

**Arbuscular Mycorrhizal Colonization of *Larrea tridentata* and *Ambrosia dumosa* Roots
Varies with Precipitation and Season in the Mojave Desert**

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Abstract

The percentage of fine roots colonized by arbuscular mycorrhizal (AM) fungi varied with season and with species in the co-dominant shrubs *Larrea tridentata* and *Ambrosia dumosa* at a site adjacent to the Nevada Desert FACE (Free-Air CO₂ Enrichment) Facility (NDFF) in the Mojave Desert. We excavated downward and outward from the shrub bases in both species to collect and examine fine roots (< 1.0 mm diameter) at monthly intervals throughout 2001 and from October 2002 to September 2003. Fungal structures became visible in cleared roots stained with trypan blue. We quantified the percent colonization of roots by AM fungi via the line intercept method. In both years and for both species, colonization was highest in fall, relatively low in spring when root growth began, increased in late spring, and decreased during summer drought periods. Increases in colonization during summer and fall reflect corresponding increases in precipitation. Spring mycorrhizal colonization is low despite peaks in soil water availability and precipitation, indicating that precipitation is not the only factor influencing mycorrhizal colonization. Because the spring decrease in mycorrhizal colonization occurs when these shrubs initiate a major flush of fine root growth, other phenological events such as competing demands for carbon by fine root initiation, early season shoot growth, and flowering may reduce carbon availability to the fungus, and hence decrease colonization. Another possibility is that root growth exceeds the rate of mycorrhizal colonization.

Keywords: Arbuscular mycorrhizae, Mojave Desert, creosote bush, AM fungi, root colonization

Running head: Mycorrhizal colonization in the Mojave Desert

Introduction

Arbuscular mycorrhizae (AM) is an important phenomenon in the Mojave Desert of the southwestern United States, where establishment of mycorrhizal symbiosis plays a vital role in increasing the likelihood of survival in this harsh environment (Stutz et al. 2000). The hyphal filaments of mycorrhizal fungi effectively increase the surface area of a root system, thereby increasing water and nutrient uptake in the desert, where water and nutrients are often scarce (Mather and Vyas 2000). Titus et al. (2002a) found that the quantity of hyphae varied between the spring and fall and that mycorrhizae were more prevalent in perennial than in annual plant species in the Mojave Desert.

Desertification is increasing globally (Dregne and Chou 1992) and results in a net loss of carbon sequestration and an increase in soil erosion. Disturbance of natural plant communities is the first visible indicator of this process, which occurs simultaneously with soil degradation (Requena et al. 2001). The presence of mycorrhizal symbionts increases the likelihood of survival by plants and thus is an important factor in stabilizing plants and soils in areas susceptible to desertification (Honrubia et al. 2003). The establishment of key plant species, hydrostable soil aggregates, organic matter, and soil nitrogen content all increased when arbuscular mycorrhizal fungi and rhizobial nitrogen-fixing bacteria were added to a desertified Mediterranean ecosystem (Requena et al. 2001).

Naturally occurring mycorrhizal symbioses in the Mojave Desert help sustain desert vegetation, maintain soil structure, and move nutrients. The *Larrea tridentata-Ambrosia dumosa* community on Mojave Desert soils has heterogenous nutrient distribution with higher nutrient levels in soils around perennial shrubs (Titus et al. 2002b). These higher nutrient levels may be perpetuated by mycorrhizae because mycorrhizae have the potential to influence nutrient distribution by acting as a conduit between soil and roots. The presence of spores, albeit at low levels (<0.2 spores g⁻¹ soil), suggests that mycorrhizae are self-perpetuating in the Mojave Desert and that no artificial inoculation is necessary.

Although others have observed that the extent of mycorrhizal colonization varies with species and with season (Sanders and Fitter 1992, Mullen and Schmidt 1993, Klironomos 2003), we investigated variations in the extent of colonization in terms of both seasons and species in order to relate patterns of mycorrhizal abundance with environmental factors such as precipitation and to explore the dynamics of mycorrhizal colonization in the context of the desert environment. Specifically, we quantified the percent of arbuscular mycorrhizal colonization of fine roots (<1 mm diameter) for *Larrea tridentata* and *Ambrosia dumosa* over the course of two years in the Mojave Desert of Nevada, where these two shrubs are co-dominant at a site adjacent to the Nevada Desert FACE (Free Air Carbon Enrichment) Facility (NDFF). We asked the following questions: 1) Does arbuscular mycorrhizal colonization vary with season? 2) Does arbuscular mycorrhizal colonization increase with precipitation? and 3) Does the extent of arbuscular mycorrhizal colonization vary between *Larrea tridentata* and *Ambrosia dumosa*?

Materials and Methods

Study Site Location: The field study site was situated on an alluvial fan at an elevation of 970 m in an undisturbed Mojave Desert ecosystem, where the co-dominant shrubs in the vegetation are *Larrea tridentata* (DC.) Cov., an evergreen shrub that grows over 1 m in height, and the smaller but abundant *Ambrosia dumosa* (A. Gray) Payne. Precipitation averages ~135 mm annually, and temperatures range from winter minimum of -10 C and a summer maximum of 48 C. This site

was approximately 100 m from the Nevada Desert FACE Facility (NDFF) on the Nevada Test Site (Jordan et al., 1999).

Samples: Fifty-four randomly selected shrubs per species had nine plants randomly assigned to one of six groups. Roots were collected from one group each month from January 2001 – December 2001 and from October 2002 – November 2003 by excavating outward 25 cm from the bases of *L. tridentata* and 12.5 cm from the bases of *A. dumosa* (generally the smaller species), to a depth of 10 – 15 cm for a soil/root sample volume of approximately 750 cc, a sample size that almost always contained fine roots (< 1 mm diameter). Only 6 of the 432 samples did not contain fine roots.

Roots were separated from their surrounding soil via root elutriation and cleared for 4-6 h in 10% KOH. Fungal structures were stained with 0.01% Trypan Blue (Brundrett et al. 1996). Approximately five 1-4 cm long segments of fine roots (< 1 mm diameter) were mounted on 2 mm gridded slides (Electron Microscopy Supply, Inc., Ft. Washington, PA). Percent mycorrhizal colonization was quantified via the line intercept method (Tenant 1975) with a compound light microscope (American Optical Co., New Haven, CT) at 100X magnification.

Statistical Analyses: Data were analyzed using Proc Mixed in SAS (SAS Institute, Inc., Cary, NC). A repeated measures ANOVA experimental design was used, with year and species (*Larrea* or *Ambrosia*) as fixed factors and month as the repeated measures factor. In order to normalize the data, we had to remove 2 *Ambrosia* data points from the total of 426 data points that appeared to be statistical outliers and use a square root transformation. $P<0.05$ was considered significant, but we also indicate results where $0.05<P<0.10$.

Results

Arbuscular mycorrhizal colonization varied both in extent and in seasonality for *Larrea* and *Ambrosia*, two co-dominant shrubs of the Mojave Desert (Fig. 1A, B). These variations occurred in 2001 and again in 2002-03, two years with different seasonal patterns of precipitation. During the first year, a number of precipitation events occurred from January through mid-April 2001 (Fig. 1E), and only small precipitation events occurred after mid-April until a 16 mm event in late-October 2001. Thus in 2000-2001, most of the precipitation (Fig. 1G) occurred before or during the early portion of the time period when these two species typically grow roots (Figs. 1C, D). During the second year, the first major precipitation event occurred about a month later than in 2000-2001 (Fig. 1F), and precipitation continued to accumulate into May 2003 (Fig. 1H), which is near the middle of the time period that these species typically produce new roots (Figs. 1C, D). By mid-May, total cumulative precipitation in 2002-2003 was approximately 10% greater than in 2000-2001. In addition, two large precipitation events occurred in summer 2003.

Averaged over all months, mean AM colonization of *Ambrosia* (16%) was significantly greater than that of *Larrea* (12%) (Table 1). However, this difference was significant only in certain parts of the growing season, as indicated by the significant interaction between species and month (Table 1, Figs. 1A, B). The difference between species was significant at $P<0.05$ in the middle of the growing season (May) and in autumn (September and October) and at $0.05<P<0.010$ in winter (January $P=0.08$ and February $P=0.06$).

In 2000-2001, both species had low levels of colonization during April (Fig. 1A), which corresponds to the beginning of the period of new root growth for these two species (Figs. 1C,

D). Mycorrhizal colonization increased during the rest of the growing season for both species, followed by a small decline during the summer drought period. Mycorrhizal colonization increased during August for both species in response to several small rain events and was highest in November, following the large October precipitation event. In 2002-03 as in 2000-2001, the highest levels of colonization were in autumn and lowest in spring, and colonization gradually increased during the growing season and was followed by a decline during the summer drought. Colonization increased for both species following the summer precipitation in 2003. Although these general seasonal patterns were similar between years, the exact timing of increases, decreases, and peaks in colonization differed between the two years, as indicated by the significant Year X Month interaction term (Table 1). Averaged across both species, colonization in early spring (February and March), late summer (August and September) and late fall (November and December) of 2000-2001 was significantly greater than that of 2002-2003, whereas 2002-2003 was significantly greater than 2000-2001 only in the middle of the growing season (May).

Discussion

Arbuscular mycorrhizal colonization had significant variation with season and with species in *L. tridentata* and *A. dumosa* in the Mojave Desert. These results are in accord with those of Titus et al. (2002a), who found that perennial plant species of the Mojave Desert were colonized by arbuscular mycorrhizal fungi, and that the proportions of hyphae, arbuscules, and vesicles changed between spring and autumn in the Mojave Desert. Seasonal and specific change in AM colonization is not limited to the desert and occurs in other ecosystems. For example, Sanders and Fitter (1992) found seasonal and species changes in an English grassland.

Although the seasonal patterns of AM colonization in *L. tridentata* and *A. dumosa* were similar, they were not identical for both shrub species. Both species had their highest percent AM colonization in autumn, had low colonization rates when root growth began in the spring, and declines during summer drought periods. Roots of both species also had increased colonization after summer rain events, but only *Ambrosia* maintained high colonization rates through late-summer and early fall in 2001.

These seasonal changes in AM colonization were likely influenced in part by environmental factors such as rainfall as well as by the timing of root growth. For example, the rainfall events in summer of both years may have contributed to the subsequent spike in colonization during August. Similarly, autumn peaks in colonization followed large rain events for both years. Because of the time interval between precipitation events and the root sampling dates, we could not determine whether a spike in colonization occurred immediately after precipitation or whether there was a delay of several weeks. Although fungi may have responded to the rainfall quickly, their increased presence would not have been detected until the next sampling date after rainfall. During spring, however, colonization declines with the onset of root growth in both species, despite the greater abundance of precipitation and peaks in soil water availability (Nowak et al. 2004). We suspect that soil temperature does not limit AM growth during the spring, as daily mean soil temperatures at 10 cm soil depth increase from ~13 C in March to ~30 C in June (unpublished data). Thus, other factors, such as allocation of carbon within plants that is tied to phenological events associated with a major flush of root growth, initiation of shoot growth, and flowering, may reduce carbon availability to mycorrhizae

(Gianinazzi 1991; Brundrett 2002), or the rate of root growth simply may exceed the rate of AM colonization (Bruce et al. 1994).

Mojave Desert arbuscular mycorrhizal spore densities were generally low (0-0.2 spores g⁻¹ soil, Titus et al. 2002b). AM colonization existed continuously throughout our study, albeit sometimes at low levels. Therefore, a sufficient quantity of spores must have been in place in order to initiate the establishment of arbuscular mycorrhizae once environmental conditions proved favorable, if we assume that the spores were colonizing new fine roots produced after precipitation or following precipitation in combination with other favorable environmental events such as warming of the soil in spring. Oak seedlings inoculated with ectomycorrhizal fungus showed a 4 – 12% colonization rate at five months post-inoculation (Tateishi et al. 2003). This 4 – 12% rate is similar to what was found in *Larrea* and *Ambrosia* and suggests that even relatively low levels of spores in the soil are sufficient in quantity to generate mycorrhizal colonization at a rate similar to that found in inoculated plants in controlled settings.

These findings on arbuscular mycorrhizal colonization in Mojave Desert shrubs will be useful in future investigations of the under-explored topic of mycorrhizal dynamics in desert ecosystems. Knowledge of changes in magnitude and seasonality of mycorrhizal associations can be linked with investigations of the physiology of the initiation of mycorrhizal colonization with respect to such seasonal environmental factors as precipitation and soil moisture. Seasonal changes in host plant physiology and phenology such as fine root initiation, early season growth, and flowering are important factors in mycorrhizal symbioses in the desert. Lastly, an understanding of the mycorrhizal dynamics in desert ecosystems will be useful in tracking the movement of carbon from the host plants to the rhizosphere and outward throughout the ecosystem.

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Table 1. Results from the repeated measures ANOVA of root colonization by arbuscular mycorrhizae for *Larrea tridentata* and *Ambrosia dumosa*. In order to normalize the data, two statistical outliers, the highest value and one of the zero values for *A. dumosa*, were removed from the total data set of 424 data points, and data were transformed with a square root transformation.

Factor	Numerator		Denominator		
	d.f.		d.f.	F value	P value
Species	1		284	22.70	<0.001
Year	1		284	42.81	<0.001
Species X Year	1		284	0.05	0.820
Month	11		97	11.82	<0.001
Species X Month	11		284	1.91	0.038
Year X Month	11		284	8.26	<0.001
Species X Year X Month	11		284	1.21	0.277

Fig. 1. A, B. Percent mycorrhizal colonization of *Ambrosia dumosa* (circles) and *Larrea tridentata* (inverted triangles), averaged across January – December, 2001 (A) and October 2002 – September 2003 (B). Asterisks (*) indicate significantly ($P < 0.05$) greater percent colonization in *Ambrosia* than in *Larrea* based on the Species X Month interaction term; pound signs (#) indicate $0.05 < P < 0.10$. Error bars are standard errors. **C, D.** Typical period of root growth for *Larrea* and *Ambrosia*, based on minirhizotron observations near the area (unpublished data, Wilcox et al. 2004). **E, F, G, H.** Individual precipitation events during October 2000 – December 2001 (E) and October 2002 – December 2003 (F) and cumulative precipitation over those same time periods (G and H, respectively).

