Mycorrhizal Inoculation of Big Sacaton: Implications for Grassland Restoration of Abandoned Agricultural Fields

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Abstract

Grasslands dominated by *Sporobolus wrightii* (big sacaton) once covered riparian floodplains in southwestern United States and northern Sonora, Mexico but now occupy less than 5% of their historic range, mostly due to clearing for agriculture. Many agricultural fields have been abandoned because of changing land uses, and efforts are under way to restore native grassland habitat. Arbuscular mycorrhizal (AM) fungi are known to form associations with *S. wrightii* and can be a potential factor in grassland restoration efforts. The goal of this study was to determine the effects of mycorrhizal inoculation on *S. wrightii* during transplant production and in a restoration trial. *Sporobolus wrightii* was grown with and without AM fungi in 2.8-L tall pots and 150-mL nursery containers under greenhouse conditions for 8 weeks and then transplanted into an abandoned agricultural field. Plants were monitored for growth, survival, and mycorrhizal infection. Seedling emergence in the greenhouse was higher in pots with mycorrhizal inoculation, but inoculation had little effect on growth except more tillers were produced by pre-inoculated plants grown in the smaller containers. In the abandoned field, pre-inoculated plants had greater survival, basal diameter, and tiller and panicle production through the first two growing seasons. Plants started in smaller containers also had greater survival, height, basal diameter, and tiller production than those started in tall pots. Root colonization was detected in all plants by 2 months after transplanting but was not consistent throughout the experiment except for pre-inoculated plants started in the smaller containers. These results indicate that mycorrhizal inoculation can benefit restoration efforts in abandoned agriculture fields in semiarid regions.

Key words: **agriculture, arbuscular mycorrhizae, grassland, restoration, riparian, sacaton, *Sporobolus wrightii*.**

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Introduction

One of the leading causes of losses in biodiversity worldwide is the conversion of habitat to agriculture and subsequent abandonment (Vitousek et al. 1986; Dobson et al. 1997). In the past 50 years, over 96,356,000 ha of farmland have been abandoned in the United States alone (USDA 1998). Rates of secondary succession of abandoned agricultural fields vary, but in arid and semiarid regions natural recovery can be slow, with abandoned fields showing little recruitment of perennial species, or any vegetation, even after 30 to 40 years (Reichhardt 1982; Jackson et al. 1991; Jackson & Comus 1999). Sacaton grasslands, dominated by the perennial bunch grass *Sporobolus wrightii* (big sacaton grass), are an example of this type of pattern. These semiarid grasslands once occupied riparian floodplains throughout the southwestern United States and northern Sonora, Mexico but have been reduced to less than 5% of their historic range because of clearing for agriculture (Humphrey 1958; Bahre 1991). Historically, the floodplain areas of southern Arizona were prime locations for the development of agriculture because of accessibility to water, flat topography, and buildup of rich sediments from past floods. Water availability, however, has become limiting because water tables have fallen beyond levels that can be economically pumped and urban water demands have risen, prompting transfers of water rights to urban uses (Cox et al. 1983; Cox & Madrigal 1988). As a result, many farms are being abandoned, contributing to problems with run-off and dust storms (Cox et al. 1983; Cox & Madrigal 1988). Efforts to restore some of this land to native grassland habitat have met with variable success (USFWS 1995). Among those efforts, direct seeding has met with virtually no success (Gori et al. 1997), whereas transplanting has been more effective (Tiller et al. 1999).

Sporobolus wrightii is known to form associations with arbuscular mycorrhizal (AM) fungi (Kennedy et al. 2002), and mycorrhizal inoculum has been a potential factor in the success or failure of other grassland revegetation efforts (Smith et al. 1998; Thorne et al. 1998). AM fungi can benefit plants by enhancing mineral uptake, specifically P, N, and Zn, and by improving drought tolerance (Smith & Read 1997), factors that could be critical to the survival of transplants in semiarid areas such as southern Arizona. Non-mycorrhizal plants often dominate abandoned fields in southern Arizona (Cox & Madrigal 1988) and could be indicative of impoverished soils. Studies have demonstrated that agricultural practices alter AM fungal levels, species composition, and colonization rates (Kurle & Pfleger 1994; Miller et al. 1995).

Although many studies have looked at the occurrence of AM fungi after restoration efforts (Allen & Allen 1980; Johnson & McGraw 1988; Corbett et al. 1996; Gould et al. 1996; Lovera & Cuenca 1996; Koske & Gemma 1997), relatively few have examined the effects of AM fungal inoculation as a part of restoration efforts. Most of these studies focused on reclamation of abandoned mine lands and found mycorrhizal inoculation to be beneficial in those efforts (Noyd et al. 1996; Johnson 1998; Thorne et al. 1998). Gemma and Koske (1997) found that the introduction of soil containing mycorrhizal propagules into transplant holes increased growth and panicle production in beachgrass plantings in coastal sand dunes. Field inoculation with mycorrhizal propagules from pot cultures also has been shown to benefit revegetation of degraded tropical lands in Venezuela with an introduced grass (Cuenca et al. 1998) and restoration of native grass species in a recently disturbed Minnesota tallgrass prairie (Smith et al. 1998).

This study was conducted to assess the potential for using mycorrhizal inoculation in grassland restorations of abandoned agricultural fields in semiarid regions. In addition, the effect of container type was assessed, because an earlier effort to restore big sacaton in abandoned agriculture fields had found that transplants produced in larger containers had greater survival (Tiller et al. 1999), but no mycorrhizal treatment was included. Three research questions were addressed: (1) Does mycorrhizal inoculation affect seedling emergence and growth of *S. wrightii* during the transplant production stage in the greenhouse? (2) Does pre-inoculation of sacaton seedlings with AM fungi during greenhouse production affect growth and survival of *S. wrightii* transplanted into an abandoned agricultural field? (3) What effect does container type have on the survival and growth of inoculated and uninoculated transplants?

Materials and Methods

A two-phase experiment was conducted to test the effects of mycorrhizal inoculation on greenhouse grown *Sporobolus wrightii* seedlings and post-transplant survival and productivity. The first phase evaluated effects of mycorrhizal inoculation on seedling emergence, early survival, and growth under greenhouse conditions in two differently sized nursery containers. The second phase evaluated survival and productivity of transplanted *S. wrightii* seedlings in an abandoned agricultural field.

To produce fungal inoculum for this experiment, soil was collected from a southern Arizona riparian lower floodplain terrace on the San Pedro River at the Bureau of Land Management Bennet site (32° 20′N, 110° 24′W) and used to establish pot cultures with *Sorghum sudanese* (sudan grass) as the host (Stutz & Morton 1996). Inoculum consisted of soil/sand media from a second-generation pot culture that contained spores, hyphae, and colonized root pieces. AM fungi propagule density of the inoculum was determined using the most probable numbers method (Alexander 1982) and was calculated to be 817 propagules/cm^3. Species composition of the inoculum was determined by spore extraction from 100-cm^3 samples, using wet sieving followed by a sucrose gradient centrifugation (Daniels & Skipper 1982). *Glomus mosseae* comprised approximately 50% of the spores extracted, and *Glomus eburneum* comprised approximately 30%. Other species present, in order of prevalence, were *G. microaggregatum*, *Acaulospora delicata*, *Glomus spurcum*, and *Glomus macrocarpum.*

Seed used in this experiment was collected in 1996 from sacaton plants growing along Sonoita Creek, approximately 3 km downstream of the transplant site, and was screened to 600 μm. Germination percentage was determined to be 93%.

Greenhouse Experiment

Two differently sized nursery containers (Stuewe & Sons, Inc., Corvallis, Oregon, U.S.A.) were used to grow *S. wrightii* seedlings, Treepots ("Tall One," 10 × 10 × 36 cm, 2.8 L) and Ray Leach "Cone-tainers" ("Super Cell," 3.8 × 21 cm, 164 mL). Tall pots were sterilized in 10% commercial bleach (>1 hr), and a sterile aluminum grate was placed in the bottom of each. A 200-cm^3 layer of no. 12 coarse silica sand (autoclaved at 121°C for 1 hr) was added to each pot, followed by 1,500 cm^3 of soil/sand mix consisting of two parts sieved field soil (collected at transplant site, sieved to 2 mm, autoclaved twice at 101°C for 1 hr) to one part no. 20 fine silica sand (autoclaved at 121°C for 1 hr). To half the pots 90 cm^3 of fungal inoculum (approximately 7.4×10^4 propagules) was added in a band application, followed by 600 cm^3 of the autoclaved soil/sand mix. The remaining pots received 690 cm^3 of the autoclaved soil mix. A total of 40 pots were prepared: 20 inoculated and 20 uninoculated. Seed was surface sterilized for 3 minutes in 10% com-

mercial bleach, and 10 seeds were placed on each of 40 sterile 7-cm filter paper circles. The filter papers were placed in each pot and covered with 0.5 cm soil/sand mix. Pots were placed in the greenhouse in plastic crates (40 × 32 × 27 cm), four inoculated and four uninoculated pots per crate, with four empty sterilized pots acting as spacers between treatments, and watered to flow through (400 mL). To reduce algal growth on the soil surface, 0.5 cm of sterile no. 12 sand was added to the top of each pot. On day 2, uninoculated pots received a sieved washing of the inoculum to reestablish as much of the non-mycorrhizal microflora as possible (Koide & Li 1989). One hundred cubic centimeters of the inoculum was mixed with 3 L of water and sieved three times through a 35-μm screen, and 100 mL of the washing was added to each of the 20 uninoculated pots.

Cone-tainers and 2.2-cm glass marbles were sterilized in 10% bleach. A marble was placed in the bottom of each cone and covered with 10 cm^3 of autoclaved no. 12 silica sand, followed by 60 cm^3 of the autoclaved 2:1 soil/sand mix. A 10-cm^3 band of inoculum (approximately 8.2×10^3 propagules) was added to half the cones and topped with 30 cm^3 of the soil/sand mix. Uninoculated cones received 40 cm^3 of the soil mix. A total of 170 cone-tainers were prepared: 85 inoculated and 85 uninoculated. Five cones from each treatment were used to replace cones with no emergence or were harvested for mycorrhizal examination, leaving 160 to be transplanted into the field. Three surface-sterilized seeds were placed on the surface of each cone and covered with 0.5 cm of soil/sand mix. Cones were placed in racks in alternating blocks of 14 (two rows of seven), with two rows left empty between treatments, and watered to flow through (20 mL). A layer of 0.5 cm of no. 12 sand was added to the top of each cone. On day 2, 10 mL of the filtered washing was added to each of the 80 uninoculated cones.

Plants were arranged in the greenhouse in a block design consisting of five tall pot blocks and two cone-tainer blocks. Each crate of tall pots constituted a block, containing four inoculated and four uninoculated containers. Each pair of cone-tainer racks constituted a block, containing either 28 or 29 cones of each treatment. All containers were watered to flow through daily for the first 16 days, and then the watering frequency was reduced to three times a week. Plants were maintained under natural light conditions in the greenhouse (524 $\mu mol\ m^{-2}\ s^{-1}$ mean reading at midday). Daytime temperatures in the greenhouse were approximately 31°C, and night temperatures ranged from 16 to 22°C. Plants were maintained in the greenhouse for a total of 9 weeks.

Plant emergence data from tall pots were recorded weekly for 8 weeks. At 8 weeks data on all plants were collected. For tall pots height of the tallest flag leaf and number of tillers were recorded. The treatment mean height was calculated, and the plant nearest the treatment mean for each pot was marked. All other seedlings were clipped at the surface and oven dried, and aboveground dry weights were recorded. Core samples were taken from each tall pot with a no. 6 borer, and root material was removed from these cores for mycorrhizal examination. Roots were wrapped in permanent wrap papers, placed in tissue capsules, and stored in 50% ethanol. For plants in cone-tainers, heights and number of tillers were recorded, and the tallest plant in each cone was retained whereas others were clipped at the surface. Complete root systems from five inoculated and three uninoculated cones were harvested and stored, as above.

Transplant Experiment

The transplant site was established in an abandoned agricultural field owned by the Nature Conservancy in Patagonia, Arizona (31°32′40″ N, 110°4′30″ W, 1,237-m elevation) on the upper terrace riparian floodplain at the confluence of Sonoita and Harshaw creeks. The plot was located on Pima loam soil (fine-silty, mixed, thermic Anthropic Torrifluvents). Annual rainfall averages 466 mm (National Climactic Data Center for the nearest locale) with a bimodal pattern (56% as monsoon thunderstorms in July through September and the remainder as winter rainstorms). Mean annual temperatures range from 4°C (minima) to 23°C (maxima), with an average of 245 frost-free days. The field had been unplanted for 7 years. Previously it had supported a variety of vegetable crops and alfalfa grown with conventional farming techniques, including use of commercial fertilizers and pesticides. In the years since farming operations had ceased, the site was used for small-scale cattle grazing. The predominant vegetation on the site at the time of this experiment was *Salsola kali* (Russian thistle) and *Chenopodium album* (lambsquarter). Soil samples were collected from the top 30 cm of the plot area at 15 regularly distributed points. Subsamples from each point were bulked and sent to a commercial laboratory (Laboratory Consultants, Ltd., Tempe, Arizona, U.S.A.) for analysis (pH and electrical conductivity in 1:1 water paste; nitrate-N by ion selective electrode; extractable phosphorus by Olsen method; organic matter by Walkley-Black method; soil texture by hydrometer). The soil was classified as a loam, with 48.8% sand, 30.6% silt, and 20.6% clay, with a pH of 8.0. Organic matter was 2%, nitrate was 12 ppm, bicarbonate phosphorus was 15 ppm, and exchangeable sodium was 42 ppm. Separate samples were collected and evaluated for mycorrhizal inoculum potential using the mean infection percentage method (Moorman & Reeves 1979; Koske & Gemma 1997). Two samples were taken along the center line of

the plot area, one-third of the way in from the north and south ends. *Zea mays* L. (corn) was grown as a host in a one-fourth dilution of the sample soil, and roots were stained in trypan blue (Koske & Gemma 1989). One hundred 1-cm root segments were examined for colonization using a light microscope. The mean infection percentage values ranged from 31.9% in the south end of the field to 9.7% in the north end.

Transplants were made on 22–23 July 1998, 2 weeks after the onset of the summer monsoon rains. A blocked design was used for planting, consisting of four blocks of 10 tall pot plants and eight blocks of 21 cone-tainer grown plants. Plants were spaced at 1-m intervals with 2 m between blocks. Six mycorrhizal and six non-mycorrhizal blocks were arranged in alternating treatments. Water was supplemented only when plants showed signs of severe drought stress (leaf rolling and die-back) during the remainder of the growing season. This occurred twice: On 8 September each plant received 1 L of water by hand watering, and on 15 October each plant received 1.8 L. Weeds were managed during the growing season by hand removal within blocks but not in the corridors between blocks.

Data were collected 2, 4, and 10 months after transplanting. Height, basal diameter, and number of tillers were recorded for all tall pot plants and a subsample of 80 cone plants (all plants in the interior of each block), and survival was recorded for all plants. Five tall pot plants and three cone plants in each block were designated for collection of root cores. Cores 2 cm in diameter and 12 cm deep were taken 10 cm from the center of the plant. The same plants were cored at each data collection; cores were taken from different sides of the plants each time. Cores were placed in plastic vials and taken back to the laboratory, where roots were removed and stored in 50% ethanol for mycorrhizal examination. At 12.5 months, final data were collected on height, basal diameter, and number of panicles for every plant.

Mycorrhizal Examination

Root material from the greenhouse-grown plants and transplant cores was evaluated for AM fungal colonization using the magnified intersections method (McGonigle et al. 1990). Roots were cleared in 4% KOH and stained with 0.05% trypan blue (Koske & Gemma 1989), and segments were mounted horizontally on glass slides and examined under a light microscope.

Statistical Analyses

Analysis of variance (Systat 7.0, 1997 SPSS, Inc., Chicago, IL, U.S.A.) was used to identify significant differences among treatments at each time period. When percent infection data failed to meet normality, arcsine transformation was used. Repeated-measures analysis was conducted on weekly emergence and height data from the 8-week transplant production period. Unless noted, significance level was set at $\alpha = 0.05$ for all tests. Final data collected from plants started in cone-tainers from the field site were also analyzed separately for position effects. Comparisons were made between plants growing in the single row next to the 2-m corridor between blocks, designated edge plants ($n = 14$ for each block), and those growing in the center of the block, designated center plants ($n = 6$).

Results

Greenhouse Experiment

Over the first 8 weeks of growth in the greenhouse, mycorrhizal treatment had no significant effect on seedling emergence (Table 1). Repeated-measures analysis of variance showed a between-subjects p value of 0.196 for treatment effect. The within-subjects p value was 0.030, showing a significant change in treatment effect by week (Fig. 1). For height of the tallest flag leaf, repeated-measures analysis showed a significant mycorrhizal treatment effect ($p = 0.066$) and a significant treatment by time effect ($p = 0.019$) (Fig. 1).

At 8 weeks there was a significant difference in number of seedlings per pot, showing higher emergence in inoculated pots (Table 1). Mean emergence in both inoculated and uninoculated pots was less than the 93.0% seed germination percentage. Inoculation had no significant effect on height, tiller production, or aboveground biomass (as dry weight) of seedlings grown in tall pots at 8 weeks. There was no block effect in tall pots for any variable measured.

Among seedlings grown in cone-tainers, height was unaffected by inoculation, but inoculated plants did have significantly higher tiller production (Table 1). The only significant difference in growth between seedlings grown in cone-tainers and those grown in tall pots was that cone-tainer grown plants were significantly shorter than tall pot grown plants (Table 1).

Transplant Experiment

Inoculation with AM fungi during the transplant production stage had a significant effect on plant growth after plants were transplanted into an abandoned agriculture field (Table 2). Tiller production and basal diameter were significantly greater in pre-inoculated plants than in uninoculated plants at all measurement dates (Table 2). Heights were significantly greater in pre-inoculated plants at the 2-month and 1-year measurements and were significant at $p = 0.057$ at 10 months.

Table 1. Treatment means for height, tiller production, emergence, and aboveground biomass of *Sporobolus wrightii* after 8 weeks in a greenhouse (± SEM).

Treatment	*Height (cm)*	*Tillers*	*Emergence*	*Dry Weight (g)*
TM	68.4 ± 2.1	3.4 ± 0.2	70.5%	0.44 ± 0.03
TN	67.5 ± 2.1	3.4 ± 0.2	62.0%	0.46 ± 0.04
p Value				
Mycorrhizal treatment	N.S.	N.S.	0.06	N.S.
CM	45.5 ± 1.1	3.3 ± 0.1	—	—
CN	47.1 ± 0.9	2.8 ± 0.1	—	—
p Value				
Mycorrhizal treatment	N.S.	0.011	—	—
Container treatment	<0.001	N.S.	—	—

TM, pre-inoculated plants in tall pots; TN, uninoculated plants in tall pots; CM, pre-inoculated plant in cone-tainers; CN, uninoculated plants in cone-tainers; N.S., not significant.

The type of container used during the transplant production stage also had a significant effect on plant growth after plants were transplanted into an abandoned agricultural field (Table 2). Plants started in cone-tainers had significantly greater heights and tiller production at all measurement dates and greater basal diameters at all but the 1-year measurement (Table 2).

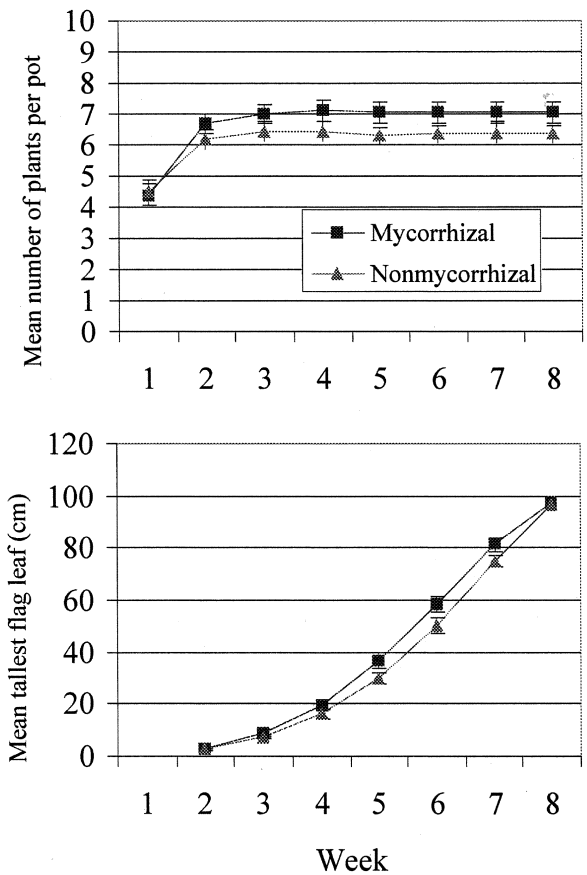

Figure 1. *Sporobolus wrightii* seedling emergence and survival and height of tallest flag leaf per pot over an 8-week greenhouse growth period. Bars indicate the standard error of the mean.

There were no treatment × container interaction effects for height, tillers, or basal diameter at any measurement time.

Plant survival was high for all treatments, but the only treatment with 100% survival at final data collection (12.5 months) was pre-inoculated plants started in cone-tainers (Fig. 2). Pre-inoculation had a significant effect on mean panicle production (Table 3). The percent of plants with panicles and the mean number of panicles on those plants that produced panicles also appeared to be greater in pre-inoculated plants, but differences were not significant.

Pre-inoculation appeared to have a greater effect on the survival of plants at the edges of blocks in comparison with the center (Fig. 2). When survival percentage data were arcsine transformed, there was a significant interaction effect between treatment and position; however, even with transformation the data set was not normal. Interaction effects (treatment × position) were significant for final basal diameter ($p = 0.023$) and panicle production ($p = 0.047$). Pre-inoculation had a greater effect on basal diameters and panicle production in the center of blocks, whereas plants at the edge were uniformly smaller with lower panicle production (Fig. 3).

Mycorrhizal Colonization

At the end of the greenhouse period examination of roots collected in soil cores from plants growing in tall pots found all plants to be colonized, with a 40.2 mean percent colonization (total colonized intersections/100). Colonization was absent in roots from uninoculated plants growing in tall plots except for one infected segment found in one pot. The five inoculated plants growing in cone-tainers harvested at the end of the greenhouse phase had a 44.6 mean percent colonization. No colonization of roots was detected in the three uninoculated plants examined.

Roots of both pre-inoculated and uninoculated plants were colonized by AM fungi two months after trans-

Table 2. Means of height, basal diameter, and number of tillers of *Sporobolus wrightii* at 2, 4, 10, and 12.5 months after transplanting into an abandoned agricultural field (± SEM).

	Height (cm)	*Basal Diameter (cm)*	*Tillers*
2 Months			
TM	51.7 ± 3.6	4.6 ± 0.3	35.6 ± 4.3
TN	46.7 ± 3.4	3.7 ± 0.2	27.8 ± 3.9
CM	67.1 ± 2.7	5.4 ± 0.2	53.2 ± 4.6
CN	57.3 ± 3.2	4.1 ± 0.2	37.6 ± 3.8
p Value			
Mycorrhizal treatment	0.024	<0.001	0.006
Container treatment	<0.001	0.015	0.002
4 Months			
TM	23.6 ± 1.9	4.4 ± 0.4	36.8 ± 5.1
TN	19.9 ± 1.9	3.9 ± 0.3	27.1 ± 3.9
CM	33.9 ± 1.6	5.8 ± 0.3	62.1 ± 5.4
CN	31.0 ± 2.0	4.8 ± 0.2	48.8 ± 5.1
p Value			
Mycorrhizal treatment	N.S.	0.013	0.024
Container treatment	<0.001	<0.001	<0.001
10 Months			
TM	26.0 ± 2.4	3.8 ± 0.4	42.9 ± 5.9
TN	20.7 ± 1.9	3.3 ± 0.2	34.2 ± 4.4
CM	44.4 ± 2.0	6.1 ± 0.4	75.4 ± 6.0
CN	40.7 ± 2.7	5.3 ± 0.3	57.6 ± 5.0
p Value			
Mycorrhizal treatment	0.057	0.037	0.019
Container treatment	<0.001	<0.001	<0.001
12.5 Months*			
TM	79.4 ± 4.5	7.2 ± 0.6	—
TN	62.8 ± 7.0	5.3 ± 0.4	—
CM	85.2 ± 3.1	6.9 ± 0.3	—
CN	78.6 ± 2.9	6.6 ± 0.2	—
p Value			
Mycorrhizal treatment	0.016	0.007	—
Container treatment	0.025	N.S.	—

p Values for effects of mycorrhizal inoculation and container from two-way analyses of variance tables, treatment × container type. TM, pre-inoculated plants started in tall pots; TN, uninoculated plants started in tall pots; CM, pre-inoculated plant started in cone-tainers; CN, uninoculated plants started in cone-tainers; N.S., not significant.
*12.5-Month means include all surviving plants; others are calculated from a subsample.

planting (Table 4). The percentage of plants with colonization was similar across treatments. There were no significant differences in infection levels by either treatment ($p = 0.765$) or container type ($p = 0.253$). Data were arcsine transformed to meet normality; transformation did not affect significance results.

Four months after transplanting both the percent of plants infected and mean percent colonization had fallen in all treatments except pre-inoculated plants started in cone-tainers, where the percent of plants infected was only slightly reduced compared with infection at two months after transplanting and mean percent colonization was unchanged (Table 4). There was a significant interaction effect between treatment and container type. Roots collected from pre-inoculated plants started in cone-tainers had much greater mean infection than roots for plants in the other treatments.

Ten months after transplanting root cores yielded less root material than at other sampling periods, and data presented are for samples that yielded greater than 40 intercepts in the gridline-intercept analysis of infection level. There was a trend indicating a treatment × container interaction effect with ($p = 0.057$) and without ($p = 0.099$) arcsine transformation. The percentage of plants with colonized roots was greater for all treatments than that detected at 4 months (Table 4). Mean infection was at the highest detected levels for all pre-inoculated plants and for uninoculated plants started in cone-tainers. The mean infection levels remained low for uninoculated plants started in tall pots.

Discussion

We found that during transplant production of *Sporobolus wrightii* in the greenhouse there was a trend toward greater emergence among inoculated than uninoculated plants grown in tall pots and significantly greater tiller production among inoculated than uninoculated

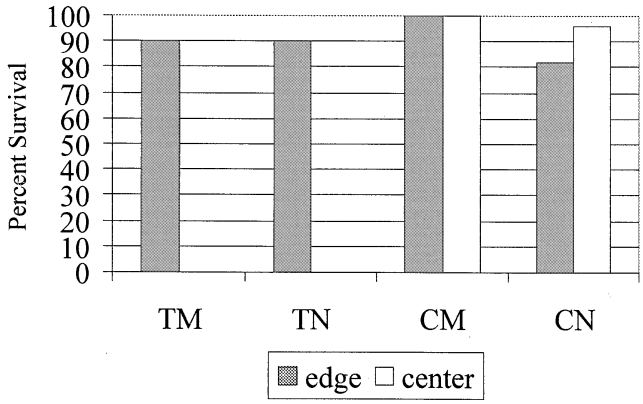

Figure 2. Percent survival of *Sporobolus wrightii* transplants at 12.5 months after transplanting into an abandoned agricultural field. TM, pre-inoculated tall pots; TN, uninoculated tall pots; CM, pre-inoculated cone-tainers; CN, uninoculated cone-tainers.

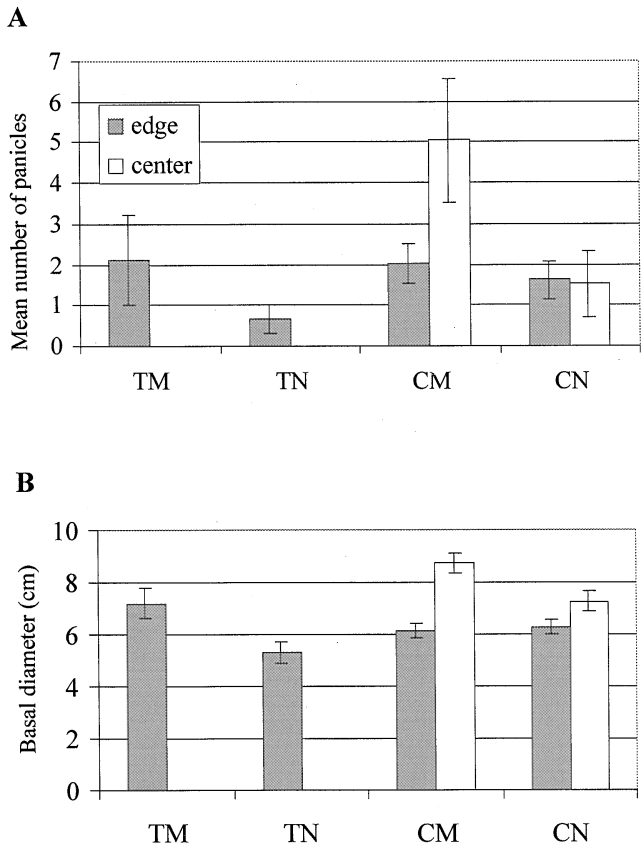

Figure 3. (A) Mean number of panicles and (B) basal diameters of *Sporobolus wrightii* transplants at 12.5 months after transplanting into an abandoned agricultural field. TM, pre-inoculated tall pots; TN, uninoculated tall pots; CM, pre-inoculated cone-tainers; CN, uninoculated cone-tainers. Bars indicate standard error of the mean.

plants grown in cone-tainers. These results differ from some previous experiments (Bethlenfalvay et al. 1982; Koide 1985) that found mycorrhizal inoculation caused a growth depression response in early plant developmental stages. In these cases C allocation to fungal development represents a relatively greater cost to the young seedling, and the plant is not yet benefiting from improved nutrient uptake. In contrast, Wilson and Hartnett (1998) found significantly increased dry mass in inoculated *Sporobolus airoides* and *S. heterolepis* plants relative to uninoculated plants after 16 weeks of growth in a greenhouse and calculated high mycorrhizal responsiveness indices ([dry mass inoculated − dry mass uninoculated] / dry mass uninoculated × 100) of 91.0 and 98.1 for these plants. In *Sporobolus virginicus*, Corkidi and Rincon (1997) found increased leaf area but calculated a negative mycorrhizal responsiveness index (based on dry weight) in 9-week-old greenhouse grown plants.

We also found that pre-inoculated plants showed greater growth and survival rates than uninoculated plants 1 year after they were transplanted into an abandoned agricultural field. These results are consistent with the observations of Gemma and Koske (1997) in restoration projects with the beachgrass *Ammophila breviligulata*, in which inoculated plants were found to

Table 3. Panicle production in *Sporobolus wrightii* transplants 1 year after transplanting into an abandoned agricultural field.

Treatment	*Percent with Panicles*	*Mean Panicle Production*[a]	*Mean Panicles per Plant*[b]
TM	33.3	2.1	6.3
TN	22.2	0.7	3.0
CM	43.7	2.9	6.7
CN	33.3	1.6	4.7
p Value			
Mycorrhizal treatment	N.S.	0.031	N.S.
Container treatment	N.S.	N.S.	N.S.

p Values for effects of mycorrhizal inoculation and container from two-way analyses of variance tables, treatment × container type. TM, preinoculated plants started in tall pots; TN, uninoculated plants started in tall pots; CM, preinoculated plant started in cone-tainers; CN, uninoculated plants started in cone-tainers; N.S., not significant.
[a]Mean of all plants.
[b]Mean among only those plants that had panicles present.

Table 4. Mycorrhizal colonization of *Sporobolas wrightii* seedlings at 2, 4, and 10 months after transplanting into an abandoned agricultural field.

Treatment	*Percent of Plants Infected*	*Mean % Colonization (total)*	*Mean % Colonization in Colonized Plants*
2 Months			
TM	90.0	10.9	12.2
TN	90.0	9.9	11.0
CM	91.7	14.3	15.6
CN	91.7	13.8	15.0
4 Months			
TM	30.0	2.5	8.2
TN	40.0	4.6	11.5
CM	83.3	15.0	18.0
CN	58.3	3.6	5.9
10 Months			
TM	100	35.7	35.7
TN	60.0	6.1	10.2
CM	91.7	32.0	34.9
CN	100	33.8	33.8

TM, preinoculated plants started in tall pots; TN, uninoculated plants started in tall pots; CM, preinoculated plant started in cone-tainers; CN, uninoculated plants started in cone-tainers.

have greater culm production than uninoculated plants after 42 and 81 weeks, even though roots at 47 weeks showed no significant differences in incidence or extent of colonization. In the present experiment most plants became infected with AM fungi within 2 months of transplanting, but both number of samples showing infection and levels of infection fell by 4 months in all plants except those that had been pre-inoculated and started in cone-tainers. It appears that many new infections were formed in the uninoculated plants but did not persist, and infections established in inoculated plants before transplanting persisted to varying degrees. In-depth analysis of the AM fungal species present in the transplant site has not been conducted, and it is unknown whether after transplanting roots were colonized by fungi in the riparian grassland inoculum or by fungi present at the transplant site. It is possible that much of the variation seen in colonization levels may be a product of the sampling technique and sample size. If the distribution of inoculum in the field was patchy or varied over time, core sampling would result in a biased estimate of colonization. Boerner et al. (1996), in a study of mycorrhizal inoculum potentials from a chronosequence of abandoned fields, found infectivity rates to vary spatially within 5- and 10-year-old fields. Thus, colonization results presented here should be interpreted conservatively.

The continued benefit of pre-inoculation seen in this study, even after uninoculated plants became colonized, could indicate that the AM fungal assemblage present in the abandoned field transplant site did not confer the same benefits as the assemblage that was collected from an existing grassland and used to inoculate plants in the greenhouse. It is also possible that the sustained differences between pre-inoculated and uninoculated plants are the result of a "head start" in pre-inoculated plants. In this case, pre-inoculation enabled plants to recover from transplant shock more quickly than uninoculated counterparts, and the differences in growth that were established during the first 2 months lingered. The head start hypothesis would not, however, explain differences in survival, because most mortality occurred over the winter and early spring.

Most surprising among the results of this study was the effect of container type on transplant growth. Monitoring of an earlier sacaton transplant project that had no mycorrhizal treatment showed that plants started in tall pots had out-performed plants started in cone-tainers (Tiller et al. 1999). In the present study plants grown in the smaller cone-tainers consistently out-performed plants grown in tall pots. The differing results between the two transplant projects could be due to differences between the studies during the transplant production stage. The greenhouse growth period was shorter in this study than in previous transplant experiments. Plants grown in cone-tainers became completely established under these conditions, forming a solid root mass within the cones, whereas those grown in tall pots had not filled their containers with roots by the transplant date. The plants started in tall pots, with a less stable root mass, might have suffered greater disruption during transplanting. Also, the roots of the tall pot plants would not have been immediately in contact with the surrounding field soil with its higher clay content and water-holding capacity. It is also possible that differences in growth after transplanting between plants started as tall pots and those started in cone-tainers were due to field plot design. All plants started in tall pots were located as the edge of a block, whereas plants started in containers

were located at the edge or center of a block. Basal diameters and panicle production of pre-inoculated plants started in tall pots were similar to that of plants started in cone-tainers that were located at the edge of the treatment block. These differences between plants growing at the edge of the treatment block and the center of the block could be due to competition with weeds at the edge of the blocks, because hand weeding was applied during the growing season within blocks but not in the 2-m corridor between blocks.

In conclusion, the benefits to plants of pre-inoculation with AM fungi before transplanting into an abandoned agricultural field were similar to those found in the restoration of highly disturbed habitats such as mine tailings (Noyd et al. 1996; Johnson 1998; Thorne et al. 1998) or coastal sand dunes (Gemma & Koske 1997). Unlike these highly disturbed habitats, which typically have a low number or no viable AM fungal propagules, we did detect viable AM fungal propagules in the soil at our study site that were within the range found in extant sacaton grasslands (Richter et al. 2002). Despite the presence of AM fungal propagules, the dominance at the abandoned field site of two non-mycorrhizal plants in the Chenopodiaceae indicates an altered soil community. Agricultural practices are known to alter AM fungal levels, species composition, and colonization rates (Kurle & Pfleger 1994; Miller et al. 1995) and result in the selection of more aggressive and less effective AM fungal communities (Johnson 1993). Because of these factors mycorrhizal restoration should be considered in future restoration efforts of abandoned agriculture fields located in arid and semiarid regions as well as in other habitats with altered AM fungal communities.

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