Ann. Bot. Fennici 45: 435–447 ISSN 0003-3847 (print) ISSN 1797-2442 (online)
Helsinki 30 December 2008

Influence of soil water status on arbuscular mycorrhizas in three perennial grasses in central Argentina

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Received 30 Apr. 2008, revised version received 15 Aug. 2008, accepted 7 Oct. 2008

Busso, C. A., Bolletta, A., Flemmer, A. C. & Montani, T. 2008: Influence of soil water status on arbuscular mycorrhizas in three perennial grasses in central Argentina. — *Ann. Bot. Fennici* 45: 435–447.

In this field study, we determined the effects of water stress, rainfed or irrigated conditions on the arbuscular mycorrhizal fungal (AMF) root colonization in the late-seral *Stipa clarazii*, the earlier-seral *S. tenuis* and the early-seral *S. gynerioides* at different developmental stages and sampling dates. We had two goals: (1) to document the levels of AMF root colonization, and (2) to examine the plant stress responses during imposed water stress in the three *Stipa* species. We tested the hypothesis that the effects of water stress on *Stipa* grasslands depend upon the interactions between plant AMF status and plant species. No consistent patterns in AMF root colonization frequency were observed relative to soil water levels. This suggests that AMF root colonization is highly plastic and responds to environmental factors that we do not yet understand. Mycorrhiza colonization was greater in the late-seral than in the earlier-seral species. Higher AMF root colonization levels in *S. clarazii* may partly explain its higher competitive ability in comparison with the earlier-seral species.

Key words: ecology, mycorrhiza, perennial grasses, soil water regimes, successional stages

Introduction

Giorgetti *et al*. (1998, 1999, 2000) demonstrated the replacement of early- by late-seral, more competitive species in long-term exclosures to domestic livestock and in well managed grasslands of central Argentina (H. D. Giorgetti pers. comm.). This has associated a change in floristic composition with a change in plant community structure. Higher levels of AMF root colonization in the late-seral, perennial grass *Stipa clarazii* than in the earlier seral *S. tenuis* and *S. gynerioides* (Saint Pierre *et al*. 2004b) could help explain the observed species replacement process in the study plant community.

Mycorrhizal symbiosis generally increases host-plant nutrient uptake, drought tolerance, resistance to soil-borne pathogens and grazing tolerance, and may also bind soil into stable aggregates that resist erosion (Wallace 1981, Hayman 1983, Menge 1986, Allen *et al*. 1989, Allen 1991). Under water stress, mycorrhizal

fungi can improve plant water economy (Sieverding 1981). At the same time, however, water stress can reduce formation of arbuscular mycorrhizal fungal (AMF) root colonization in several grass species, which is in part dependent on the stress intensity (Mohammad *et al*. 1982, Bethlenfalvay & Dakessian 1984, Bethlenfalvay *et al*. 1985, Allen *et al*. 1989, Auge *et al*. 1995, Busso *et al*. 2003). Augé (2001) reviewed several mechanisms as to how water stress intensity may influence AMF root colonization levels in several plant species.

Stipa clarazii, *S. tenuis* and *S. gynerioides* were chosen for this study because they are abundant in the semiarid rangelands of central Argentina under different management regimes. *Stipa clarazii* is a late-seral, palatable, dominant and highly competitive species under exclosure or light grazing conditions (Saint Pierre *et al*. 2004a, 2004c). Under moderate grazing, this species is replaced by the palatable *S. tenuis*. Selective grazing of both species results in their replacement by the early-seral, unpalatable, and less competitive *S. gynerioides*. In this study, we examined the AMF root colonization and plant water status in the three species of *Stipa* in response to soil moisture availability.

Under soil water deficit, plants colonized by AMF can recover more rapidly than uncolonized plants (Allen & Boosalis 1983, Henderson 1997). A variety of mechanisms can help colonized plants to recover from water stress. Mycorrhiza can alter root morphology, allowing the plants to explore a greater soil volume and acquire a greater share of the soil resources (i.e. water and nutrients) in comparison with non-mycorrhizal plants (Kothari *et al*. 1990). Mycorrhizal hyphae can increase root water uptake, providing an adequate water supply to preserve the plant's physiological activity, particularly under conditions of severe water stress (Read 1984). Mycorrhizal hyphae outside the roots can also adhere soil to the roots, thus maintaining a better root-soil contact during water stress, which may facilitate root water uptake.

It has been shown that plants can compete better when they are mycorrhizal (Bethlenfalvay & Linderman 1993, Hetrick *et al*. 1994), which is associated with the greater resource acquisition of mycorrhizal plants. Since *Stipa clarazii* is competitively superior to *S. ambigua* and *S. tenuis* (Saint Pierre *et al*. 2004a), it might be that the greater competitive ability of *S. clarazii* could, at least in part, be due to a greater degree of AMF root colonization. However, mycorrhizas can actually have a range of interactions with plant hosts although they are most well known for their mutualistic assistance in nutrient acquisition for the hosts. The net costs in some instances may outweigh the net benefits provided by mycorrhizae depending on the nutrient availability (Fitter & Hay 1983), which could provide an obstacle in competition with other plants.

Cool season C_3 perennial tussock grasses, like those in our study, generally have well-developed, highly branched root systems and are only weakly dependent on AMF root colonization for nutrient uptake (Baylis 1972, Hetrick *et al*. 1988). Consistent, high percentages of AMF root colonization in C_4 perennial grasses, however, support the interpretation that they may function as obligate mycotrophs in semi-arid as well as in mesic environments (Hetrick *et al*. 1990, Hartnett *et al*. 1994, Wilson & Hartnett 1997). High AMF root colonization in these C_4 perennial grasses may preclude, compared with C_3 species, AMF root colonization from being one of the mechanisms contributing to competitive ability in C_4 late-seral species (Busso *et al*. 2001). However, higher values of AMF root colonization in C_3 perennial grasses could be one of the mechanisms contributing to competitive ability in late-seral species such as *S. clarazii*. This is because of the above-mentioned beneficial effects of mycorrhiza on the mechanisms which can provide a greater competitive ability (*see* also Sieverding 1981, Kothari *et al*. 1990, Augé 2001).

We had two goals for this study: (1) to document the levels of AMF root colonization, and (2) to examine plant stress responses during imposed water stress in the three *Stipa* species. We used these data to test the hypothesis that the effects of water stress in *Stipa* grasslands depend upon the interactions between plant AMF status and the plant species. Studies like this are a vital foundation for subsequent understanding of how ecological communities work. This study contributes to highlight the role of AMF root colonization in establishing competitive hierarchies

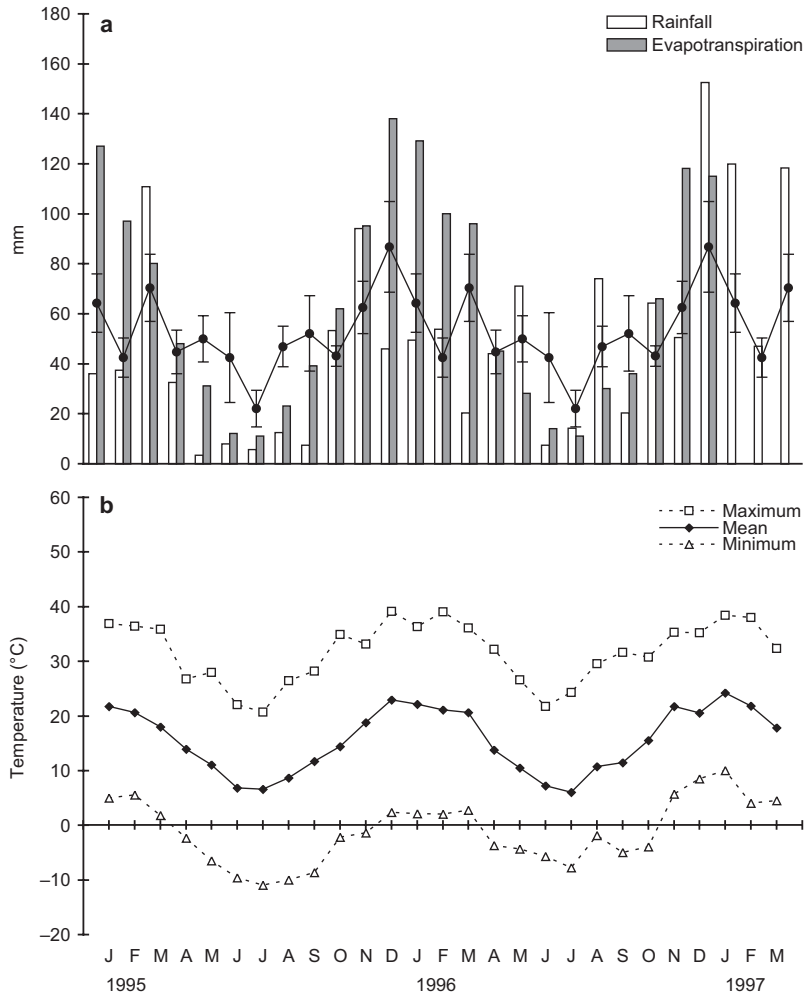

Fig. 1. — **a**: Monthly rainfall, long-term (1987–1996) mean (± 1 SE of the mean) monthly rainfall, and mean monthly potential evapotranspiration (thornthwaite: De Fina & Ravelo 1973). — **b**: Absolute minimum and maximum and mean monthly air temperatures to 0.25 m above the soil surface during 1995, 1996 and early 1997. Measurements were taken using a meteorological station located 100 m away from the experimental plots.

among related plant species, and adds to our broader understanding of the factors determining the abundance and distribution of organisms.

Material and methods

Study area

The studies were conducted at the research site nearby the Departamento de Agronomía-CERZOS in Bahía Blanca (38°48´S, 62°13´W). The soil is a typical *haplustol* with a petrocalcic horizon at 1.8 m depth. It has a loam-sandy texture; 1.9% organic matter; 24 to 50 mg kg^{-1} extractable P (Olsen & Sommers 1982); 0.10% total N and a pH of 7.4. Climatic information during the study period (1995, 1996 and early 1997) was provided through a meteorological station located at the research site (Fig. 1).

Experimental design

Between December 1993 and April 1994, 28 experimental plots (1.8 × 1.8 m; 2 m distance one from each other) were established in the field on unploughed, weeded soil. These plots covered a total of 325 m^2 within a plain, even soil surface area. Plants were obtained from a 20 year-exclosure to domestic animals located southeast of La Pampa Province (38°45´S, 63°45´W). Within each plot, transplants were placed 30 cm apart from one another in seven horizontal and verti-

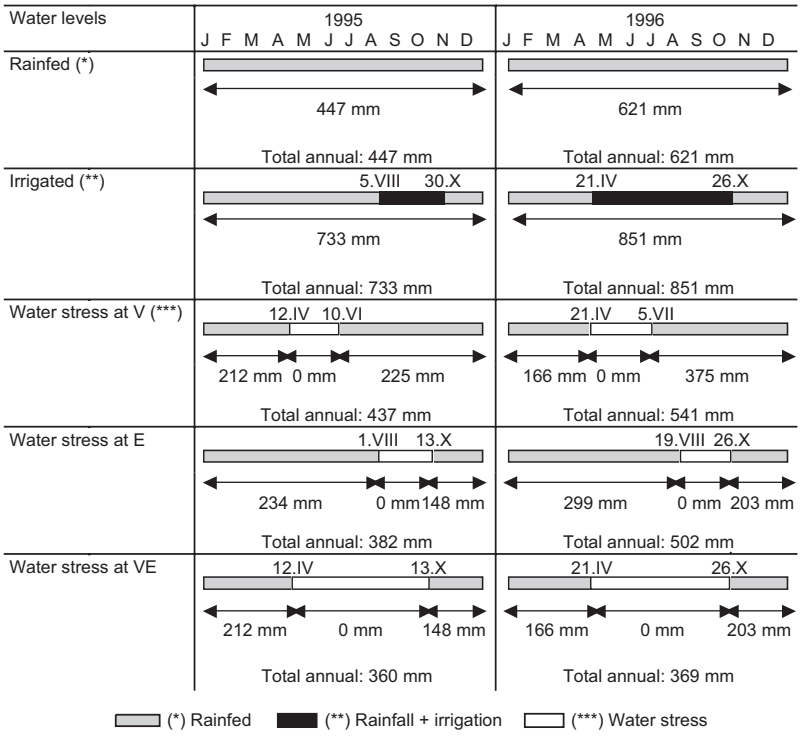

Fig. 2. Periods of imposition of the different water levels [natural (Rainfed plots), Irrigation (Irrigated plots) or Water-stress (Water-stressed plots)] at the vegetative (V), internode elongation (E) or both (VE) phenological stages in 1995 and 1996. Numbers below horizontal, bold lines are water fallen during each water level treatment. Numbers above horizontal histograms indicate initiation and finalization of a specific water level treatment.

cal rows such that each plant of *S. clarazii* or *S. tenuis* was surrounded by four plants of *S. gynerioides*. Disposition of plants within a uniform matrix contributes to reduce potentially confounding effects on plant responses as a result of plant competition. A total of 1372 transplants were used for the whole study. Crown-level plant diameters ($n = 56$) were similar among species at the time of transplanting: 13.47 ± 0.56 cm (mean ± 1 SE) for *S. clarazii*, 10.02 ± 0.51 cm for *S. tenuis*, and 12.27 ± 0.61 cm for *S. gynerioides*.

All tussocks of *S. clarazii* and *S. tenuis* were hand-clipped to a 5-cm stubble height in January 1995, during the plant quiescent period. From a total of 28 experimental plots, eight were randomly assigned to the irrigated and eight to rainfed treatments, and four plots to each of the water stress treatments.

Water regimes

Plants were exposed to rainfed, irrigated or water stress conditions. Rainfed plots received rainfall all year round (Fig. 2). A drip irrigation system watered the irrigated plots, which were additionally rainfed. Soil tensiometers installed in the irrigated plots allowed watering of these plots to saturation whenever they reached 60% of field capacity. Periods of irrigation and imposition of water stress during 1995 and 1996 are depicted (Fig. 2). The water-stressed plots were covered with plastic sheets (0.2 mm thick) whenever rain fell at periods when these species are often exposed to water stress in their native environment (Busso 1997): vegetative or early internode elongation, or both phenological periods (Fig. 2). The plastic sheets were mounted on a specially designed wooden structure that provided an open-sided rain shelter. Studies which used rain shelters similar to ours demonstrated slight changes in air temperature and relative humidity between the environments inside and outside them while the plots were covered (Jacoby *et al.* 1986). Water-stressed plots were surrounded with plastic sheets up to 1.8 m soil depth to prevent lateral movement of water into these plots (cf. Asay & Johnson 1990, Johnson *et al.* 1990, Johnson & Rumbaugh 1995, Asay *et al.* 2002, Jensen *et al.* 2002).

All 28 experimental plots received 313.7 mm water from mid-October 1995 to late April 1996, and 487.8 mm from late October 1996 to March 1997. Water-stressed plots were thus alleviated from water stress during these periods by receiving natural rainfall.

Sampling procedures

Leaf water potentials were periodically determined at midday in all treatments to provide a measure of plant water status during the study period. Measurements were done using a pressure-chamber on sunny days only between noon and 13:00. Youngest, fully expanded leaf blades were taken for these measurements using one tiller per species within each replicate plot and sampling date. From excision to the end of each determination, leaves were cut one at a time and maintained in a plastic bag to reduce water loss (Turner 1987).

A total of 460 soil plus root samples were obtained from between 0–15 cm soil depth using a soil corer (8.4 cm diameter, 15 cm height: 831.3 cm^3 volume) during 1996 and early 1997. Samplings were conducted on 18 April (mid-autumn), 6 June (late autumn), 21 September (early spring) and 20 October (mid-spring) 1996, and 28 February (late summer) 1997. Parallel, labor-intensive studies on these plots prevented us from taking soil plus root samples during 1995. Samples were obtained diagonally from the plant periphery to the plant center to assure that sampled roots corresponded to the sampled plant. One plant of each species was used per replicate at each sampling date. Sampled plants of *S. clarazii* and *S. tenuis* were always surrounded by four plants of *S. gynerioides*. Also, sampled *S. gynerioides* plants were always surrounded by two individuals each of *S. tenuis* and *S. clarazii*. Roots were obtained after washing the soil samples through a 60 mesh screen (Williams & Baker 1957), and they were maintained at 4 °C in a solution of formaldehyde, glacial acetic acid and ethanol (Phillips & Hayman 1970). Roots were cut into 15 mm segments, cleared and stained for determination of AMF root colonization at 100× magnification (Busso *et al*. 2001). Three fields on each of thirty root segments were scored in each water level treatment for presence or absence of hyphae, vesicles and arbuscules for each plant.

Statistical analysis

Leaf water potentials were analysed using a three-way ANOVA (5 water levels × 3 species × 4 sampling dates: April, June and September 1996, and February 1997) in split plot. Soil water levels were the main factor applied to randomly distributed plots, in an imbalanced but proportional manner; there were four replicates for the irrigated and rainfed treatments, and two replicates for each of the water stress treatments (vegetative, internode elongation and vegetative plus internode elongation). There were plants of the three species within each plot; one plant of each species was assigned to be sampled within each sampling date. Secondary factors were the sampling dates and sampled species. Interactions were analyzed to evaluate the effects of water levels, dates and species. Since interactions involved water levels, species and sampling dates, comparisons were conducted (1) among water levels for each sampling date, and (2) among sampling dates for each water level on each of the study species individually. Means were compared using LSD at 5%, when the *F*-test indicated that the variables were different at 5% (Steel & Torrie 1981).

At first, AMF root colonization data were analyzed using a three-way split plot ANOVA (5 water levels × 3 species × 5 sampling dates: April, June, September and October 1996, and February 1997). Soil water levels acted as main factors, applied to randomly distributed plots, in a proportional but imbalanced manner. Eight replicates were used for the irrigation and rainfed treatments, and four replicates were utilized for each of the water stress treatments (vegetative, internode elongation, vegetative plus internode elongation). Plants of the three species were within each plot, assigning one plant of each species for analysis at each sampling date. Secondary factors were sampling dates and species. Within each plot, plants were assigned for sampling previous to the sampling dates. This allowed avoiding measurements of root param-

eters corresponding to nearby plants previously sampled. However, this rigid scheme did not allow replacement of loss plants (i.e., plants which died as a result of treatment application) during the study. Because of this, it was necessary to adapt the statistical analysis when the loss of sampling units resulted in an imbalance not proportional among the species within each plot. This phenomenon occurred mainly in June and September.

In this way, we have a 'Design 1' using a three-way ANOVA for those months where information was complete: April, October and February. Months with missing data (June and September) were analyzed with a 'Design 2': a split-plot two-way ANOVA with the same main factor (soil water levels) and a unique secondary factor (the species). Incomplete plots, which lack information on the three species, were eliminated to apply this analysis, leaving an imbalanced, proportional design (2a). When this procedure eliminated too many replicates, maintenance of the design was considered more adequate, but only between pairs of species (2b).

Interactions were analyzed to evaluate the effects of water levels, dates and species. Means were compared with Fisher's protected LSD at 5% when the *F*-test indicated that the variables differed at that significance level (Steel & Torrie 1981).

Results

Leaf water potentials

In April 1996 and February 1997, the leaf water potentials of *S. clarazii*, *S. gynerioides* and *S. tenuis* did not differ ($p > 0.05$) among soil water levels (data not shown). However, the leaf water potentials were lower ($p < 0.05$) under water stress at the (1) vegetative and (2) vegetative plus internode elongation developmental stages, than under rainfall or irrigated conditions in June 1996 for all three species (Table 1 and Fig. 3). Also, leaf water potentials of *S. clarazii*, *S. tenuis* and *S. gynerioides* were lower ($p < 0.05$) under water stress in the internode elongation and vegetative plus internode elongation stages than under the other water levels in September of the same year.

Lowest leaf water potentials ($p < 0.05$) were found in September 1996 and February 1997 under rainfed and irrigated conditions in *S. clarazii* and *S. gynerioides* (data not shown). In June 1996, leaf water potentials were lower ($p < 0.05$) than in September of the same year when water stress occurred at the vegetative stage in *S. clarazii* and *S. gynerioides*. In September 1996, leaf water potentials were lower ($p < 0.05$) than values in June in the internode elongation stage,

Table 1. Analysis of variance for leaf water potential (June and September 1996) and AMF root colonization data (%) (April and October 1996, and February 1997) in plants of *Stipa clarazii*, *S. tenuis* and *S. gynerioides* exposed to water stress, rainfed or irrigated conditions.

	Leaf water potential		AMF root colonization	
Variation source	df	MS	df	MS
Water levels	4	5.6905***	4	261.9630*
Error 1	9	0.1716	23	108.3834
Dates	3	4.1446***	2	328.4391*
Species	2	0.4974*	2	5402.2337**
Water levels × dates	12	3.4678***	8	79.9179^{ns}
Water levels × species	8	0.2260^{ns}	8	547.8707**
Dates × species	6	0.6333***	4	125.5059^{ns}
Water levels × dates × species	24	0.1116^{ns}	16	82.6935^{ns}
Error 2	99	0.1292	184	78.2305
Weighed error	108	0.1327	207	81.5808

df = degree of freedom; MS = mean square, ns = $p > 0.05$; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$.

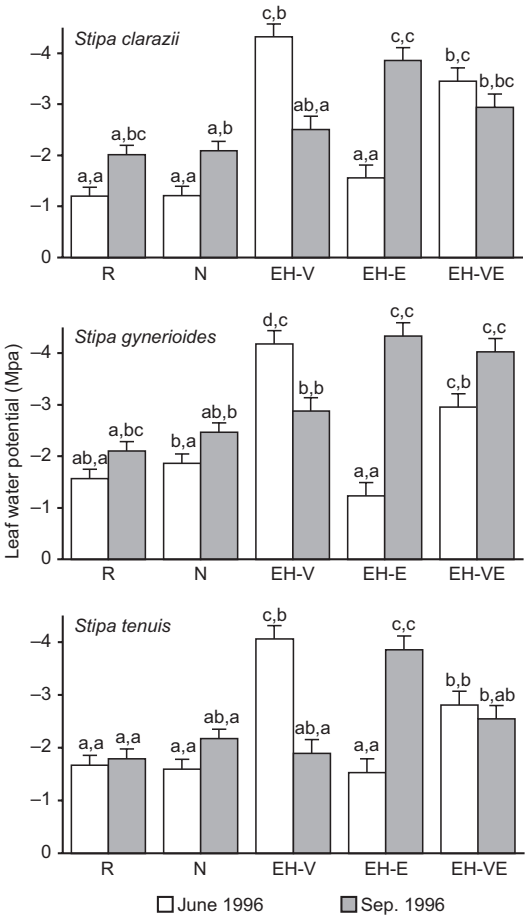

Fig. 3. Mid-day leaf water potential (MPa) of plants of which were exposed to irrigated (I), rainfed (R) or water stress (WS) conditions at the vegetative (V), internode elongation (E) or both (VE) phenological stages during June and September 1996. Each histogram is an average of n = 2–4. Vertical bars represent 1 SE of the means. Different letters to the left of the comma indicate significant differences ($p < 0.05$) within each species, and those to the right of the comma indicate significant differences ($p < 0.05$) among water levels.

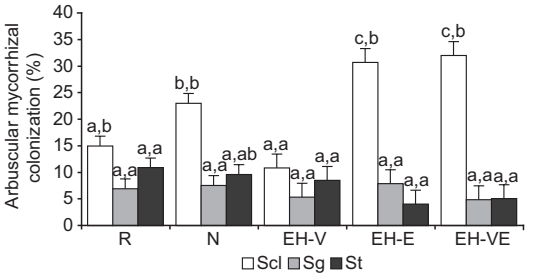

Fig. 4. AMF root colonization (%) of *Stipa clarazii* (Scl), *S. gynerioides* (Sg) and *S. tenuis* (St) exposed to irrigated (I), rainfed (R), or water stress (WS) conditions during the vegetative (V), internode elongation (E) or both phenological stages (VE) in April and October 1996, and February 1997. Each histogram is the mean of n = 12–24. Vertical bars represent 1 SE of the means. Statistical analysis was performed using Design 1-complete data April/Oct/Feb. Different letters to the left of the comma indicate significant differences ($p < 0.05$) among water levels within each species. Different letters to the right of the comma indicate significant differences ($p < 0.05$) among species within a soil water treatment.

and greater ($p < 0.05$) than values in June at the vegetative stage in all three species (Table 1 and Fig. 3).

AMF root colonization

Levels of colonization were significantly different among species ($p < 0.05$) after analyzing the significant ($p < 0.05$) interaction between water levels and species (Table 1). AMF root colonization levels were greater ($p < 0.05$) in *S. clarazii* than in the other two species under irrigated, rainfed and water stress in the internode elongation and vegetative + internode elongation phenological stages during April and October 1996, and February 1997 (Table 1 and Fig. 4). At this time, only *S. clarazii* showed significant differences ($p < 0.05$) among water levels in percentage AMF root colonization. This species showed the greatest ($p < 0.05$) values for this parameter under rainfed and water stress in the internode elongation and vegetative + internode elongation phenological stages (Fig. 4).

In June 1996, percentage AMF root colonization was greater ($p < 0.05$) in *S. clarazii* than *S. tenuis* under water stress in the vegetative + internode elongation phenological stage (Table 2 and Fig. 5). In addition, the plants of *S. clarazii* reached, on average for all water levels, a 30% higher ($p < 0.01$) AMF root colonization than *S. gynerioides* (6%) (Fig. 5). Percentage AMF root colonization in *S. tenuis* was greater ($p < 0.05$) under irrigation than under rainfed and water stress conditions in the vegetative and vegetative + internode elongation phenological stages (Fig. 5). In addition, percentage AMF root colonization was greater ($p < 0.05$) in *S. tenuis* than in *S.*

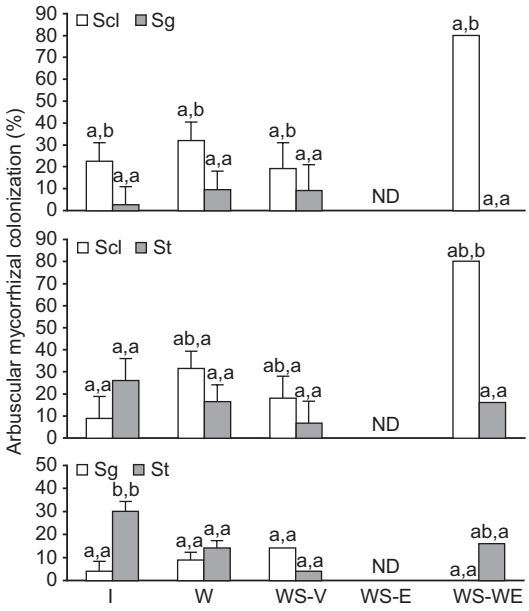

Fig. 5. AMF root colonization (%) of *Stipa clarazii* (Scl), *S. gynerioides* (Sg) and *S. tenuis* (St) exposed to irrigated (I), rainfed (R) or water stress (WS) conditions during the vegetative (V), internode elongation (E) or both (VE) phenological stages in June 1996. Each histogram is the mean of n = 1–5. Vertical bars represent 1 SE of the means. Histograms without SE bars come from n = 1. Statistical analysis was performed using Design 2-imbalanced/proportional data. Different letters to the left of the colon indicate significant differences (p < 0.05) among water levels within each species. Different letters to the right of the colon indicate significant differences (p < 0.05) between species within a soil water treatment. ND = not determined.

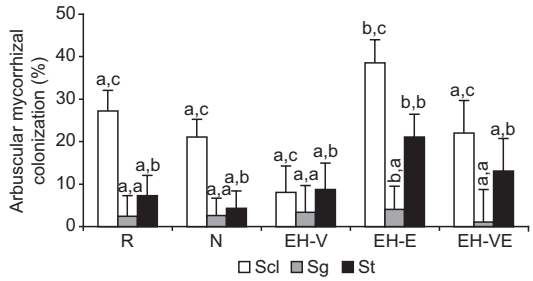

Fig. 6. AMF root colonization (%) of *Stipa clarazii* (Scl), *S. gynerioides* (Sg) and *S. tenuis* (St) exposed to irrigated (I), rainfed (R) or water stress (WS) conditions during the vegetative (V), internode elongation (E) or both (VE) phenological stages in September 1996. Each histogram is the mean of n = 2–7. Vertical bars represent 1 SE of the means. Statistical analysis was performed using Design 2-imbalanced/proportional data. Different letters to the left of the colon indicate significant differences (p < 0.05) among water levels within each species. Different letters to the right of the colon indicate significant differences (p < 0.05) between species within a soil water treatment.

gynerioides under irrigated conditions (Table 2 and Fig. 5).

In September 1996, all three species achieved highest (p < 0.05) levels of AMF root colonization when water stress was imposed in the internode elongation developmental stage (Table 2 and Fig. 6). *Stipa clarazii* showed greater (p < 0.01) AMF root colonization levels than the other two species under all soil water contents (Table 2 and Fig. 6). Additionally, levels of AMF root colonization were greater (p < 0.01) in *S.*

Table 2. Analysis of variance for AMF root colonization data (%) during June and September 1996 in plants of *Stipa clarazii*, *S. tenuis* and *S. gynerioides* exposed to water stress, rainfed or irrigated conditions.

	S. clarazii and *S. gynerioides* (June)		*S. clarazii* and *S. tenuis* (June)		*S. gynerioides* and *S. tenuis* (June)		*S. clarazii*, *S. gynerioides* and *S. tenuis* (September)	
Variation source	df	MS	df	MS	df	MS	df	MS
Water levels	3	443.4200^{ns}	3	690.1675^{ns}	3	66.2^{ns}	4	353.9546*
Error 1	7	260.5743	8	407.5898	6	51.7	16	155.5096
Species	1	3300.6400**	1	617.2218^{ns}	1	605.0**	2	2485.7908***
Water levels × species	3	599.9900^{ns}	3	879.8444*	3	218.2*	8	137.3333^{ns}
Error 2	7	304.0957	8	187.4918	6	57.1	32	98.6088
Weighed error	14	282.3350	16	297.5408	12	54.4	48	117.5758

df = degree of freedom; MS = mean square, ns = p > 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001.

tenuis than in *S. gynerioides* under all levels of soil water availability (Table 2 and Fig. 6).

The AMF% root colonization correlated with plant water potential only in *S. tenuis*. In this species, the leaf water potential was negatively correlated ($p = 0.05$) with levels of AMF root colonization in June 1996 (AMF% = 34.217–6.3323 MPa, $n = 8$, $r = –0.70$).

AMF root colonization levels were greater ($p < 0.05$) in April 1996 (early autumn; mean ± 1 SE: 14.3% ± 1.0%) than in October (early spring; 10.3% ± 0.9%) 1996 and February (summer; 11.1% ± 1.0%) 1997.

Discussion

We manipulated the soil moisture availability during the growth of three *Stipa* species that differed in their successional status. We found that percentage mycorrhizal colonization and plant water potential both demonstrated strong species-dependent responses to the imposed moisture regimes. We have to recognize that the scope of data interpretation might be limited in this study because the samplings were conducted for only one growing season (January 1996–February 1997). However, 1996 appeared to be a rather typical, average year at the study site. For example, rainfall during this year (621 mm) was fairly similar to the long-term mean (633.9 ± 58.3 mm, mean ± 1 SE) at the same research area during 1987–1996. No long-term evapotranspiration data were available.

AMF root colonization was higher in *S. clarazii* than in *S. tenuis* and *S. gyneroides*, and even increased under certain drought conditions. Simpson and Daft (1990) found that levels of AMF root colonization in maize and sorghum were not affected by low soil water availability. Sieverding (1981) and Mahmood and Iqbal (1982) indicated that colonization levels by mycorrhizal fungi were negatively correlated with soil water content. Also, Allen and Boosalis (1983) found no differences for winter wheat between wet and dry treatments.

Our data provide no definitive answers regarding the effects of soil moisture on AMF root colonization in the three tussock grasses, even though colonization in *S. clarazii* sometimes increased under water stress conditions. Few significant differences were found in all three species, and these were often not consistent among soil water levels. The importance of AMF root colonization in natural communities, therefore, may lie at the scale of individual root-fungal infections. The species-specific plasticity shown by *S. clarazii*, *S. tenuis* and *S. gynerioides* to the investigated soil moisture regimes might be a major factor to explain the persistence of these species in the plant communities of the study region.

Plants of all three species had lower leaf water potentials under water stress than under irrigated conditions during June–September 1996. The wetter 1996 than 1995 determined higher leaf water potentials during that period in the rainfed than in the water-stressed plots. Higher AMF root colonization significantly correlated with higher plant water potential only in one study species (*S. tenuis*) at a specific date. This indicates the difficulty of finding this relationship more often in field studies, where plants can freely explore the whole soil profile for resources. Higher leaf water potentials, which allow continuation of physiological activity, in highly AMF root colonized plants, might well contribute to drought tolerance of these plants. Busso *et al.* (2003) found that these species osmotically adjust under water stress; the degree of osmotic adjustment during the internode elongation developmental stage was 0.50 MPa in *S. clarazii* and 0.42 MPa in *S. gynerioides* when both species reached a midday leaf water potential of –6.15 ± 0.35 MPa.

Our results support the posted hypothesis. Higher AMF root colonization levels in *S. clarazii* than in the other two species may help to explain that *S. clarazii* is more abundant than the other two species both in well managed grasslands and in exclosures to the access of domestic livestock (Giorgetti *et al.* 1998, 1999, 2000).

Soil moisture largely determines the available nutrient supply and affects water flow through a plant, and the plant's photosynthetic C gain. Although AMF root colonization improves the water throughflow in some grasses, compared with non-mycorrhizal root systems (Allen & Allen 1986), our field data on the influence of soil water status on AMF root colonization were

inconclusive. One might speculate extensively on the reasons for the inconsistent AMF root colonization responses to severe perturbation of the host. However, it is clear that simple predictions of AMF root colonization responses to reduced host C inputs and allocation due to water stress are not adequate to explain the results we obtained. Crick and Grime (1987) postulated that plants from variable habitats show a high degree of plasticity when faced with patchy resources. Arbuscular mycorrhiza effects on grasses are sensitive to perturbations in the environment; increased water uptake with AMF occurred only during dry periods (Allen & Allen 1986). Despite the environmental control in our sampling protocol, and the severe perturbations imposed, AMF root colonization may respond to much smaller resource shifts than has been supposed. Fungal symbiosis is one of the factors which make the result interpretation between the relationship of percentage AMF root colonization and soil water levels more complicated. For example, drought effects in the vegetative developmental stage of sorghum on the root colonization by hyphae, vesicles and arbuscles depended on the fungal species (Auge *et al*. 1995). When the fungus was *Glomus intraradices*, hyphae and arbuscules colonization was reduced under drought when compared with higher soil water contents. However, no differences existed between soil water levels when the fungus was *Glomus etunicatum*. The authors concluded that in general the interaction between AMF root colonization and water stress was not significant.

Results shown by other studies sustain that annual drought dynamics influence the seasonality of the AMF root colonization. If drought periods alternate frequently enough, causing increments in root ramification densities, with wet periods, stimulating root elongation, AMF root colonization levels can be higher (Babel 1981, Kottke & Agerer 1983). In the present study, the different soil water contents were alternatively more or less exposed to water stress and rewetting periods. This might have contributed to the relatively high values for AMF root colonization, despite the high levels of soil available phosphorus (24–50 ppm).

Levels of AMF root colonization were in general greater in the C_3 *S. clarazii* than in the other two C_3 species, in agreement wih the second hypothesis. These results very likely contribute to explain the greater competitive ability in *S. clarazii* (Saint Pierre *et al*. 2002, 2004a, 2004c, 2006). Cool season C_3 perennial tussock grasses generally have well-developed, highly branched root systems and are only weakly dependent on AMF root colonization for nutrient uptake (Baylis 1972, Hetrick *et al*. 1988). Consistent, high percentages of AMF root colonization in C_4 perennial grasses, however, support the interpretation that they may function as obligate mycotrophs in semi-arid as well as in mesic environments (Hetrick *et al*. 1990, Hartnett *et al*. 1994, Wilson & Hartnett 1997). High AMF root colonization percentages in these C_4 perennial grasses may preclude, compared to C_3 species, that percentage root colonization by AMF is one of the mechanisms contributing to competitive ability in late-seral C_4 species (Busso *et al*. 2001). Results obtained in this study indicate that the higher AMF root colonization percentages in the late-seral C_3 *S. clarazii* than in the earlier-seral C_3 *S. tenuis* and *S. gynerioides* may contribute to determine the competitive hierarchies among the study species, leaving *S. clarazii* as the superior competitor as compared with the other two species.

Our results, however, differ from those of Saint Pierre *et al*. (2004b), who did not detect significant differences between *S. clarazii* and *S. tenuis* in their AMF root colonization percentage. The experimental design, history of use and edaphic characteristics were different in those studies. This emphasizes the importance in considering these characteristics when comparing different studies. In addition, the fact that some arbuscular mycorrhizal fungi are ecologically distinct in their distribution with, and their species richness can be positively related to, soil moisture content (Miller 2000, He *et al*. 2002, Beauchamp *et al*. 2006) can influence why responses may not be seen and/or are different among studies. In addition, changes in species composition of mycorrhizal fungi also make a difference in external hyphae. Hart and Reader (2002) reported that different species of mycorrhizal fungi obtained from the grasses *Poa pratensis* and *P. annua* and other forbs, produced different amounts of extra-radical hyphae, which

may not be related to the internal hyphae. That is, the impacts of water stress on mycorrhizae can be determined not only by the internal but also by the external hyphae. The importance of extra-radical hyphae to water transport was recently emphasized by Querejeta *et al*. (2007). Colonization levels found in *S. clarazii*, *S. tenuis* and *S. gynerioides* were similar to those found in other annual and perennial C_3 and C_4 grasses (Busso *et al*. 2001).

AMF root colonization levels have been associated with soil nutrient acquisition in perennial grasses (Jackson & Caldwell 1996). These parameters contribute to a greater competitive ability in these species (Jackson & Caldwell 1996). This is very important in low productive environments, such as that of our study species, where competition for soil resources is high (Jackson & Caldwell 1996). Because of this, mycorrhizal fungi can affect competitive interactions among plant species by improving soil nutrient uptake (Marschner & Dell 1994, Mohammad *et al*. 1998). *Stipa clarazii*, for example, showed a greater ^{15}N uptake than *S. tenuis* and *S. ambigua* under rainfed conditions (Saint Pierre *et al*. 2004c, 2006). Given the general similarity in root length densities between *S. clarazii*, *S. tenuis* and *S. gynerioides* (Busso *et al*. 2003), differences in soil nutrient acquisition might then be associated, at least in part, with differences in AMF root colonization in these species.

Levels of AMF root colonization decreased from April to October 1996 and February 1997 in all three species. Variation in colonization levels during the year were also reported by Busso *et al*. (2001), where those parameters decreased from early to late spring in other C_4 perennial grasses. Similar to our results in the southern hemisphere, Saint Pierre *et al*. (2004b) found a different pattern of AMF root colonization, with AMF root colonization increases from October to December in *S. clarazii* and from September to October in *S. tenuis*. The differences between our study and that of Saint Pierre *et al*. (2004b) outlined in the previous paragraph, may contribute to explain the observed differences in the obtained results.

Knowledge of a seasonal colonization pattern is necessary to quantify the functional and ecological significance of mycorrhiza fungi (Sanders & Fitter 1992). Periods when AMF root colonization is high are those when the fungi highly influence the plant nutritional state and exert a plant carbon demand. Highest AMF root colonization in *S. clarazii*, *S. tenuis* and *S. gynerioides* were obtained during the vegetative developmental stage in early fall. It is likely that this was due to the high growth rates that these species have during that period (Busso *et al*. 2003), which requires an adequate supply of water and nutrients. These soil resources may have been supplied, at least in part, throughout an adequate association with mycorrhizal fungi.

Greater mycorrhizal colonization in the late- than earlier-seral C_3 study perennial grasses may be due to the fact that mechanisms of competition (i.e., root proliferation, root length density, nutrient uptake rates and shoot relative growth rates: Moretto & Distel 1997, 1999, Saint Pierre *et al*. 2002, Saint Pierre *et al*. 2004a, 2004c, 2006, Busso & Bolletta 2007) are greater in the late-seral *S. clarazii* than in the other two *Stipa* species. This ended up with more than 15% greater above-ground biomass in the late- than earlier seral *S. tenuis* under irrigated, rainfed and water stress conditions, which might have provided a greater share of photoassimilates to the associated mycorrhizal fungi (Bolletta 2006).

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