Arbuscular Mycorrhizae and Water Stress Tolerance of Wyoming Big Sagebrush Seedlings

Peter D. Stahl,* Gerald E. Schuman, Sandra M. Frost, and Stephen E. Williams

ABSTRACT

Although Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *wyomingensis* Beetle and Young) is widespread in the western USA, reestablishment of this native shrub on disturbed lands by direct seeding is problematic. A number of theories have been proposed to explain this difficulty. Included are the hypotheses that seedlings are unable to obtain adequate moisture and are handicapped by reduced levels of mycorrhizae in perturbed soils. We conducted a greenhouse study to examine the influence of vesicular arbuscular mycorrhizae (VAM) and seedling age on soil moisture stress tolerance of Wyoming big sagebrush seedlings. Results demonstrated greater survival of mycorrhizal seedlings than nonmycorrhizal seedlings as soil dried down past soil water potential values of −2.5 MPa to as dry as −3.8 MPa. For all different aged seedlings tested (30, 45, 60, 90, 120, 150 d), the degree of soil dryness resulting in death of mycorrhizal seedlings was significantly greater ($P < 0.01$) than that causing death of nonmycorrhizal seedlings. Analysis of variance indicated a significant interaction of seedling age and mycorrhizae on moisture stress tolerance. Experimental data suggest that as sagebrush seedlings age, the beneficial influence of arbuscular mycorrhizae on soil water stress tolerance increases.

THE IMPORTANCE OF SHRUBS, including Wyoming big sagebrush, in arid and semiarid environments is well documented (McKell, 1975). On millions of hectares of western North America, shrubs are essential to ecosystem function and soil stability (McArthur and Welch, 1985). Shrubs are also important as wildlife habitat and serve as preferred feed for many types of domestic and wild animals (Roundy et al., 1995). It seems obvious that to restore productivity and stability to disturbed shrublands, shrub reestablishment is critical.

Although big sagebrush is one of the most common and widespread shrubs in the western USA, reestablishment of this species on disturbed lands by direct seeding has proven difficult for a number of reasons (Cockrell et al., 1995). This poses important concerns in Wyoming, where restoration of sagebrush is required by law.

A number of hypotheses have been proposed to explain the difficulty in successfully establishing sagebrush from seed on disturbed sites. These include the idea that reduced levels of arbuscular mycorrhizae on roots of sagebrush seedlings in perturbed soils decrease their ability to survive stressful environmental conditions (Call and McKell, 1982; Stahl et al., 1988). This hypothesis is based on the fact that arbuscular mycorrhizae can improve a host plant's ability to extract nutrients and water from the soil and observations that sagebrush appears to be particularly dependent on mycorrhizal symbiosis to reach full growth potential (Allen, 1984).

Indirect evidence indicates that soil water availability is one of the most critical factors involved in big sagebrush seedling establishment (Jones, 1991). On both local and regional levels, sagebrush distribution has been shown to be related to soil water availability (Burke et al., 1989; West, 1979). Because water availability is a key factor in sagebrush establishment, mortality of sagebrush seedlings is high; however, once established, mortality among adult plants is low (Daubenmire, 1974: Cawker, 1980). In addition to mycorrhizae, adult sagebrush plants employ various physiological and morphological mechanisms to deal with moisture stress (DePuit and Caldwell, 1973; Romo, 1984; Campbell and Harris, 1977) that are not developed in seedlings. Formation of arbuscular mycorrhizal symbiosis may enable sagebrush seedlings to obtain more moisture from soil than nonmycorrhizal seedlings and may play an important role in seedling establishment.

The objectives of this study were to: (i) determine if mycorrhizal sagebrush seedlings are more tolerant of soil moisture stress than nonmycorrhizal sagebrush seedlings and (ii) determine if there is an interaction between seedling age and mycorrhizae on soil moisture stress tolerance.

METHODS

A greenhouse experiment was conducted to test two null hypotheses: (i) mycorrhizal sagebrush seedlings are equally tolerant of soil moisture stress as are nonmycorrhizal sagebrush seedlings, and (ii) there is no interaction between seedling age and mycorrhizae on soil moisture stress tolerance in big sagebrush.

The soil used in this study, classified as a Ustic Torriorthent, was collected from an undisturbed sagebrush–grassland site on the North Antelope Coal Mine in the Powder River Basin of northeastern Wyoming. Selected physiochemical characteristics of this soil are given in Table 1.

All soil was passed through a 1.0-cm sieve before use. Arbuscular mycorrhizal fungi were eliminated from soil for the nonmycorrhizal treatment by pasteurizing at 115°C for 4 h. The pasteurized soil was subsequently treated with a finely sieved (24 μm) water extract of unautoclaved soil to restore indigenous soil microorganisms other than arbuscular mycorrhizal fungi. The mycorrhizal treatment utilized fresh untreated soil. Each soil treatment (−VAM and +VAM) was used to fill 108 15-cm pots with about 1.4 L of soil. Approximately 10 sagebrush seeds were placed on the surface of each pot. After germination and emergence, sagebrush seedlings were thinned to two per pot. All seedlings were planted on the same date.

To test these hypotheses, different aged mycorrhizal and

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Abbreviations: ANOVA, analysis of variance; VAM, vesicular arbuscular mycorrhizae.

Table 1. Physical and chemical characteristics of soil used in the experiment.

pH	Electrical conductivity	Cation-exchange capacity	Texture	Soluble cations				Kjeldahl N	Inorganic P
				Mg	Ca	Na	K		
	$\mathrm{dS\ m^{-1}}$	$\mathrm{cmol_c\ kg^{-1}}$		$\mathrm{mg\ kg^{-1}}$				$\mathrm{mg\ kg^{-1}}$	
7.2	2.3	15.7	sandy clay loam	47	449	10	20	362	2.6

nonmycorrhizal sagebrush seedlings were subjected to increasing levels of soil moisture stress by discontinuing water application to the pots. Moisture stress evaluations were conducted on 30, 45, 60, 90, 120, and 150-d-old seedlings. At each sampling interval, 18 pots (each with two sagebrush seedlings) of both soil treatments were subjected to the moisture stress evaluation. Pots were allowed to dry until the seedlings died (seedlings were subjected to water stress one time only) and were then harvested immediately to quantify the amount of mycorrhizae on root systems.

Soil moisture status of all 36 pots subjected to moisture stress at each sampling interval was monitored daily. Soil water potential was estimated gravimetrically based on a soil moisture retention curve established for the soil used in this study (Fig. 1). Pots and soil were weighed and soil moisture was determined using the initial weight of the pot and dry soil. The soil moisture retention curve was generated by the method of Klute (1986). Soil water potential was then estimated by relating the soil moisture percentage to the moisture retention curve using the following regression formula:

$$\text{soil water potential} = 24.2 - 1.7(\text{soil moisture content, \%}) \qquad [1]$$

Dead sagebrush seedlings were harvested by first soaking pots and soil in water until they were completely saturated. Pots were then removed from the water so the excess moisture could drain. Dead sagebrush seedlings were then carefully excavated from the soil using a gentle stream of water. After harvest, roots were prepared for examination of mycorrhizal status by washing with distilled water and clearing in warm (≈60°C) 10% KOH for 30 min. Root samples were then stained with 0.166% trypan blue in lactoglycerol (equal parts glycerin, lactic acid, and distilled water) for 1 h and then destained in clear lactoglycerol. Levels of arbuscular mycorrhizal infection were quantified using the method of Allen and Allen (1980).

This study was designed as a completely randomized 2 × 6 factorial experiment with experimental factors being mycorrhizal status (−VAM or +VAM) and seedling age (30, 45, 60, 90, 120, or 150 d old). Two hundred sixteen experimental subjects (pots containing sagebrush seedlings) were included in this test with 18 replicates per treatment. Paired *t*-tests were used to compare the responses of mycorrhizal and nonmycorrhizal sagebrush seedlings in each age group to soil moisture stress (i.e., the level of soil dryness resulting in death). Analysis of variance (ANOVA) was used to test the hypotheses of main effects (the influence of arbuscular mycorrhizae and seedling age on sagebrush drought stress tolerance) and the interaction of these two factors.

RESULTS AND DISCUSSION

Sagebrush seedlings from the mycorrhizal treatment developed arbuscular mycorrhiza on 65 to 86% of root segments examined while seedlings from the nonmycorrhizal treatment formed mycorrhizae on only 1 to 2% of the root segments examined (Table 2). The observed differences in levels of mycorrhizae on sagebrush roots in the two treatments were statistically significant at $P < 0.001$ for all age groups.

Sagebrush seedlings in the mycorrhizal treatment were able to tolerate significantly drier soil conditions than nonmycorrhizal seedlings (Fig. 2, Table 3). For all of the different aged seedlings tested (30, 45, 60, 90, 120, and 150 d), the degree of soil dryness causing death of mycorrhizal seedlings was significantly greater ($P < 0.01$, based on paired *t*-tests) than that causing death of nonmycorrhizal seedlings. For example, after 45 d of growth, the average soil water potential resulting in death of mycorrhizal seedlings was −3.22 MPa, compared with an average of −2.77 MPa causing death of nonmycorrhizal seedlings, a difference of 0.45 MPa. As soils dried, the soil water potential that caused first deaths of nonmycorrhizal seedlings was about −2.5 MPa, compared with a dryness value of −2.8 MPa causing the first deaths of mycorrhizal seedlings (Fig. 3). Further, no nonmycorrhizal seedlings survived in soils

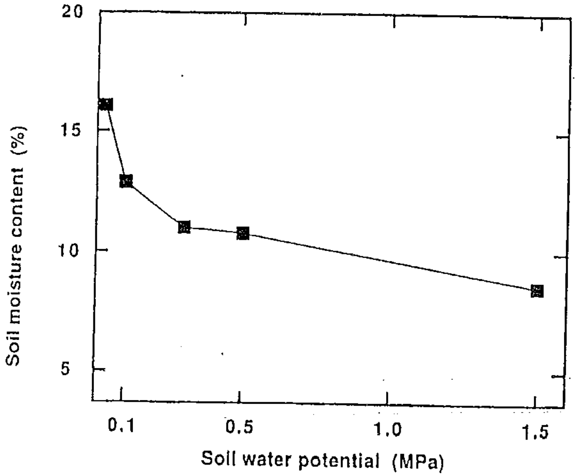

Fig. 1. Soil moisture retention curve.

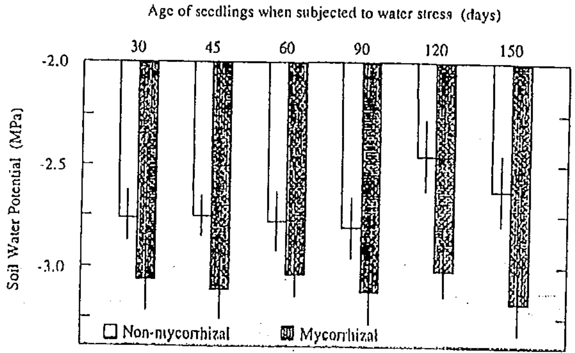

Fig. 2. Average soil water potentials resulting in death of mycorrhizal and nonmycorrhizal sagebrush seedlings.

Table 2. Amount of mycorrhizae on different aged seedlings.

Treatment	30 d	45 d	60 d	90 d	120 d	150 d
Nonmycorrhizal	2 ± 3†	1 ± 1	1 ± 2	1 ± 2	1 ± 1	1 ± 1
Mycorrhizal	86 ± 8	69 ± 17	77 ± 14	74 ± 12	70 ± 11	65 ± 20

† Values indicate percentage of observed 1-mm root segments inhabited by arbuscular mycorrhizal fungi ± standard deviation. Differences in mycorrhizae formation in the two treatments were statistically significant at the $P < 0.001$ for each age group.

with water potential values less than −3.3 MPa, whereas some mycorrhizal seedlings survived in soil as dry as −3.7 MPa (Fig. 3).

Analysis of variance indicated that seedling age, regardless of mycorrhizal treatment, also significantly affected water stress tolerance in sagebrush (Table 3). Additionally, ANOVA showed that the influence of mycorrhizae on soil moisture stress tolerance in sagebrush was disparate for different aged seedlings; that is, there was a significant interaction of plant age and mycorrhizal status on moisture stress tolerance ($P < 0.024$, Table 3).

Experimental data suggested that as sagebrush seedlings age, the beneficial influence of arbuscular mycorrhizae on soil moisture stress tolerance by sagebrush increases (Fig. 2). The data showed clearly that 120- and 150-d-old nonmycorrhizal seedlings are much less tolerant of soil moisture stress than younger nonmycorrhizal seedlings and that the disparity between mycorrhizal and nonmycorrhizal seedlings is greater for 120- and 150-d-old seedlings than for younger plants. On average, for seedlings 90 d old or younger, soil moisture levels resulting in death of mycorrhizal plants were about 12% drier than those causing death of nonmycorrhizal plants. For 120- and 150-d-old seedlings, soil moisture levels at the time of death of mycorrhizal plants were about 24% drier than those causing death of nonmycorrhizal plants This indicates that sagebrush seedlings become more dependent on the benefits of mycorrhizae as they age.

Research on the effects of arbuscular mycorrhizae on the water relations of a number of different plant species show generally improved water relations and greater drought resistance of mycorrhizal compared with nonmycorrhizal plants (Safir et al., 1971; Allen et al., 1981; Hetrick et al., 1987; Kothari et al., 1990; Bethlenfalvay, 1992). Researchers have observed a number of beneficial changes in the water relations of arbuscular mycorrhizal plants including altered rates of water uptake, hydraulic conductivity, leaf and stem water potentials, stomatal resistances, and transpiration rates. Several mechanisms have been proposed to explain the observed effects of mycorrhizae on host plant water relations. One explanation is that changes in host plant water relations are simply a secondary response due to improved nutrition, especially P uptake, provided by the arbuscular mycorrhizal fungus. Improved P nutrition due to mycorrhizal infection may have a direct effect on membrane resistance to water flow, probably the greatest limiting factor to water movement in plants (Nobel, 1974; Nelsen, 1987). Other explanations include: (i) external fungal hyphae may increase the total surface area of the host root system and increase the volume of soil exploited for water, in effect making more water available to the host plant; (ii) hyphae penetrating the root cortex to the endodermis may provide a low resistance pathway for water movement throught the root; and (iii) fungae may alter of root and shoot hormone levels that affect host plant water relations (Allen, 1982; Gogala, 1991; Murakami-Mizukami et al., 1991). For a number of reasons, George et al. (1992) concluded that water transport throught the hyphae is probably not the major cause of the greater rate of water uptake per unit root length of arbuscular mycorrhizal plants.

Improved ability to obtain water from soil and increased drought tolerance in Wyoming big sagebrush seedlings may have critical consequences in reestablishment of this important shrub on disturbed lands such as surface mine reclamation sites. This is especially important considering the arid and semiarid habitat in which this species occurs and is being planted. A number of studies have demonstrated that increasing the amount of soil moisture available to big sagebrush seedlings increases their survival and establishment (Young et al., 1990; Jones, 1991; Cockrell et al., 1995). One reason for this response is that sagebrush seedlings, compared with herbaceous species, appear to be poor competitors for water (Blaisdell, 1949; Sturges, 1977). Cockrell et al. (1995) showed that big sagebrush establishment was significantly reduced by the inclusion of herbaceous species in the seed mixture on surface mine reclamation sites.

Our data also showed that sagebrush seedling survival across a wide range of soil water potentials is greater for mycorrhizal than for nonmycorrhizal plants (Fig. 3). This may be critically important during early stages of seedling establishment when sagebrush root development is quite limited. Cockrell et al. (1995) reported finding significantly fewer sagebrush seedlings on plots treated with >5-yr-old stockpiled topsoil compared with plots treated with stripped and directly placed topsoil. The stockpiled topsoil used in their study had significantly lower numbers of arbuscular mycorrhizal fungal spores than did the fresh topsoil. Examination of root

Table 3. Summary of analysis of variance results.

Source	Sum of squares	df	Mean square	*F*-ratio	*P*
Mycorrhizal treatment	978.580	1	978.580	132.130	0.000
Seedling age	265.959	5	53.192	7.182	0.000
Mycorrhizae × age	98.092	5	19.618	2.649	0.024
Error	1421.988	192	7.406		

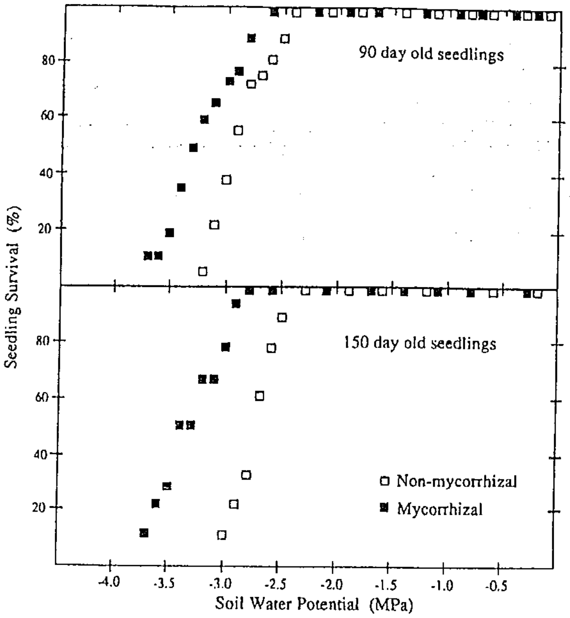

Fig. 3. Survival rates for 150- and 90-d-old mycorrhizal and nonmycorrhizal seedlings at different levels of soil dryness.

systems at the end of the first growing season, however, showed that surviving seedlings in both treatments had similar levels of mycorrhizal roots. The researchers hypothesized that the majority of seedlings germinating in stockpiled topsoil failed to develop mycorrhizae and did not survive; only those seedlings that did establish a mycorrhizal root system lived through the first growing season. The observation of similar levels of mycorrhizae on all surviving sagebrush seedlings led to this investigation to further assess the role of mycorrhizae in drought stress tolerance of big sagebrush.

Another environmental factor that makes moisture stress tolerance critical to sagebrush reestablishment is the degraded edaphic conditions often encountered on reclamation sites. Reconstructed soils on these sites usually have reduced infiltration rates and less water-holding capacity than undisturbed soils (Pederson et al., 1978; Smith and Sobek, 1979). This implies that sagebrush seedlings establishing on revegetation sites may have to survive greater moisture stress than those on undisturbed sites.

Results of this and other studies lead us to conclude that mycorrhizal big sagebrush seedlings are generally more vigorous, stress tolerant, and competitive than nonmycorrhizal seedlings. This may be a critical advantage in reestablishment of this species on disturbed lands. We recommend that sagebrush revegetation efforts include a strategy to ensure an opportunity for formation of the mutually beneficial mycorrhizal symbiosis. This can be accomplished by planting sagebrush seed in freshly placed (as opposed to stockpiled) topsoil containing sufficient levels of viable propagules of effective mycorrhizal fungi.

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Manuring and Soil Type Influence on Spatial Variation of Soil Organic Matter Properties

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ABSTRACT

The spatial variability of microbial biomass C and three humification indexes (the degree of humification, DH; the index of humification, HI; and the humification rate, HR) were investigated in order to evaluate their usefulness as indicators of the response of soil organic matter to altered soil environments. The investigation was carried out by examining the topsoil layer of an experimental field characterized by the presence of four phases of one soil series and, except for the addition of cattle manure in part of the field, by fairly uniform agricultural practices. A rectangular area of 350 by 800 m was sampled on a square grid scheme coupled with nesting. The experimental objective was to determine if the investigated properties were influenced by changes in soil type and by the addition of cattle manure. All properties measured showed spatial dependence at the sampling distances adopted. Two groups of properties were identified based on their degree of spatial variability: microbial biomass C, DH, and HI exhibited short-range variability, whereas HR varied across longer distances. Microbial biomass C and HR showed a spatial pattern related to soil amendment and soil types, respectively. Therefore, these variables provided complementary information about the condition of soil organic matter in the study area.

AN INTENSE DEBATE currently exists regarding the definition and quantification of the overall quality of a soil (Doran et al., 1994). Organic matter is involved in the enhancement of soil quality since it acts on soil structure, nutrient storage, and biological activity. It can be considered as constituted by a readily decomposable fraction and a stable fraction (Tate, 1987). The former, sensitive to short-term modifications, influences biological activity and behaves as a nutrient reservoir for plants and soil organisms. The latter, represented by humic substances, is involved in some physicochemical process such as those that influence soil structure and ion exchange. It is possible that estimates of the status of the decomposable and the stable fractions of soil organic matter could be obtained by measuring the size of microbial biomass and the rate of humification of the soil, respectively.

The size of the soil microbial pool is often expressed in terms of microbial biomass C (Powlson, 1994). Field experiments conducted to detect changes in surface soil organic matter attributable to differing management of barley (*Hordeum vulgare* L.) straw (Powlson et al., 1987) and sorghum [*Sorghum bicolor* (L.) Moench] residues (Saffigna et al., 1989) revealed that, in the short to medium term, microbial biomass C is much more sensitive to soil management than is soil organic matter as a whole. Fromm et al. (1993) investigated the influence of land use (grassland or winter wheat [*Triticum aestivum* L.] cultivation) and soil type on soil fauna and soil microbial biomass. They attributed observed changes in the spatial distribution of microbial biomass C to land use rather than to soil type. However, this conclusion cannot be generalized because the physicochemical conditions of the surface soil layer under grasses differ from those of a plowed layer, and such difference might mask any possible effect of soil type.

Unlike soil microbial biomass, little experimentation has been done in the last two decades to quantify the rate of humification of soil organic matter. The transformation of organic materials into humified compounds — i.e., pigmented polymers formed by secondary synthesis reactions (Stevenson, 1994) — is linked to the stabilization of soil organic matter. The amount of humic substances extracted from the soil with alkali might be related to the level of stabilization reached by organic matter in a given soil. However, humic extracts also contain known classes of organic compounds that are not humified. Humic substances can be separated from other organic compounds by means of insoluble polyvinylpyrrolidone (PVP), which selectively adsorbs polyphenols at low pH (Lowe, 1975). Watanabe and Kuwatsuka (1992) demonstrated that carbohydrates and N-containing compounds were concentrated in the fraction of humic extract not adsorbed by PVP. An index

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Abbreviations: DH, degree of humification; H, humified fraction; HI, index of humification; HR, humification rate; NH, nonhumified fraction; PVP, polyvinylpyrrolidone.