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GROWTH, PHYSIOLOGY, AND CHEMISTRY OF MYCORRHIZAL AND NONMYCORRHIZAL *TYPHA LATIFOLIA* SEEDLINGS

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Abstract: We investigated the impact of arbuscular mycorrhizae fungi on common cattails (*Typha latifolia*), a ubiquitous wetland plant species. The mycorrhizal relationship, which involves the exchange of fungal-acquired nutrients and plant-produced carbon, has been shown to elicit a range of physiological and biochemical responses in host plants. Growth, photosynthetic activity, biomass accumulation, and nutrition were compared between seedlings inoculated with viable mycorrhizal fungal spores and control seedlings given a sterilized inoculum. Plants were grown in inundated soils at three levels of phosphorous availability under glasshouse conditions for 11 weeks. The presence of arbuscules and hyphae was confirmed in all inoculated plants with levels of infection reaching 23.1 and 34.1%, respectively. Control plants were taller and had greater above- and below-ground biomass. Photosynthetic rates measured at week 11 of the experiment were significantly higher in mycorrhizal plants than in control plants. Mycorrhizal plants had higher concentrations of N, P, and C in their shoots and higher concentrations of N in their roots. Our results suggest that under greenhouse conditions, the fungus acts to reduce host plant growth despite increased mineral nutrition and photosynthetic activity.

Key Words: vesicular arbuscular mycorrhizae, *Typha latifolia*, growth depression

INTRODUCTION

The mycorrhizal symbiosis, a relationship involving the bi-directional exchange of resources between plants and fungi, is one of the most common and widely investigated biological associations between plants and microorganisms. It has been suggested that as many as 95% of all plant families contain species that are mycorrhizal (Smith and Read 1997) and that as many as 80% of all terrestrial plant species are mycorrhizal (Harley 1989). It has further been speculated that this symbiosis may have acted as the vehicle for vascular plants to colonize and exploit terrestrial habitats (Selosse and LeTacon 1998, Blackwell 2000). Despite the importance of this symbiosis, and its likely role in shaping terrestrial plant communities and structuring ecosystem function, our understanding of the mycorrhizal symbiosis in wetland habitats is limited. In fact, much of the work dedicated to improving our understanding of mycorrhizal fungi in wetlands has been restricted to surveys of infection (Wetzel and van der Valk 1996, Turner et al. 2000) or examinations of infection across natural or artificial hydrologic gradients (Rickerl et al. 1994, Stevens and Peterson 1996, Miller and Bever 1999, Miller and Sharitz 2000).

Miller and Sharitz (2000) experimentally examined the effect of mycorrhizae on wetland plant nutrition using two semi-aquatic grass species. Consistent with research from the terrestrial literature, there was a positive relationship between mycorrhizal infection and plant nutrition. The correlation that they reported between the extent of mycorrhizal infection and tissue phosphorus suggests that the presence of mycorrhizae in wetlands may affect the partitioning of nutrients in wetland plant tissues.

Just as the host plant benefits by improved nutrition, the fungal symbiont is dependent on the host plant, and therefore represents a sink for the products of photosynthesis (Douds et al. 1988, Wright et al. 1998a). One hypothesis on sink strength suggests that the demand for carbon is sufficient to increase carbon assimilation in mycorrhizal plants (Fitter 1991). Using terrestrial plant species, mycorrhizal infection has been shown to stimulate photosynthesis (Lovelock et al. 1997, Wright et al. 1998a), and up to 20% of fixed carbon may be allocated to mycorrhizae (Jakobsen and Rosendahl 1990, Wright et al. 1998b).

Whether or not the mycorrhizal symbiosis enhances photosynthetic activity or nutrient uptake, improvements in plant fitness (performance) are often observed. Changes in plant performance can be described by an increase in primary production that can be measured for an individual, community, or ecosystem. As reported by Miller and Sharitz (2000), mycorrhizal

colonization of grasses in an experimental wetland led to increases in primary production and nutrient uptake. If these processes are sufficient to affect the growth and nutrition of plants, then the presence of mycorrhizae may significantly alter nutrient dynamics by leading to the accumulation of nutrients in standing live plant material and litter, while accelerating nutrient removal from water and soils.

To address the impact of mycorrhizae on wetland primary production and nutrient cycling, we examined the effect of mycorrhizal fungi on greenhouse-grown common cattails (*Typha latifolia L.*). Our specific objectives were to compare the patterns of growth and biomass accumulation, photosynthetic rates, and tissue nutrients (C, N, and P) of cattail seedlings receiving viable soil inoculum with those receiving sterilized inoculum.

MATERIALS AND METHODS

The experiment consisted of a full factorial design using *Typha latifolia*, grown with and without viable fungal inoculum at three levels of phosphorus addition. *Typha* seeds were germinated in a sterilized sphagnum peat moss medium. Seedlings were transplanted into $10 \times 10 \times 35$ cm pots (3.5L) containing a sterile 1:1 mixture of sand:perlite.

Trap cultures (Stutz and Morton 1996) using field-collected soils were maintained for 12 weeks to increase the biomass of mycorrhizal fungi. After 12 weeks, viable mycorrhizal inoculum taken directly from these cultures was introduced into the inoculated treatment at a ratio of 1:4 inoculum:soil. A similar quantity of inoculum was added to the non-mycorrhizal treatment after being autoclaved at 121° C for 2 hours to kill fungi and spores.

Pots, each containing one *T. latifolia* seedling, were placed in 60-L plastic tubs. Three tubs each received 12 inoculated plants, and three tubs each received 12 control plants, resulting in a total of 72 plants in six tubs. Tubs were kept filled with a dilute (10% strength) modified (without P) Hoagland's solution (Taiz and Zeiger 2002) that was changed weekly.

The three phosphorus treatments were maintained by adding different amounts of phosphorus to the Hoagland's solution in the tubs. One tub of each mycorrhizal treatment received no P, one tub received sufficient P to bring the Hoagland's solution to 50% strength with respect to P, and one tub of each mycorrhizal treatment received enough P to bring the Hoagland's solution to 100% with respect to P. During week four of the experiment, approximately 120 mg of nitrogen, in the form of ammonium nitrate, was added to each 60-L tub to stimulate growth.

Plants were initially kept in a growth chamber maintained at a temperature of 27° C. A 16-hr photoperiod was established with peak light intensity of 450 mol m^{-2} s^{-1} (200 μmol m^{-2} s^{-1} at the beginning and end of the illumination period). After three weeks, plants were moved to a greenhouse bay where maximum intensities reached as high as 700 μmol m^{-2} s^{-1}.

We measured the height of the tallest leaf of each plant each week. Growth rates were calculated using the following equation to determine differences in growth between dates:

$$R = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

The equation, as described by Fitter and Hay (2002) uses plant biomass as W, however, since non-destructive measurements were used to track growth in our experiment, we used plant height (cm) in place of mass. There was a significant relationship between plant height and total plant biomass at the time plants was harvested ($R = 0.752$, $P < 0.001$).

At the end of week 10, six plants from each tub were randomly selected and harvested. Shoots were cut at the soil surface, dried, and weighed. Roots were washed clear of soil. Two root sections of approximately 0.15 g (wet weight) were clipped during the harvesting process. One section was preserved in 70% ethanol and set aside to examine the extent of arbuscular mycorrhizal (AM) colonization. The other section was dried to constant mass at 60°C and used to determine the wet weight to dry weight conversion. This conversion was then used to account for the removal of both sections (preserved and dried) and include this mass in total biomass calculations.

Preserved roots were clipped into 1- to 2-cm segments and were suspended in a 1-L beaker and stirred using a magnetic stirring bar. Root fragments were randomly selected from the beaker (Stenlund and Charvat 1994) until approximately 1 to 2 m of roots (measured using the line intersect method described by Tennant 1975) were obtained from the larger sample. Selected roots were fixed with formyl acetic alcohol (28% weight to volume formaldehyde, glacial acetic acid, and 95% ethanol in the ratio 1:1:18 by volume), cleared with 10% potassium hydroxide, and stained with 0.05% chlorazol black E solution (Brundrett et al. 1984) to detect the presence of arbuscular mycorrhiza. Root infection, described as the percentage of root length with arbuscules, was calculated using a line intercept approach and determined using procedures described by McGonigle et al. (1990).

Dried root and shoot material was ground in a Wiley mill. Phosphorus concentrations were measured using a persulfate digestion technique, which hydrolyzes phosphorus to orthophosphate. Orthophosphate concentrations were determined colorimetrically using a flow analyzer. Carbon and nitrogen concentrations

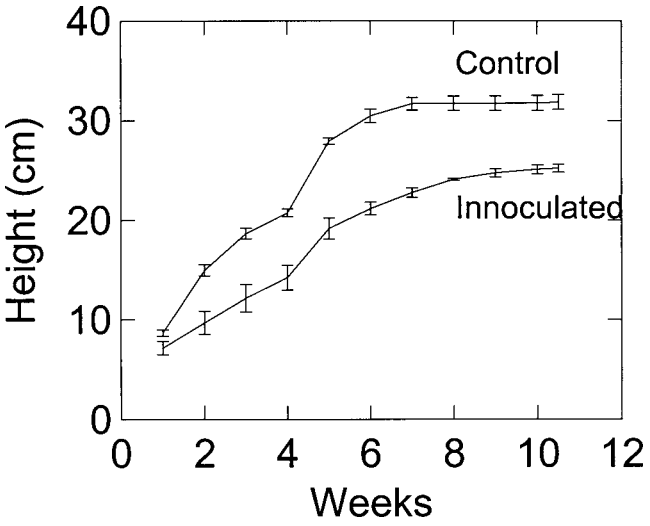

Figure 1. Mean (± 1 S. E.) heights of control and innoculated plants over the 10-week research period. Heights were measured to the nearest millimeter using the longest leaf for each plant.

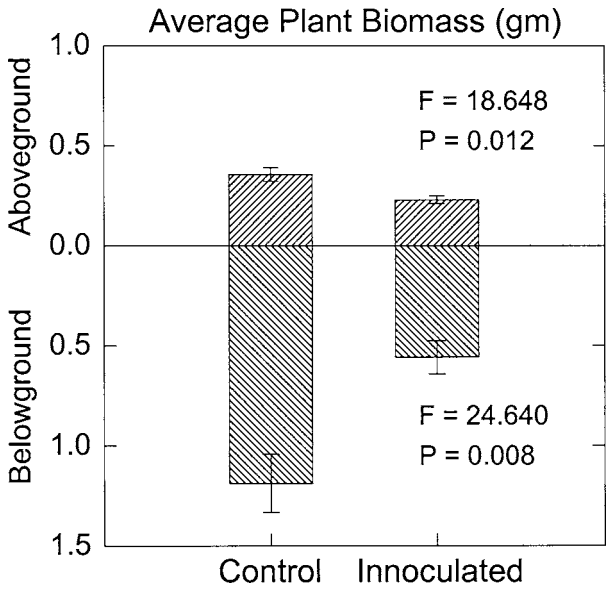

Figure 2. Mean (± 1 S. E.) above and belowground biomass for each treatment reported as grams of dry mass. Total plant mass was determined following harvest at the end of week 10.

were determined using a Fisons model 1500 NC combustion gas chromatograph.

Photosynthetic measurements were taken during week 11. Photosynthetic carbon assimilation rates (A_{CO2}), stomatal conductance of water vapor (g_s), and sub-stomatal carbon dioxide (CO_2) concentrations (C_i), reported as a ratio of internal to ambient CO_2 (C_a), or Ci:Ca, were measured with a portable gas exchange system (LCA-4 system, Analytical Development Company, Limited, Herts, England) using a narrow chamber cuvette (PLC4N). Readings were taken at ambient CO_2 levels (approximately 350 ppm), at light levels varying between 400 and 600 moles m^{-2} s^{-1} in a greenhouse, and at temperatures near 25° C. Measurements were made using three leaves with potted plants in standing water at the time of harvest. Photosynthetic measurements were corrected to account for differences in leaf area between plants.

Differences in biomass, tissue chemistry, and photosynthetic rates were first examined using a 2-way ANOVA and comparing the average values for each tub. There were no significant differences between P levels, so we combined the three P treatments for each of the two innoculum treatments. Here, we present results from 1-way ANOVAs and Student's t-tests comparing innoculated (n=3) and control plants (n=3), using the average value for plants in each tub as the experimental unit.

RESULTS

All plants inoculated with viable mycorrhizal fungal spores stained positive for infection. Closer examination of infected roots showed extensive colonization of the cortical tissue. Recognizable structures included hyphae, arbuscules, vesicles, and spores. All 18 plants receiving viable inoculum contained hyphae and arbuscules. Mean levels of infection for these plants were 34.1, 23.1, and 2.6 % of the examined root length for hyphae, arbuscules, and vesicles, respectively. Two of the 18 plants given the sterile inoculum (control plants) stained positive for infection. Only one of those plants had arbuscules and infection levels (arbuscules = 8.9 %, hyphae = 9.0%, and vesicles = 0.0 %) were much lower than the means reported for inoculated plants.

Average height of control plants was greater than that of inoculated plants (Figure 1). Growth of control plants was greater than that of inoculated plants during the first week ($t = -9.50$, $P < 0.001$). There were no differences in the growth of plants during weeks 2–5; however, both inoculated and control plants increased in height following the addition of ammonium nitrate in week 4. Mycorrhizal plants showed marginally higher rates of growth in weeks 6 through 8 ($P < 0.10$ in all cases) and significantly higher rates in week 9 ($t = 5.98$, $P = 0.004$). No differences were detected between treatments during week 10.

At the end of week 10, both shoot and root biomass of control plants were greater than those of inoculated plants (Figure 2). The difference between the two treatments was particularly striking for root biomass; control plants had nearly double the root biomass of inoculated plants. The difference in shoot biomass was

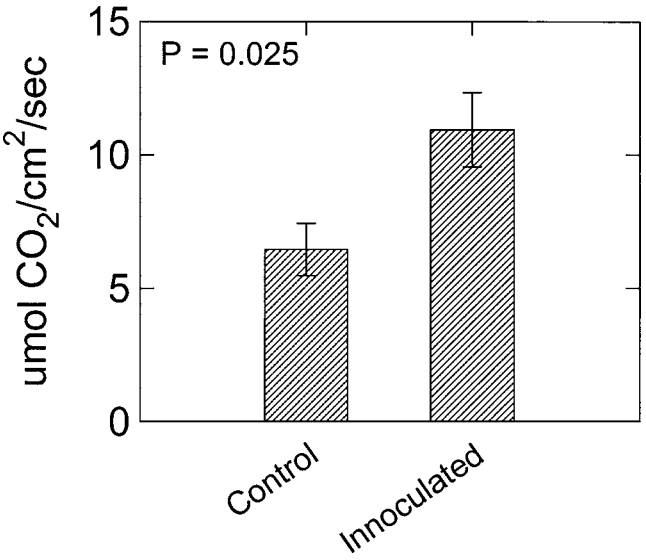

Figure 3. Mean (± 1 S. E.) photosynthetic rates for control and innoculated plants during week 11 of the experiment. Measurements were made using three representative leaves from each plant.

roughly 35%. Root:shoot of control plants was significantly greater than that of inoculated plants ($F_{1,4}$ = 12.86, P = 0.023).

Photosynthetic rates for inoculated plants were nearly double those of control plants (Figure 3). The Ci:Ca for inoculated plants was significantly lower than that of controls (t = −3.11, P = 0.035). The lower Ci of inoculated plants, coupled with greater rates of photosynthesis, indicates that a greater CO_2 deficit existed inside the leaves of inoculated plants.

There were significant differences between control and inoculated plants in tissue nutrient concentration and in total plant nutrient content (Figure 4). Inoculated plants had higher shoot concentrations of N, P, and C, and their roots had a higher concentration of N compared to control plants. Because they were larger, the total content of N and P in control plants was greater than that of inoculated plants. There was not a significant relationship between P concentration or total P content and P treatment.

DISCUSSION

It is apparent from this and other work with cattails (see Stenlund and Charvat 1994, Turner et al. 2000)

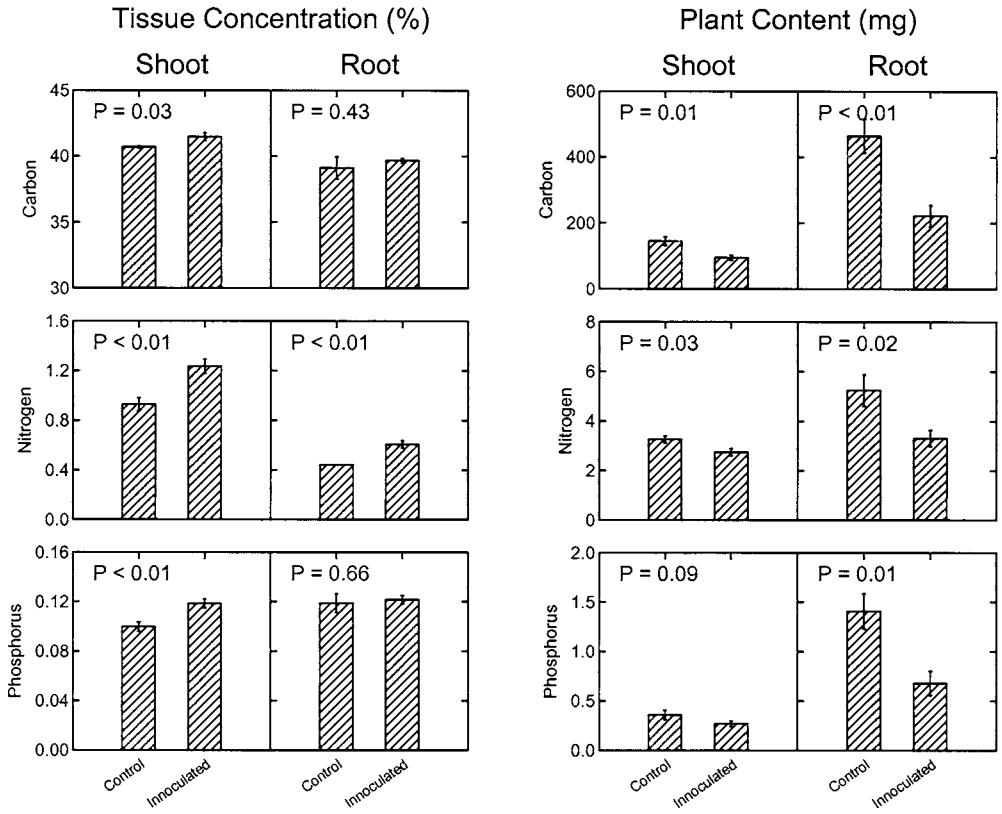

Figure 4. Mean (± 1 S. E.) carbon, nitrogen, and phosphorus concentrations (left panels) and content (right panels) of control and mycorrhizal (innoculated) plants. Concentrations are reported as percentages and content is reported in milligrams of each nutrient per plant.

that arbuscular mycorrhizal (AM) fungi readily infect *Typha* species. Graham and Eissenstat (1994) put mycorrhizal dependency in an evolutionary context and hypothesized that highly mycorrhizal dependent plants do not have variable levels of colonization because they rarely occupy environments where there is no benefit to the symbiosis. This contradicts the existing dogma on mycorrhizae in wetlands, which states that levels of infection are reduced in these habitats because of the inability of mycorrhizal fungi to tolerate anoxic conditions (Peat and Fitter 1993). Recent evidence demonstrated that mycorrhizal infection potential was greater in wetland than upland habitats in the Great Lakes region (Miller et al. 1999), and Miller and Bever (1999) found that the total number of fungal spores was greatest at the wettest portion of a hydrologic gradient at a Carolina bay wetland.

Our work with both field and greenhouse grown plants has identified some form of AM fungal infection in the roots of all plants grown with viable wetland soil inoculum. Plants in this greenhouse experiment were grown in containers with standing water to the soil surface and nutrients were replenished weekly. Even after eliminating the limitation of both water and nutrients, mycorrhizal infection was maintained. Mycorrhizal plants had an average level of arbuscular infection of approximately 20% of examined root length. These plants also had significantly higher photosynthetic rates than the nonmycorrhizal plants. Mycorrhizal fungi have the ability to create a flux of C to the point of infection and, therefore, to increase the C demand of the root. This demand, when not supported by nutritional benefits, results in a direct impact on the C assimilation of the infected plant (Smith and Read 1997). The elimination of nutrient deficits makes this carbon drain even more apparent, and the relationship moves towards one that more closely resembles a parasitism (Marschner and Dell 1993, Johnson et al. 1997) or exploitation by one of the mutualists (Bronstein 2001).

The drain on plant carbon was most obvious at the beginning of the experiment (Figure 1) when growth rates for nonmycorrrhizal plants were significantly greater than mycorrhizal plants. However, after the first week, plant growth rates did not differ for several weeks, and there were several weeks where the growth rates of mycorrhizal plants surpassed controls, indicating that the drain on the host plant was greatest during early stages of seedling growth. It is likely that the presence of an additional carbon drain is more deleterious to seedling carbon budget than to a mature plant. Johnson et al. (1997) reported that depression in seedling growth due to mycorrhizal infection often diminishes as a plant matures. This type of initial drain has been reported for citrus plants (Peng et al. 1993), sunflowers (Koide 1985), and soybeans (Bethlenfalvay et al. 1982) and may account for the early depression in cattail seedling growth. Since this experiment was carried out for only 10 weeks, longer experiments involving cattails are necessary to ascertain the net effect of the mycorrhizal association on plant performance over a growing season.

Plants grown with viable inoculum were significantly smaller than controls using measurements of both above and belowground biomass. This is especially significant when one considers that, in mycorrhizal plants, the fungal additions to the root represent some 3 to 20 % of the total root weight (Harris and Paul 1987). There is little agreement in the literature regarding the effect of mycorrhizae on resource allocation and biomass partitioning, suggesting that effects are site-specific. This experiment reinforced the fact that there is a real C expenditure for the host plant that is associated with infection and this led to growth depression.

In this experiment, mycorrhizal infection led to increased carbon assimilation. Mycorrhizal plants were characterized by higher photosynthetic rates that were not matched with similar increases in growth. This may have been induced and exacerbated by light limitation in an environment that rarely exceeded light levels of 700 $\mu mol\ m^{-2}\ s^{-1}$. Light limitation has reportedly reduced growth in several mycorrhizal agronomic species (Hayman 1974, Daft and El-Giahmi 1978, Son and Smith 1988). The work by Hayman (1974), Daft and El-Giahmi (1978), and Son and Smith (1988) suggests there was a positive relationship between irradiance, mycorrhizal infection and plant productivity. Cattails most commonly grow in open marshes, free of shade, and therefore, the imposed light levels may have acted to reduce plant growth in mycorrhizal plants.

Mycorrhizal infection is thought to be most prevalent when nutrients and/or water is limiting, and this is often demonstrated by showing a positive correlation between infection and tissue concentrations of nutrients. Our mycorrhizal plants had higher concentrations of nutrients (N and P) in both above- and belowground tissues. However, mycorrhizal plants were significantly smaller than controls. By examining the total mass of nutrients, we determined that, in all cases except for root P, non-mycorrhizal plants had a greater mass of nutrients than mycorrhizal plants. This indicates that P nutrition may have been improved by the presence of mycorrhizae even in an environment where P was not limiting. Our data are consistent with observations that mycorrhizae improve plant nutrition, but because the overall growth rates were lower for mycorrhizal plants, they ended up with less total N and P.

From this work, it is apparent that, under greenhouse conditions, mycorrhizae impact the carbon budget of cattail seedlings. It appears that the fungal part of the association benefited at the expense of the host plant by disrupting a mutualistic exchange of biochemical currency. This resulted in a relationship that more closely resembled a parasitism. At the ecosystem level, mycorrhizal infection, resulting in depressed growth of seedlings, decreased total plant biomass, and elevated carbon assimilation, could upset a competitive interaction in favor of non-mycorrhizal or less commonly mycorrhizal marsh species (e.g., *Schoenoplectus* spp.). Light levels imposed by the greenhouse may have amplified these effects, and therefore, it is imperative that plant/fungal interactions be explored under varying degrees of irradiance, including conditions similar to those experienced by this semi-arid ecotype. Experimental manipulations of this type will enable us to fully consider the effects that mycorrhizae have on the carbon budgets of wetland ecosystems.

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