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Interactions between *Tamarix ramosissima* (saltcedar), *Populus fremontii* (cottonwood), and mycorrhizal fungi: Effects on seedling growth and plant species coexistence

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Abstract

Little is known about the composition and function of the mycorrhizal fungal community in riparian areas, or its importance in competitive interactions between *Populus fremontii*, a dominant tree in southwestern United States riparian forests which forms arbuscular and ectomycorrhizas, and *Tamarix ramosissima*, an introduced tree species that has spread into riparian areas. The objectives of this study were to determine the mycorrhizal status of *Tamarix* and to evaluate the effect of mycorrhizal fungal inoculation on *Tamarix* growth and on the coexistence between *Tamarix* and *Populus*. Arbuscular mycorrhizal fungal colonization of *Tamarix* was very low in both field and greenhouse grown roots, but levels of colonization by dark septate endophytes were high. Fungal inoculation had little effect on *Tamarix* seedling growth in monoculture. When *Populus* and *Tamarix* were grown together in a greenhouse pot experiment, fungal inoculation reduced the height and biomass of *Tamarix* but had no effect on *Populus*. Fungal inoculation shifted coexistence ratios. When *Tamarix and Populus* were grown together, *Tamarix* plants averaged 20% of pot biomass in the uninoculated control but only 5% of pot biomass in the inoculated treatment. These results indicate that *Tamarix* is non-mycotrophic and that in this greenhouse experiment inoculation altered patterns of coexistence between *Populus* and *Tamarix*.

Introduction

Populus fremontii S. Wats. (Fremont cottonwood), the dominant overstory species in most riparian areas of the western United States, is an early successional species whose regeneration is tightly tied to natural flood cycles. Pioneer tree species, such as *Populus,* require disturbance in the form of large spring floods which clear streambanks of vegetation and deposit fresh alluvium, creating the open, bare germination beds needed for recruitment (Braatne et al., 1996; Karrenberg et al., 2002; Scott et al., 1997). The seed dispersal period for *Populus* is short and has evolved to occur in conjunction with natural high flow events (Braatne et al., 1996). When flood timing, magnitude or frequency are altered, regeneration of these trees usually decreases (Friedman et al., 1998; Johnson 1992; Rood and Mahoney, 1990, 1995). Throughout the western United States, riparian forests dominated by *Populus* are declining due to disruptions in natural flood cycles and groundwater levels by dams, groundwater pumping and water diversions (Busch and Smith, 1995; Patten, 1998; Stromberg et al., 1993).

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The regional decline of riparian gallery forests has occurred concomitantly with the spread of *Tamarix ramosissima* Ledeb. (saltcedar) into riparian areas. These trees were introduced to the United States from Europe in the 1800's for windbreaks and erosion control and now occupy over 1.6 million acres of riparian and wetland habitat throughout the western United States (Brock, 1994; Zavaleta, 2000). *Tamarix* ecology is similar to that of *Populus*, except that it disperses seed over a period of months, rather than weeks (Horton et al., 1960; Shafroth et al., 1998), which enables *Tamarix* to take advantage of floods or flow releases occurring throughout the summer. *Tamarix* is also more deeply rooted and more tolerant of drought and salinity that *Populus*, allowing it to survive in de-watered areas no longer capable of supporting *Populus* forests (Cleverly et al., 1997; Horton et al., 2001; Shafroth et al., 1998).

In many areas of the western United States, extensive restoration projects have focused on clearing *Tamarix* dominated riparian areas and re-introducing native riparian species including *Populus* (Sprenger et al., 2001, 2002; Taylor and McDaniel, 1998; Taylor et al., 1999). One aspect that has not been considered in these restoration efforts is the importance of mycorrhizal fungi, root-inhabiting soil microbes that form symbiotic associations with many plant species (Smith and Read, 1997) and supply the plant with nutrients in exchange for photosynthetic carbon.

Mycorrhizal fungi are important in plant community interactions, are able to alter ecosystem productivity and plant diversity (Gange et al., 1999; Grime et al., 1987; Klironomos et al., 2000; van der Heijden et al., 1998) and are important determinants of community structure in many ecosystems (Grime et al., 1987; Hartnett and Wilson, 1999; van der Heijden et al., 1998). *Populus fremontii* is known to form tripartite relationships (sensu Lodge; 2000) with arbuscular mycorrhizal fungi (AMF) and ectomycorrhizal fungi (EMF) (Jacobson, 2004; Lodge, 1989; Neville, et al., 2002; Vozzo and Hacskaylo, 1974). The mycorrhizal status of *Tamarix* has not been well studied; however Titus et al., (2002) reported that *Tamarix ramosissima* roots sampled in the Mojave Desert were non-mycorrhizal.

Extended occupation of habitats by non-mycorrhizal species could reduce local mycorrhizal inoculum potentials, creating a positive feedback that inhibits establishment of mycorrhizal plant species (Bever, 2003; Goodwin, 1992; Vogelsang et al., In prep). In riparian areas, positive feedback between *Tamairx* and soil fungi may give *Tamarix* a competitive advantage over native mycorrhizal riparian trees and shrubs. An understanding of the interactions between *Tamarix*, *Populus* and mycorrhizal fungi may improve the success of riparian restoration efforts.

The objectives of this study were to (1) determine if *Tamarix ramosissima* exhibits mycotrophy; (2) evaluate the effect of AMF inoculation on *Tamarix* growth; and (3) determine how mycorrhizal fungal inoculation influences patterns of coexistence between *Tamarix* and *Populus*. Based on the previous report by Titus et al., (2002), we hypothesized that *Tamarix ramosissima* is non-mycorrhizal and predicted that root colonization of *Tamarix* by mycorrhizal fungi will be low or absent. If so, we predict that *Tamarix* growth will be minimally or negatively affected by mycorrhizal fungal inoculation. We also hypothesized that fungal inoculation will alter patterns of coexistence between species and predicted that when *Tamarix* and *Populus* are grown together, the proportional contributions of *Populus* and *Tamarix* to the plant community will differ when plants are inoculated with mycorrhizal fungi.

To test these hypotheses, mycorrhizal fungal colonization levels were examined in roots from field and greenhouse grown *Tamarix* plants. We also conducted greenhouse experiments to measure the growth of *Tamarix* grown alone and with *Populus*, with and without mycorrhizal fungi. Preliminary investigations indicated that *Tamarix* might also be colonized by dark septate endophytes (DSE), another class of fungal-root associates whose function is largely unknown (Jumpponen, 2001). To investigate this further, all *Tamarix* and *Populus* roots were also examined for DSE colonization.

Methods

Collection of field-grown Tamarix *roots*

Root samples of *Tamarix* were obtained from four locations in the southwestern United States

including sites along the Rio Grande, Big Bend National Park, TX; Verde and Little Colorado Rivers, AZ; and Owens River, CA (total n = 18). Approximately 0.5 L of roots and soil were collected from directly underneath *Tamarix* trees growing in riparian areas in locations where the understory was free of most other vegetation. Each sample was sealed in a plastic bag and shipped to Arizona State University where fine roots were separated from the soil and fixed in 70% ethanol. *Tamarix* roots were cleared in 5% KOH and stained in trypan blue and sudan IV (Barrow and Aaltonen, 2001; Koske and Gemma, 1989). Colonization of AMF and EMF were assessed with the magnified intersections method (McGonigle et al., 1990). Dark septate endophytes were poorly visible in most samples with standard light microscopy. Infection was determined by the presence of stained lipid bodies within the hyphae or by the presence of melanized microsclerotia (Barrow, 2003). For every intersection used to assess mycorrhizal fungal colonization, the entire field of view (400×) was also used to record DSE infection. One hundred intersections per sample were recorded to give a measure of percentage root length colonized.

Experiment 1: Response of Tamarix *to AMF inoculation*

Inoculum production. Soil was collected from *Populus* stands at three locations on the upper Verde River in Arizona (Perkinsville 34° 53′ N, 112° 12′ W; Tapco 34° 47′ N, 112° 02′ W; Dead Horse Ranch 34° 45′ N, 112° 01′ W) and inoculum was generated from field soil through pot cultures (Stutz and Morton, 1996). Collected soil was bulked together and mixed in a 1.25:1 ratio with autoclaved #12 and #20 mesh size (1:1 v/v) silica sand. Surface sterilized 650 mL cone-tainer pots (Stuewe and Sons D40 cone-tainers, Corvallis, OR) were filled with 600 cm^3 of this sand–soil mixture. Each pot was seeded with approximately 75 seeds of *Sorghum sudanese* (Sudan grass) that were surface sterilized by soaking in 10% commercial bleach for 5 min followed by deionized water rinse. Trap cultures were grown in the greenhouse for 12 weeks, top watered to flow through daily, and fertilized monthly (Peter's No-Phos fertilizer solution, Scotts-Sierra Horticultural Products Co., Marysville, OH; 25-0-25). After 3 months irrigation was discontinued and the cultures were allowed to dry. The resulting inoculum was removed from all pots, homogenized, and stored at 4 °C. Inoculum consisted of the soil/sand media containing AMF propagules as well as propagules from soil biota.

Experimental design. River sand (50:50 sand/silt mixture) obtained from a local sand and gravel mining company was autoclaved at 121 °C for 1 hour and used to fill 20 surface sterilized 30 L tree-pots (Stuewe and Sons, Corvallis, OR). Each pot was placed in a 20 L bucket that served as a water-reservoir during the experiment. Pots were arranged in a randomized block design of five blocks of four pots each to control for varying light levels throughout the greenhouse. Two pots in each block received 100 cm^3 of fungal inoculum, produced as described above. Inoculum contained approximately 60 spores/cm^3 and was dominated by *Glomus claroideum* Schenk & Smith (21%) and *G. spurcum* Pfeiffer, Walker & Bloss (18%). *Paraglomus occultum* (Walker) Morton & Redecker (10%), *Archaeospora trappei* (Ames & Linderman) Morton & Redecker (10%), *G. eburneum* Kennedy, Stutz & Morton (7%), *G. intraradices* Schenck and Smith (7%), *G. microaggregatum* Koske, Gemma and Olexia (6%) and an undescribed *Acaulospora* species (14%) as well as 11 other species with contributions < 5% were detected in the inoculum. These pots also received 1 g of fresh *Populus* root tips to provide EMF. *Populus* roots were collected from five trees at Dead Horse Ranch State Park, Arizona (DHR), chopped into 1 cm pieces and homogenized before addition to the pots. The inoculum was mixed into the top 3 cm of soil in each pot and covered with an additional 200 cm^3 of sand.

Three hundred cubic centimetres of sterile sand was added to the remaining 2 pots in each block to create 2 treatments (inoculated and an uninoculated control) of 10 pots each. The surface of each pot was covered with 100 cm^3 of sterile coarse sand (#12) to control algal growth. On August 2, 2002 all pots were seeded with fresh *Tamarix* seeds collected from the Verde River at DHR. Three *Sorghum* seeds were planted in each pot to test for the success of

mycorrhizal inoculation in the event that *Tamarix* roots remained completely uncolonized. All pots were top-watered daily for one month and then thinned to a target density of 15 seedlings per pot (243 seedlings/m^2) and bottom watered through bucket reservoirs. The water level in each bucket was maintained 15 cm for the remainder of the experiment. All pots were treated with 0.5 L of Gnatrol (*Bacillus thuringiensis* ssp. *israelensis*; 5 mL/L) as needed to control fungus gnats.

The length of the longest stem and standing height of the seven tallest seedlings in each pot were measured weekly and the number of stems per plant and the length of all stems of each plant for all 15 seedlings in each pot were measured monthly. Four months after thinning (week 18) the shoots of all seedlings were harvested, dried at 60 °C for 1 week and weighed. These data are reported as above-ground biomass. Distal roots of *Tamarix* and *Sorghum* were harvested from each pot, rinsed in water and fixed in 70% ethanol. Only roots that could be directly traced back to a *Tamarix* or *Sorghum* seedling were harvested. The large volume of soil in the pots and extensive intermixing of *Tamarix* and *Sorghum* roots made measurements of *Tamarix* seedling root biomass impractical. Collected roots were stained and assessed for colonization as described above.

Data analysis. Means and standard errors of the means were calculated for root colonization levels in each of the treatments. Final standing height, length of longest stem, number of stems per seedling and above-ground biomass of *Tamarix* seedlings were averaged within each pot and checked for normality with the Shapiro–Wilk test and homogeneity of variances with Levene's test before analysis. The final standing height, length of longest stem, and number of stems were compared between treatments using ANCOVA (SAS PROC GLM) with standing height, stem length, or stem number at 6 weeks after thinning (October 11) as a covariate to control for slight differences in germination date. Above-ground biomass values were 4th root transformed for normality and compared between groups using ANOVA. Block (position in greenhouse) was also included as a main effect in all analyses. Significance was set at 0.05 for all tests.

Experiment 2: Effect of AMF inoculation on coexistence of Populus *and* Tamarix

Experimental design. The experimental design for Experiment 2 was similar to that for Experiment 1. Fresh river sand was obtained and autoclaved for the experiment and all pots were washed and re-sterilized in bleach. Thirty pots were arranged in five blocks of six pots each. The pots were inoculated as in Experiment 1 with four inoculated and two uninoculated (control) pots per block. Inoculum produced for Experiment 1 was mixed with sterile sand (1:3 inoculum:sand) and used in a second generation of pot cultures to generate inoculum for Experiment 2. The resulting inoculum contained approximately 15 spores/cm^3 and was dominated by *G. claroideum* (50%) and *G. eburneum* (15%). *Glomus luteum* Kennedy, Stutz and Morton (12%), *G. spurcum* (6%) and *Ar. trappei* (5%) as well as seven other species with contributions $<5\%$ were detected in the inoculum. Each pot also received one gram of fresh *Populus* root tips (collected and processed as described above) to provide EMF.

On May 3, 2003 all pots were seeded with fresh *Populus* seed collected from DHR. On June 5, 2 of the inoculated pots in each block were drenched with 3 L (2 g active ingredient/m^2) of benomyl (Benlate Du Pont, Wilmington, Delaware) to produce a low-level mycorrhizae treatment, creating 3 experimental treatments (inoculated, inoculated + benomyl and a uninoculated control) of 10 pots each. All pots in the experiment were thinned to three *Populus* seedlings per pot (48 seedlings/m^2) 4 weeks after planting. *Populus* seedling height was measured weekly.

Fresh *Tamarix* seeds collected from DHR were added to all pots on June 23. The timing of seed introduction was intended to mimic natural patterns of seed release in these species, which is usually separated by four to six weeks (Horton et al., 1960; Shafroth et al., 1998). *Tamarix* seeds were thinned to 15 seedlings per pot on July 31. Pots were treated with Gnatrol and Marathon (5 mL/L), as needed, to control fungus gnats, aphids and mites. The length and number of all *Tamarix* stems was measured on October 2.

Four months after the *Tamarix* seedlings were thinned, the above ground portion of all *Populus* and *Tamarix* seedlings was harvested, dried at

60 °C for 1 week, and weighed. Many *Populus* seedlings had begun to drop their leaves by the end of the experiment; so only the stem portion of the *Populus* seedlings was recorded as above-ground biomass.

Distal roots of *Populus* were harvested from each pot, rinsed in water and fixed in 70% ethanol. Only roots that could be directly traced back to a *Populus* seedling were harvested. *Tamarix* roots were not collected due to very poor root growth. *Populus* roots were stained and assessed for AMF colonization as described above. The presence of EMF mantle and Hartig net was used indicate EMF infection. The presence of other non-AMF or EMF fungal structures was also recorded at each intersection.

Data analysis. Final *Populus* height and above-ground biomass and *Tamarix* height (length of longest stem), stem number, above-ground biomass and mortality were summed within each pot and checked for normality and homogeneity of variances before analysis. *Tamarix* mortality levels differed markedly between pots, and all data are reported on a pot-basis rather than on an average plant basis to account for this.

A one-way ANOVA was used to determine the effects of fungal inoculation on *Tamarix* height, above-ground biomass and stem number and *Populus* above-ground biomass; with greenhouse block included as a main effect. *Populus* and *Tamarix* above-ground biomass values were 4th root transformed before analysis. Treatment effects on *Populus* height were examined with ANCOVA (SAS PROC GLM) on final height, using height at 6 weeks after thinning (July 10) as a covariate and block included as a main effect. *Tamarix* seedling mortality was compared between treatments using a two-way non-parametric ANOVA, performed by ranking the data, conducting a normal ANOVA, and calculating the corresponding H ratio (Sokal and Rohlf, 1995).

The effect of fungal inoculation on patterns of plant coexistence was determined using a coexistence ratio (van der Heijden et al., 2003) calculated on both a seedling weight (above-ground biomass) and length (*Populus* height, length of longest *Tamarix* stem) basis. The coexistence ratio is the height or above-ground biomass of *Tamarix* divided by the combined heights or above-ground biomass of both species and gives the proportional contribution of *Tamarix* to total pot above-ground biomass or height. Coexistence ratios were checked for normality and homogeneity of variances before analysis. A one-way ANOVA was used to compare coexistence ratios between treatments, with greenhouse block included as a main effect. All *post hoc* comparisons were performed with the Tukey-Kramer method or Dunn's test where appropriate.

Populus seedlings in one uninoculated pot and one inoculated pot died early in the experiment and these pots were dropped from the analysis, leaving $n = 9$ in the control treatment and $n = 9$ in the inoculated and $n = 10$ in the inoculated + benomyl treatment.

Results

Colonization of Tamarix *and* Populus *by AMF, EMF and DSE*

Arbuscular mycorrhizal fungal colonization of *Tamarix* was very low in the in the field samples (2.0 ± 0.8 (SE)%) with only hyphae and vesicles observed, however levels of DSE colonization were high in field collected roots (78.4 ± 4.2%). In Experiment 1, inoculated greenhouse grown *Tamarix* seedlings showed levels of AMF and DSE colonization similar to field collected *Tamarix* roots (AMF 3.2 ± 1.0%; DSE 95.9 ± 1.1%). Uninoculated seedlings remained uncolonized by AMF but had high levels of DSE colonization (90.6 ± 3.1%). Other studies have shown that DSE is carried in seeds and systemically infects plants (Barrow et al., 1997) therefore DSE colonization in the uninoculated treatment does not indicate contamination. *Sorghum* roots growing in all greenhouse pots were colonized by AMF indicating that the AMF inoculum used was viable. Ectomycorrhizal fungi did not colonize *Tamarix* roots.

In Experiment 2, AMF and EMF were all detected in *Populus* roots in the inoculated treatment (Table 1). Roots in the inoculated + benomyl treatment showed almost no AMF or EMF colonization and roots in the uninoculated treatment were not colonized by AMF and EMF. Dark septate endophyte colonization was

Table 1. Percent of *Populus* root length colonized by fungal structures

Treatment	Total AMF	EM	DSE	Other fungi
Inoculated	57.0 (8.9)	13.5 (6.6)	7.4 (2.6)	8.0 (2.2)
Uninoculated	0	0	23.4 (2.6)	0
Inoculated + Benomyl	0.3 (0.2)	1.4 (1.4)	11.0 (3.6)	3.0 (0.9)

Values are means (1 SE).

found in all treatments, including the uninoculated control

Experiment 1

No significant difference in final standing height, length of longest stem, number of stems per seedling, or above-ground biomass was found between inoculated and uninoculated *Tamarix* seedlings (Figure 1 presents distributions of plant height and biomass by treatment). *Tamarix* mortality was low, with only one seedling dying in the control treatment and three dying in the inoculated treatment.

Experiment 2

Inoculation had a significant negative effect on plant size of *Tamarix* when grown with *Populus* (Figure 2). *Tamarix* above-ground biomass ($P < 0.001$) and height ($P = 0.0004$) were reduced in the inoculated treatment when compared to the uninoculated and inoculated + benomyl treatments. *Populus* above-ground biomass ($P = 0.0067$) and height ($P = 0.0094$) were greater in the inoculated + benomyl treatment when compared to the inoculated treatment, but neither of these treatments was significantly different from the uninoculated control (Figure 3). *Populus* seedlings experienced no mortality in the inoculated + benomyl treatment and the loss of three seedlings in each of the other two treatments. *Tamarix* mortality was much higher than that of *Populus* in all treatments and showed a trend toward the highest mortality in the inoculated treatment with almost 25% of these seedlings dying. Of the surviving *Tamarix* seedlings, there was no difference in average stem number per seedling between treatments (data not shown).

Patterns of species coexistence (van der Heijden et al., 2003) differed between treatments (Figure 4). *Populus* outperformed *Tamarix* in all

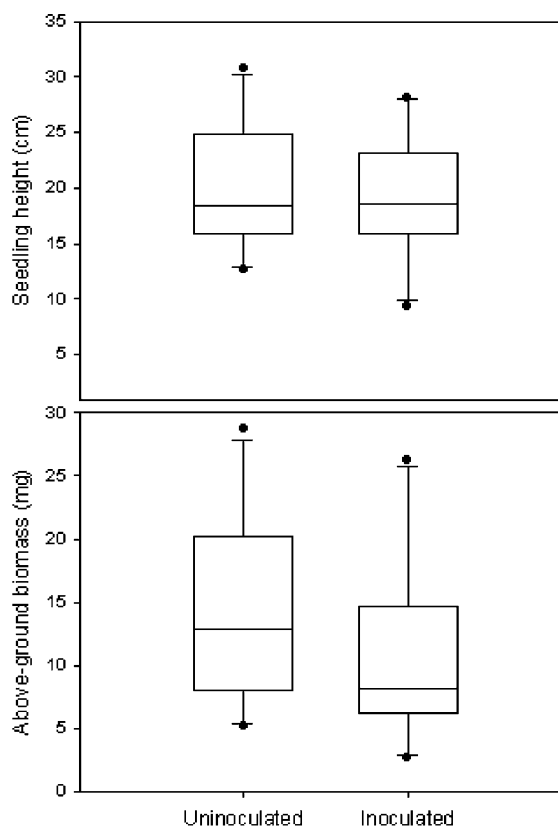

Figure 1. Box plots of *Tamarix* seedling height (top) and above-ground biomass (bottom) averaged by pot (n per pot varies due to mortality, maximum = 15) when grown in monoculture with and without fungal inoculum. n = 10 pots per treatment. In the box plots, the lower boundary of the box indicates the 25th percentile, the line within the box marks the median, and the upper boundary of the box indicates the 75th percentile. Whiskers (error bars) above and below the box indicate the 90th and 10th percentiles. Outliers (points outside the 90th and 10th percentiles) are also plotted.

treatments, but the contribution of *Tamarix* to total pot above-ground biomass and height was greatly reduced in the inoculated treatment where it made up only 5% of total pot above-ground biomass ($P = 0.0079$) and 11% of total pot height (summed heights of all plants)

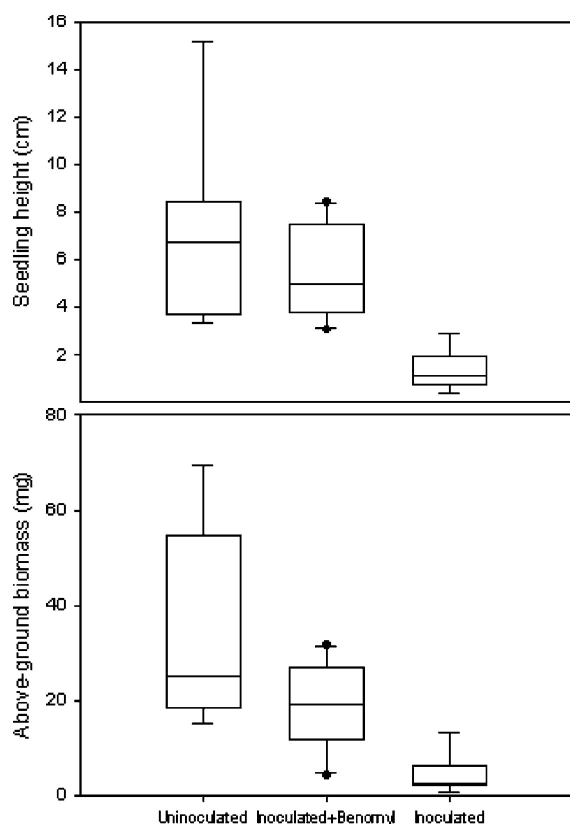

Figure 2. Box plots of *Tamarix* seedling height (top) and above-ground biomass (bottom) averaged by pot (n per pot varies due to mortality, maximum = 15) when grown in pots with *Populus* at three fungal inoculum levels. n = 10 pots per treatment. For an explanation of box plot structure, see Figure 1 legend.

($P = 0.0020$). The contribution of *Tamarix* to pot above-ground biomass was also low in the inoculated + benomyl treatment, but was not significantly different from the uninoculated control (10.7 ± 2.1% vs. 19.6 ± 3.6%). *Populus* and *Tamarix* have different height–weight relationships and in all treatments the proportional contribution of *Tamarix* measured by height was greater than when measured by above-ground biomass.

Discussion

The results of these experiments support the hypothesis that, at least in a greenhouse setting, competition between *Populus* and *Tamarix* is strongly influenced by mycorrhizal fungal inoculation. In a *Tamarix* monoculture, fungal inoculation had no direct effect on *Tamarix* biomass or height. When grown with *Populus*, *Tamarix* was the subordinate species in all treatments and *Tamarix* height and biomass were much lower in the inoculated treatment compared to the uninoculated and inoculated + benomyl treatments. The use of benomyl, which can affect plants directly or indirectly through changes in the soil fungal or bacterial community (West et al., 1993), did not have a significant effect on the plant species used in this experiment as growth of *Populus* and *Tamarix* in the inoculated + benomyl treatment was not significantly different from the control. The strong negative response of *Tamarix* to fungal inoculation when grown with *Populus* resulted in altered patterns of species coexistence, with *Tamarix* contributing the least to the community in the inoculated treatment. Studies by others investigating the

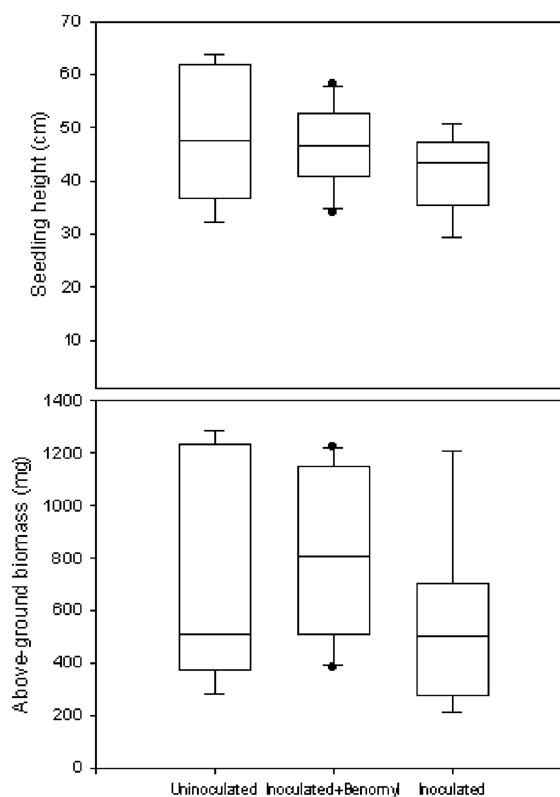

Figure 3. Box plots of *Populus* seedling height (top) and above-ground biomass (bottom) averaged by pot (n per pot = 3) when grown in pots with *Tamairx* at three fungal inoculum levels. n = 10 pots per treatment. For an explanation of box plot structure, see Figure 1 legend.

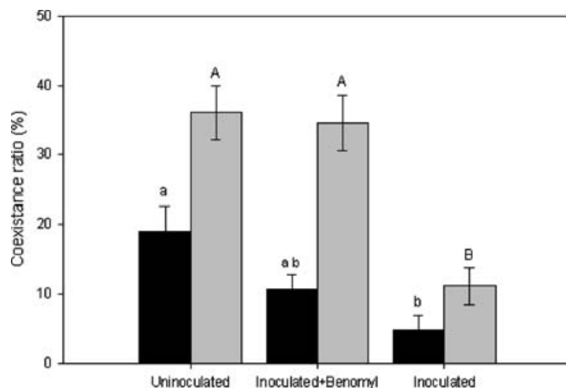

Figure 4. Tamarix coexistence ratios. Dark bars show the percent contribution of *Tamarix* to pot above-ground biomass and light bars show the percent contribution to pot seedling height (sum of heights for all seedlings) when grown in pots with *Populus* at three fungal inoculum levels . Error bars are one standard error. Treatment differences in coexistence ratio for height and above-ground biomass were analyzed separately and different letters indicate significant differences between means ($P < 0.05$).

effects of AMF on plant species competition and coexistence have also found that the presence or absence of AMF can alter competitive relationships between species (see Hart et al., 2003).

This experiment was interesting because while there were no direct effects of fungal inoculation on *Tamarix*, mycorrhizae increased the negative effect of *Populus* on *Tamarix*. Other studies showing indirect effects of fungal inoculation on plant coexistence have attributed this phenomenon to more efficient exploitation of the supply of soil phosphorus by the superior competitor, allowing it to either increase its biomass (Cavagnaro et al., 2004) or suppress the growth of other plant species in the community (Marler et al., 1999; Zabinski et al., 2002). Other mechanisms, such as increased nitrogen acquisition, inter-plant carbon transfer or protection from saprophytic fungi and other soil pathogens could operate in other systems. The mechanism behind inoculation-induced suppression of *Tamarix* by *Populus* remains to be studied. Some possible future research directions to clarify the mechanism involved in this indirect effect of inoculation would include additional greenhouse experiments to look at the influence of AMF, EMF and plant interspecific competition on tissue nutrient contents of *Populu*s and *Tamarix*.

Although *Populus* is widely reported to be mycorrhizal, the *P. fremontii* seedlings in this experiment did not have a positive survival or growth response to inoculation. We had expected to see a similar response to that found by van der Heijden (2001) in an experiment with *Salix repens* L. where cuttings experienced mortality rates of 50% in the control (uninoculated) treatment compared to only 10% mortality for the mycorrhizal plants. However, in our experiment *Populus* seedlings had mortality rates of only 10% in both the inoculated and uninoculated treatments. The difference in survival in uninoculated soil between these two species (*P. fremontii* and *S. repens*) may be the result of different degrees of mycorrhizal dependency due to the different ages of the material used (seedlings vs. cuttings) or the main reproduction strategies of these species (*P. fremontii* reproduces primarily from seed while *S. repens* is typically clonal).

Another finding of this study was that although *Tamarix* appears to be non-mycorrhizal, field collected and greenhouse grown *Tamarix* roots were heavily colonized with DSE. These fungi form melanized hyphae and microsclerotia (Jumpponen and Trappe, 1998) and thin, non-chitinous hyaline intracellular and intercellular hyphae that stain with sudan IV (Barrow and Aaltonen, 2001). The taxonomic affinity of this group is unclear although many appear to be ascomycetes anamorphic fungi including members of *Phialcephala* and *Phialophora* (Jumpponen and Trappe, 1998; Newsham, 1999). The ecology of DSE is poorly understood, but they do appear to have a significant ecological function in extreme, particularly arid and artic, habitats where AMF are less likely to occur (Barrow and Aaltonen, 2001; Barrow and Osuna, 2002; Haselwandter and Read, 1980; Kohn and Stasovski, 1990; Read and Haselwandter, 1981). Patterns of root colonization in arid plants have been shown to shift from AMF to DSE with increasing aridity, suggesting that DSE are better adapted to arid conditions than AMF (Barrow 2003; Barrow and Aaltonen, 2001). Future research is planned to ascertain the identity of DSE associated with *Tamarix* using isolation and molecular characterization and to better understand their ecological role, especially if they play a physiological role in the high drought tolerance of *Tamarix*.

Because *Tamarix* is non-mycotrophic, extensive and extended *Tamarix* occupation in southwestern riparian ecosystems (Busch and Smith, 1995; Christensen, 1962; Everitt, 1998; Graf, 1978, 1982) may decrease the inoculum potential of these areas. Field studies comparing inoculum potential, AMF richness or spore densities in different aged *Tamarix* stands would help clarify the effect of *Tamarix* on the mycorrhizal fungal community. Positive feedbacks between *Tamarix* and AMF, where interactions between *Tamarix* and AMF alter the soil fungal community in ways which benefit *Tamarix* to the detriment of other plant species (Bever, 2003), could result in degradation of the AMF community. Positive feedbacks may reinforce the existence of *Tamarix* once it has invaded a riparian area, as has been seen with other invasive species in other ecosystems (Bever, 2003; Goodwin, 1992; Vogelsang et al., In prep). Other riparian species, such as *Populus*, which form mycorrhizal associations (Jacobson, 2004), may be unable to thrive in areas dominated by *Tamarix* if fungal communities are degraded or absent.

Manual restoration efforts used to remove *Tamarix* and establish *Populus* in riparian zones have been somewhat successful at restoring native riparian habitat (Sprenger et al., 2001, 2002; Taylor and McDaniel, 1998; Taylor et al., 1999); however, the legacy of the biological effects of *Tamarix* on mycorrhizal fungal communities may still remain. Additionally, the extensive soil disturbance involved in restoration may destroy any existing hyphal networks in the soil and decrease fungal inoculum potential and the establishment success of cuttings or pole plantings of *Populus* (Barni and Siniscalco, 2000; Evans and Miller, 1990; Read and Birch, 1988). Inoculation of restoration sites with AMF has been beneficial in restoration projects in other environments (Allen et al., 2003; Cuenca et al., 1998; Richter and Stutz, 2002) and may increase the competitive advantage of *Populus* over *Tamarix* in newly restored riparian areas. Field trials using AMF inoculum in riparian restoration experiments would help determine if the interactions between *Populus* and *Tamarix* seen in this greenhouse experiment can be repeated under field conditions.

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