The Role of Endophytic Fungi in the Survival and Establishment of Fourwing Saltbush *(Atriplex canescens* [Pursh] Nutt.) in an Arid Environment

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Abstract—A seedborne septate fungus *(Aspergillus sp.)* formed intimate non-destructive interfaces with seedling radicles of germinating fourwing saltbush, *(Atriplex canescens* [Pursh] Nutt.) seedlings. When seedlings were separated from insoluble phosphorus sources with a screen that excluded roots but allowed passage of extraradicle hyphae, which accessed plant insoluble phosphate and transported it through the barrier to the plant. The fungi enhanced phosphorus uptake, biomass production of the host plants and aggregated sand similar to functions attributed to mycorrhizal fungi. The importance of symbiotic fungi in the remediation and stabilization of arid ecosystems is discussed.

In the last century, shrubs have invaded native grasslands in the northern Chihuahuan Desert, resulting in severe disturbances in the plant and soil structure and productivity. Little is known about how these disturbances have affected the structure of soil and below-ground soil microflora populations.

Mycorrhizal fungi are directly involved in the nutrition and survival of host plants in all major ecosystems (Bethlenfalvay 1992). It is expected that mycorrhizal fungi have a major role in plant nutrition and survival in resource-stressed arid ecosystems. Classical mycorrhizal fungi non-destructively colonize roots of host plants and form interfaces between and within cortical cells for the exchange of photosynthetic carbon, mineral nutrients, and water. The fungi also extend from the root surface into the soil increasing access and absorption of nutrients and water. They indiscriminately colonize roots of widely different species, forming an underground network that potentially allows for the exchange of photosynthetic carbon, water, mineral nutrients between plants, and soil microflora (Bethlenfalvay 1992; Read 1997; Simmard and others 1997).

Barrow and others (1997a) found that in addition to classical mycorrhizal fungi, the roots of fourwing saltbush (*Atriplex canescens* [Pursh] Nutt.), and other dominant shrubs and grasses of the arid Southwestern United States are constantly and more extensively colonized by non-pathogenic septate fungi. Similary, septate fungi also non-destructively colonize the emerging radicles of fourwing saltbush at germination and enhance seedling growth and establishment by transferring nutrients in the seed capsule to the seedling (Barrow and others 1997b).

The objective of our studies is to determine if these septate fungi function similar to mycorrhizal fungi in arid ecosystems. Our hypothesis was that these fungi access immobile nutrients and transport them to the host, and that they function like mycorrhizae in arid environments.

Materials and Methods

The hard fibrous utricle and internal embryonic tissues of fourwing saltbush are naturally and consistently colonized by septate fungi that are not eliminated by standard seed sterilization or heat treatments. Because inoculated and sterile plants could not be compared, plant containers (fig. 1) were constructed with a 20 μm screen to exclude roots but allow penetration of endophytic root fungi into the lower chamber containing phosphorus sources insoluble to plants but not by fungi. Standard 7.62 cm diameter schedule 40 PVC tubing was cut into 7.62 cm lengths. A 2 mm mesh vinyl

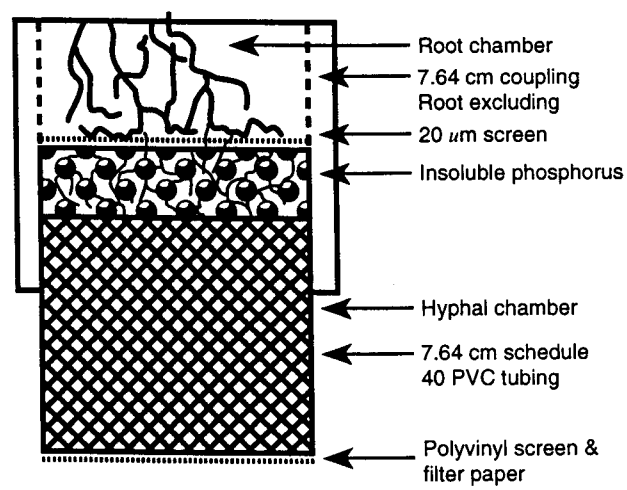

Figure 1—Root exclusion chamber

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screen was glued to the bottom of each piece, and filter paper was cut the same diameter and inserted above the screen to retain sand and allow drainage. A commercial grade of silica blasting sand was acid washed to remove all P. The sand was soaked for 30 minutes in 0.1 N HCl and copiously rinsed with distilled water before drying at 110 °C for 72 hours.

Four treatments were established: (1) zero P (0P), with no plant available P, (2) 30 ppm soluble P (SP), adequate for normal plant growth, (3) rock phosphate (RP), and (4) tricalcium phosphate (TCP). For treatments OP and SP, the above and below tube compartments were filled with acid-washed sand. For Treatments RP and TCP, the lower tube compartments were filled with acid-washed sand to 1 cm from the screen. The last cm was filled with a mixture of sand and insoluble P consisting of 1 part RP or TCP to two parts acid-washed sand. The 20 *u*m root excluding screen was glued to the top of each tube interfacing firmly with the sand beneath the screen. A standard 7.62 cm coupling was lubricated with a light petroleum jelly and placed on the tube to secure the screen in place. Acid-washed silica sand was then added to the open end of the coupling providing a plant root chamber above the screen (7.62 cm d x 4 cm h).

Seeds of fourwing saltbush were germinated on the surface of moist silica sand. Three germinants with radicles approximately 1 cm long, naturally colonized by septate fungi, were transplanted to each tube. Tubes were randomly placed in a growth chamber at 25 °C with a 12 hour light, 12 hour dark regime. Each treatment had 16 replications, 12 of which were used for root and shoot biomass and P tissue analysis at the end of the experiment. Remaining replications were used for microscopic analysis of roots and subchamber fungal colonization.

Plant containers were saturated just prior to wilting with their respective nutrient solution, which drained quickly. This interval gave plants adequate water for growth and kept tubes as dry as possible. Treatment 0P, RP, and TCP were all watered with standard Hoagland's solution with all essential nutrients except P. The SP treatment plants were watered with Hoagland's solution containing 30 ppm of plant soluble P, not limiting to plant growth. Any P utilized by seedlings in RP and TCP treatments were obtained from the insoluble P source. Distilled water was used every third watering to prevent salt accumulation. Nutrient solutions were adjusted to pH 5.5 to prevent solubilization of P from the RP and TCP treatments.

After 12 weeks, plants were carefully removed from the upper root chamber by submerging and rinsing in water to remove sand. Shoots and roots were separated, dried and weighed. Dried shoots and roots were finely ground, digested, and mg of phosphorus per gm of dry plant tissue was determined with a Jobin Yvon JY Plus Inductively Coupled Plasma Spectrophotometer.

After natural drying of the containers, feeder roots from the root chambers were harvested from additional replications, cleared and stained using the method of Brundrett and others (1984), and analyzed microscopically. One tube from the TCP treatment was infiltrated with a low viscosity resin, hardened, and cut into petrographic thin sections to observe the intact three dimensional structure of roots, screen, fungi, and sand.

Data were analyzed as a completely randomized design with phosphorus treatment as the main effect. The dependent variables were shoot, root, and plant biomass and P tissue concentrations. Mean differences were tested using a protected LSD (P 0.01).

Results

Fungi internally colonized the cortex cells of healthy roots, and considerable extraradical hyphae intimately associated with the root surface readily penetrated the 20 *u*m screen and extended into the lower chamber in all treatments. No roots penetrated the screen. When plants were approximately 25 to 35 cm tall, those treated with SP required watering every 3 days to prevent wilting while equal sized plants in both insoluble P treatments would wilt after 5 days without watering.

Shoot and root biomass of fourwing seedlings in both insoluble P treatments were equivalent to plants receiving soluble P and were greater ($P < 0.01$) than control plants receiving no P (table 1). Shoot biomass of plants grown with insoluble RP and TCP was equal to those supplemented with SP, yet P content was one-third less in these tissues compared to shoots of SP-treated plants. Root biomass of seedlings supplemented with RP or TCP was greater ($P < 0.01$) than those watered with either SP or no 0P. The root/shoot ratio was greatest ($P < 0.01$) in seedlings in the 0P treatment, intermediate for the insoluble RP and TCP treatments, and least with SP.

Screen sections approximately 2 cm in diameter were carefully cut and observed under a stereo microscope. Substantial quantities of hyphae were observed enmeshing sand particles in the region 5 mm below the screen in the RP and TCP treatments. Petrographic thin sections made from a container with TCP revealed the intact structure of the roots, screen, and the hyphal interface with the TCP-sand mixture. An airspace 1 to 3 mm thick separated the P-sand mix from the screen and the roots due to settling of sand during the experiment. An amorphous white material observed accumulating on hyphal surfaces increased fungal hyphal diameters from less than 10 to more than 200 *u*m. These hyphae formed a fibrous network similar in structure and dimensions to plant roots, which also physically aggregated a layer of the TCP-sand mixture approximately 5 mm thick. Additional settling of the sand below this aggregated layer resulted in another airspace below the aggregate, illustrating its cohesive nature. The white material on the hyphal surface did not effervesce in cold dilute HCl, suggesting it was not $CaCO_3$. We suspect this material to be extracellular polysaccharide secretion by the fungus. An isolate of the fungus was tentatively identified as *Asprilligus sp.* by Dr. Gary Samuels of the USDA-ARS Systematic Botany and Mycology Laboratory, Beltsville, MD. This isolate developed a typical endophytic, septate association with alfalfa root organ cultures.

Discussion

Fungal associations of this type have been classed as dark septate (DS) fungi, an inconclusive term because internal hyphae are often hyaline (Haselwandter and Read 1980; Odell and others 1993; Treu and others 1996). They have also been considered a part of the *mycelium radicis atrovirons*

Table 1—Mean dry weights in mg and phosphorus content of shoots and roots of fourwing saltbush grown on four different phosphorus treatments.

P treatment	Root		Shoot		Plant		Root/shoot ratio
	Dry weight	Wt P	Dry weight	Wt P	Dry weight	Wt P	
	mg		*mg*		*mg*		
Zero phosphorus	103.3c[a]	0.004b	202.3b	0.028c	305.6c	0.032d	0.820a
30 ppm soluble P	178.4b	0.385a	496.8a	2.744a	675.1b	3.129a	0.360b
Rock phosphate	263.7a	0.121b	500.3a	0.859b	764.0a	0.980c	0.622ab
Tricalcium phosphate	292.1a	0.512a	502.3a	0.967b	794.4a	1.479b	0.598ab

[a]Means within a column followed by different letters are significant at P <0.01.

(MRA) complex (Wang and Wilcox 1985). Generally the these fungi form extensive symptomless inter- and intracellular hyphal networks and microsclerotia within plant roots.

These data showed that a septate seedborne fungus (*Aspergillus sp.*) enhanced P uptake and biomass production of fourwing saltbush seedlings. Fungi intimately colonized seedling radicles, forming interfaces similar to those observed in the roots of mature grasses and other dominant shrubs harvested from native sites (Barrow and others 1997a).

These findings are also consistent with those of Haselwandter and Read (1980, 1982) who found that species of the Cyperaceae family, *Carex firma* and *C. sempervirens*, were more extensively colonized with dark septate fungi than by vesicular arbuscular mycorrhizal (VAM) fungi. They also observed that associated isolates ascribed to the genus *Rhizoctonia* differed morphologically from established mycorrhizal classes and increased yield and shoot phosphorus concentrations, typical of responses of host plants inoculated with VAM. They concluded these fungi were mycorrhizal. Sengupta and others (1989) also induced growth responses in *Cajanas cajan* with two dark septate isolates that accessed P from insoluble tricalcium phosphate under salinity stress and likewise suggested a mycorrhizal function for these isolates.

Mycorrhizal fungi also modify the size, shape, and anatomy of plant roots (Berta and others 1993) and alter photosynthetic activity of their hosts (Allen and Boosalis 1983). In this study, all seedlings in all treatments were colonized. The increase of plant and root biomass, soil aggregation, and the solubilization and transport of P would suggest increased photosynthetic rates when the plant fungal component was challenged by different phosphorus sources. The observed bridging of roots with the insoluble P source by the fungus provided a physical pathway for P to be transported to the roots via fungal hyphae, similar to observations of mycorrhizal fungi.

Equivalent shoot biomass production and greater root biomass for the insoluble P treatments compared to the SP treatment (P <0.01) (table 1) with only one-third of the phosphorus uptake suggests that the fungus not only solubilized P, but increased P use efficiency. This is consistent with the report of Brown and others (1988), who found that P use efficiency was significantly greater in VAM-inoculated plants compared to non-inoculated plants supplemented with high P. *Asprigillus niger* and other soil microbes have previously been shown to effectively solubilize P from rock phosphate (Azcon and others 1976; Khan and Bhatnagar 1977; Khasawneh and Doll 1978). In this study, an *Aspergillus sp.* fungus not only solubilized rock phosphate, but similar to mycorrhizal fungi, formed an intimate interface with root cells and the insoluble P source.

Another similarity to mycorrhizal fungi and a potentially important ecological function of these fungi is that they aggregate and stabilize sand and soil particles, enhancing nutrient and water retention (Bethlenfalvay 1992). An increase of fungal and root biomass and aggregation of sand in the chambers could explain the reduced need for watering observed in the insoluble P treatments. We agree with Trappe (1989) and Treu and others (1996) that septate fungi that do not currently fit into accepted mycorrhizal classification systems are ecologically significant and are composed of a wide assembledge of fungal species. Several of these fungi isolated from the roots of native plants formed similar nondestructive interfaces with the root cortex cells of native plants and are tentatively identified as common soil fungi (Barrow, unpublished). Their known function as pathogens or as soil saprobes may be a primary reason why their ecological significance or potential as symbiotic fungi has been overlooked. The consistent root colonization of desert plants by both VAM and septate fungi suggests that they comprise a flexible suite of organisms to meet a variety of challenges imposed by highly variable arid environments, including the enhancement of nutrient uptake and plant survival. Each fungal component may be specifically adapted to function under a specific environmental condition. Ecological roles of these fungi are varied and require innovative research approaches and measurements to understand their ecologically significant and complex multifunctional roles.

We propose that the ecological significance of these fungi as well a other mycorrhizal fungi found in arid ecosystems is to provide an underground network that allows the conservation, distribution, and transport of carbon, mineral nutrients, and water in chronically dry soils that cannot be achieved by diffusion or by mass flow. The structural integrity of this below-ground network is essential for the nutrition and survival of plant communities. If this structure is damaged by disturbances to the system, it would need to be repaired before plant establishment could be successful.

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