root segments of 9.9 mm average length for each age class in the low-P treatment. Root segments were dried after harvest for 72 h in a 65°C drying oven.

Harvested root segments were cleared in 3% KOH at room temperature for 24–48 h, and then stained in 0.25% trypan blue (Koske and Gemma, 1989). Mycorrhizal colonization of individual root segments was determined by scoring for the presence and absence of mycorrhizal fungal structures within each millimeter of root length under 40× magnification.

After 8 weeks, whole plants were harvested from each treatment, separated into roots and shoots, and dried for 72 h in a 65°C drying oven before weighing. After mylar windows were removed from all pots, a video image of the tracings on each window was recorded for analysis of root extension rates. The program MSU-ROOTS (Enslin et al., 1993) was used to measure root length produced at each tracing date; video image digitizing and analysis followed the methods described by Hendrick and Pregitzer (1992) for minirhizotron video images. The lengths of the individual root tracings at each date were measured and stored in a database file for that window. Root extension rates for individual roots were determined by dividing root length produced during an interval by the total number of days in the tracing interval.

ANOVA was carried out with the SAS MIXED procedure (Littell et al., 1996). Since pots were the unit of replication in all analyses, data from the two windows or two plants in each pot were pooled. Data on mycorrhizal colonization and root extension rates were analyzed as a mixed-model ANOVA, with P concentration and root age class as fixed effects and trays (blocks) as a random effect. Separate analyses of colonization were run for each harvest date. P effects on plant weight and root-to-shoot ratio were analyzed as a mixed-model ANOVA, with P concentration as a fixed effect and block as a random effect. Regression analyses of the dependence of colonization on root age in 8-week-old plants and the effect of plant age on colonization of 1-week-old roots were carried out with COSTAT version 3.0 (Cohort Software, Minneapolis, MN).

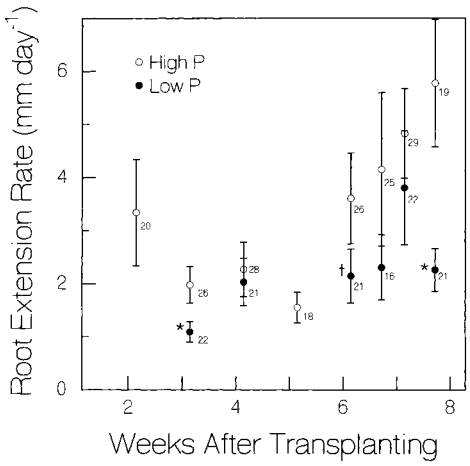

Fig. 1. Mean growth rate (mm day^{-1}) of individual roots traced on observation windows at each date. Phosphorus treatments: ○ = high-P, ● = low-P. Bars represent ±S.E.M. The number of pots included in each measurement is noted next to each point. Symbols indicate significant differences between high-P and low-P root growth rates for that date: $^{\dagger}P < 0.1$; $^{*}P < 0.05$.

3. Results and discussion

The root-to-shoot weight ratio did not vary between the two treatments (0.89 ± 0.83 for high P, $n = 11$, 0.84 ± 0.05 for low P, $n = 11$), but mean plant dry weight was significantly lower ($P_{(53,1)} < 0.001$) for low phosphorus plants (73 ± 5 mg, $n = 11$) than for high phosphorus plants (143 ± 9 mg, $n = 11$). Both plant age and P treatments had significant effects on root

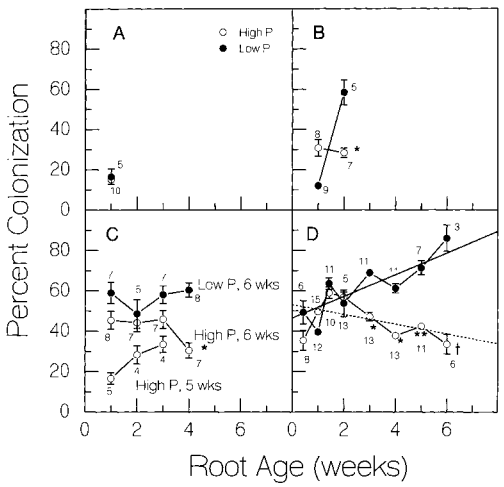

Fig. 2. Mean percent mycorrhizal colonization for different root age classes of *A. gerardii* roots sampled from plants (a) 3 weeks, (b) 4 weeks, (c) 5, 6 weeks and (d) 8 weeks since transplanting. The number of pots included in each measurement is indicated for each point. Symbols and error bars as in Fig. 1: $^{\dagger}P < 0.1$; $^{*}P < 0.05$; $^{**}P < 0.01$. Regression equations for 8-week-old transplants based on means for each age class: High-P % colonization = −0.02 (root age) + 0.52, $r^2 = 0.24$, $P = 0.22$; Low-P % colonization = 0.06 (root age) + 0.44, $r^2 = 0.70$, $P = 0.006$.

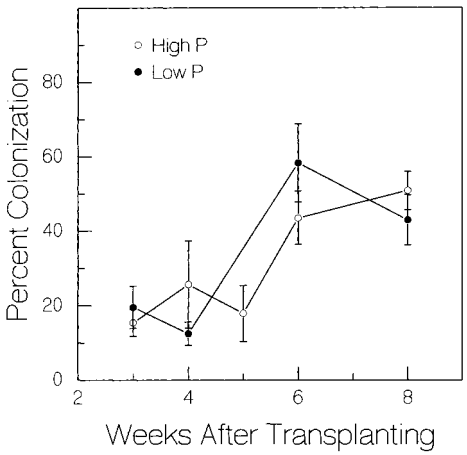

Fig. 3. Mean percent mycorrhizal colonization of one-week-old *A. gerardii* roots three to eight weeks after transplanting. Symbols and error bars as in Fig. 1.

extension rates (Fig. 1). Root extension rates increased significantly ($P_{(3.95,7)} < 0.001$) with plant age in both high-P plants and low-P plants (Fig. 1). P addition significantly ($P_{(7.89,1)} < 0.01$) increased root extension rates overall, although high-P plants did not have significantly higher rates of root extension at every date (Fig. 1).

There was a significant interaction between root age and P treatment effects on colonization of 2-week-old ($P_{(6.8,1)} < 0.05$) and 8-week-old transplants ($P_{(3.6,7)} < 0.01$). In these plants, colonization of roots increased significantly with root age in the low-P treatment, but not in the high-P treatment (Fig. 2b,d and regression equations).

As transplants aged, there was an increased rate of colonization of 1-week-old roots (Fig. 3). This increase in colonization with time is typical of that seen in other plants after initial exposure to mycorrhizal propagules (Buwalda et al., 1984). Assuming that most of the mycorrhizal inoculum in our soil mixtures came from the field soil and *A. gerardii* root fragments, the plants were exposed to much higher amounts of inoculum after transplanting.

Smith and Walker (1981) pointed out the potential importance of the balance between extension of the infection front and root growth in determining the observed rate of colonization in a root system. Although the mean root extension rate of 3-day-old roots on 8-week-old transplants in the high-P treatment was significantly higher than that of roots in the low-P treatment (Fig. 1), there was no effect of P treatment on mycorrhizal colonization in these roots (Fig. 2d). Because the differences in colonization seen between the high- and low-P treatments in 8-week-old transplants only appeared in older root segments, which did not consistently differ in extension rate (Fig.

1), they are unlikely to have been caused solely by differences in root extension rates.

There were no consistent differences between our observations of elevated P effects on the obligately mycotrophic species *A. gerardii* and previous reports of the effects of elevated P on facultative mycotrophs. High P increased rates of root extension of *A. gerardii* (Fig. 1) and cucumber (Bruce et al., 1994), but not leek (Amijee et al., 1993). Although our experimental design did not allow us to measure primary and secondary infection rates, rates of infection of older roots were probably decreased in the high P treatment; P effects on both primary and secondary infection rates have been characterized in cucumber (Bruce et al., 1994), leek, and clover (Amijee et al., 1993).

Our observations of interactions between soil P availability, plant age, root age, and mycorrhizal colonization indicate that field studies of P acquisition by *A. gerardii* and other mycotrophic plants should take all these factors into account. Root age effects on mycorrhizal colonization are especially likely to be of concern when examining exploitation of nutrient-enriched patches by mycorrhizal plants (e.g. Duke et al., 1994). Further detailed studies of the mechanisms of root age and transplant age effects on infection processes by different species of mycorrhizal fungi on obligately mycotrophic plants would be valuable for comparison with published studies of facultatively mycotrophic plants.

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