The distribution and phenology of arbuscular mycorrhizae along an inland salinity gradient

Perry C. Johnson-Green, Norm C. Kenkel, and Thomas Booth

Abstract: The distribution and seasonal patterns of arbuscular mycorrhizal fungi activity were studied in an inland boreal salt pan site in north-central Manitoba. Semipermanent study regions were set up in each of five vegetation zones along a continuous salinity gradient. Roots of *Hordeum jubatum*, *Distichlis stricta*, *Agropyron trachycaulum*, *Sonchus arvensis*, *Spartina gracilis*, and other species were collected from the study regions over six time periods: April, June, July, August, and October of 1991, and May of 1992. These roots were used to quantify mycorrhizal colonization, as well as arbuscule and vesicle formation. Arbuscular mycorrhizal fungi were prevalent in the three vegetation zones with lowest soil salinity, with >40% of the observed root pieces colonized. Colonization was <2% in the other two zones, where soil salinity was consistently greater throughout the growing season. The only common pattern in the phenology of mycorrhizal activity was a low level of activity in the early spring. Mycorrhizal activity in most plant species occurred at high levels throughout the summer and fall. Differences in patterns of activity appeared to be linked to differences in phenology of root growth, and not to edaphic differences among vegetation zones.

Key words: Manitoba, arbuscules, fungi, gradient, mycorrhiza, phenology, salinity, vesicles.

Résumé : Les patrons saisonniers et de distribution de l'activité des champignons mycorhiziens arbusculaires ont été étudiés dans une cuvette saline, à l'intérieur des terres au centre-nord du Manitoba. Des régions d'études semi-permanentes ont été établies dans chacune des cinq zones de végétation, le long d'un gradient continu de salinité. A partir des régions d'étude, et à six reprises dans le temps, les auteurs ont récolté les racines des *Hordeum jubatum*, *Distichlis stricta*, *Agropyron trachycaulum*, *Sonchus arvensis*, *Spartina gracilis* et autres espèces : les récoltes ont été effectuées en avril, juin, juillet, août et octobre 1991, ainsi qu'en mai 1992. Ils ont utilisé ces racines pour quantifier la colonisation mycorhizienne, ainsi que la formation des arbuscules et des vésicules. Les champignons mycorhiziens arbusculaires dominent dans les trois zones de végétation les moins salines, plus de 40% des morceaux de racines observés étant colonisés. La colonisation est inférieure à 2% dans les deux autres zones, là où la salinité du sol est constamment la plus forte au cours de la saison de croissance. Le seul patron commun dans la phénologie de l'activité mycorhizienne est le faible degré de colonisation observé tôt au printemps. Chez la plupart des espèces de plantes, on observe une forte activité mycorhizienne tout au cours de l'été et de l'automne. Les différences dans les patrons d'activité semblent liées aux différences phénologiques de la croissance des racines, et non aux différences édaphiques entre les zones de végétation.

Mots clés : Manitoba, arbuscules, champignons, gradient, mycorhize, phénologie, vésicules.
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Introduction

Arbuscular mycorrhizal fungi (AMF) are commonly encountered in plant communities throughout the world (Mosse et al. 1981). Although many glasshouse studies have demonstrated benefit to plant hosts from AMF associations, it has been difficult to unequivocally demonstrate benefit to plants in the field (Fitter 1991). This has created problems in assessing the role of AMF in natural communities. One approach taken to address such problems has been to collect roots from a range of species in a particular community to determine the extent of mycorrhizal colonization and the potential importance of mycorrhizae (e.g., Brundrett and Kendrick 1988). Another approach involves quantifying AMF distribution and abundance along clearly defined environmental gradients, along which both plant species and edaphic conditions vary.

Soil salinity exerts powerful effects on both individual plant species distributions and community structure (Ungar 1991; Burchill and Kenkel 1991). Salinity is also expected to strongly affect the distribution and abundance of mycorrhizal

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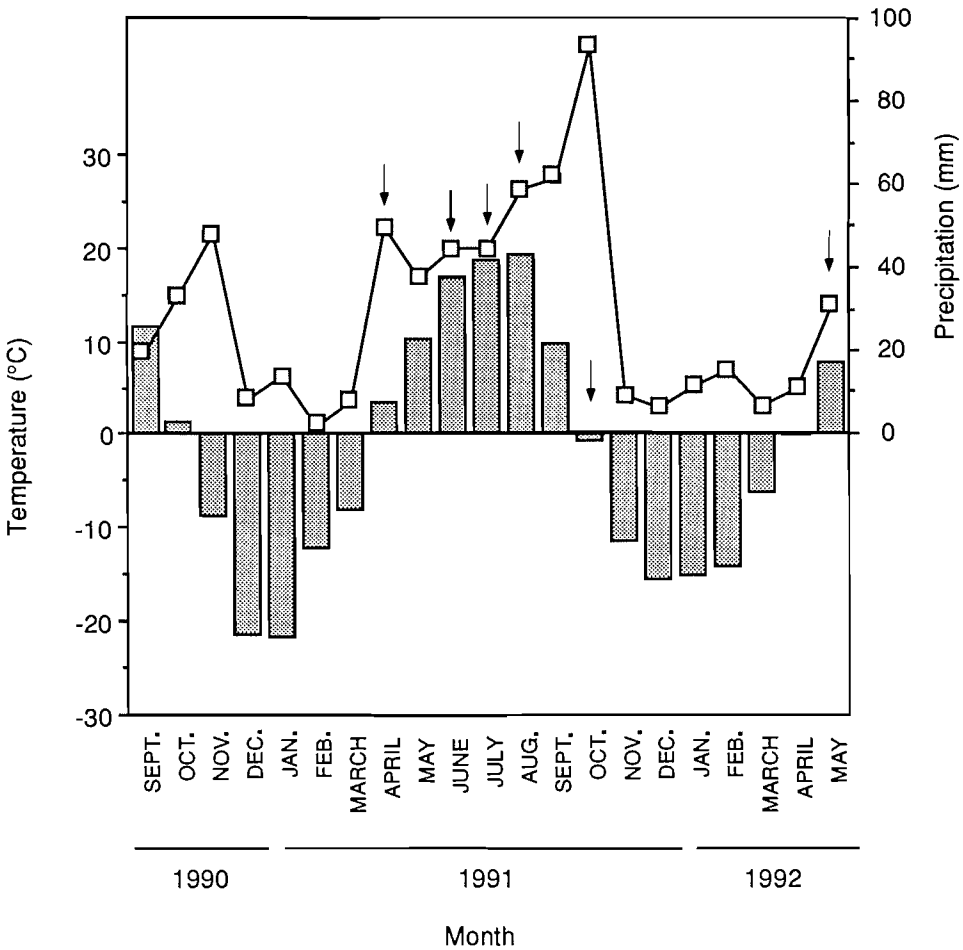
Fig. 1. Monthly mean temperature (bars) and total precipitation (□) at The Pas, Manitoba, for the period Sept. 1990 – May 1992 (from Environment Canada climate data). The Pas is 100 km north of the study area. Arrows indicate months when sampling took place.



fungi. Although soil salinity gradients are common in both coastal and inland ecosystems, such areas have rarely been the focus of studies on mycorrhizal fungi. AMF have been observed in some species of salt-marsh plants (e.g., Mason 1928; Sengupta and Chaudhuri 1990). In a coastal salt marsh in the Netherlands, Rozema et al. (1986) found that the distribution of AMF was related to host phylogeny (mycorrhizal vs. non-mycorrhizal families) rather than spatial position in the marsh.

Inland salt pans are commonly encountered in grassland and desert regions throughout the world (Ungar 1991). These saline ecosystems offer the opportunity to study the effects of soil salinity on vegetation structure and composition, without the confounding influence of tidal inundation. Two studies in North American inland saline communities have documented a negative relationship between mycorrhizal abundance and soil salinity (Kim and Weber 1985; Ho 1987). However, mycorrhizal presence and activity (presence of arbuscules) were not differentiated in these studies, nor was the potential importance of seasonal changes in mycorrhizal abundance considered. These are important factors to consider, as they provide information on the relationship between mycorrhizal phenology and host phenology, which are in turn important to the understanding of the role of AMF in natural systems (Sanders and Fitter 1992*a*).

If AMF are important to their hosts, then peak times of host growth should be accompanied by high levels of mycorrhizal activity. However, patterns in mycorrhizal activity may be linked to seasonal changes in the environment rather than patterns of host activity. The comparison of different host species helps differentiate between these two possibilities. Specifically, if phenological patterns of fungal activity vary between host species within a site, then AMF are responding to seasonal changes in host growth. However, if the phenology of fungal activity is invariant across host species, then AMF are probably reacting to environmental signals.

Our goal is to examine the seasonal distribution of AMF activity along an inland salinity gradient in boreal central Manitoba. Specific objectives are to determine the distribution of AMF along the gradient and to compare the phenology of mycorrhizal activity in five species at the site.

Study area

The study site is a large salt flat located in west-central Manitoba, Canada, 1 km from the shore of Overflow Bay, Lake Winnipegosis (53°05′N, 101°07′W). Subterranean groundwater saline seeps and springs occur all along the western shore of Lake Winnipegosis. The dominant ions of

Table 1. Dominant species and soil salinity and phosphorus of the five vegetation zones.

Vegetation zone	Dominant species	Total salts[a] (mg/mL)	Soil phosphorus[b] (mg/kg)
Salicornia	*Salicornia rubra*	78.4±7.3	11.8±13.7 (19)
Puccinellia	*Puccinellia nuttalliana*, *Suaeda depressa*, *Triglochin maritima*	55.3±5.7	19.0±8.8 (24)
Hordeum	*Hordeum jubatum*, *Distichlis stricta*, *Grindelia squarrosa*	41.9±13.4	37.8±14.4 (41)
Agropyron	*Agropyron trachycaulum*, *Sonchus arvensis*, *Aster ericoides*	23.0±9.1	33.5±14.7 (21)
Rosa	*Rosa acicularis*, *Spartina gracilis*, *Aster laevis*	19.8±6.1	53.4±13.0 (14)

[a]Mean ± SD of five soil cores from each zone in August 1991.
[b]Mean ± SD (from Burchill and Kenkel 1991), with sample size in parentheses.

the saline springs are Na^+ and Cl^- (van Everdingen 1971; McKillop et al. 1992).

Concentric zones of vegetation of differing salt tolerance occur around these hypersaline unvegetated salt pans. *Salicornia rubra* (nomenclature follows Looman and Best 1987) occurs around salt-pan margins, with vegetation zones characterized by the grasses *Puccinellia nuttalliana*, *Hordeum jubatum*, and *Agropyron trachycaulum*, and the shrub *Rosa acicularis* occurring on progressively less saline soils. A complete description of vegetation–environment relationships in the region is given by Burchill and Kenkel (1991). The soil, a rego-gleysol of the series "saline flats" (Hopkins and Smith 1982), is derived from recently exposed lake flats. Soils in the area are somewhat alkaline (pH range of 7–9) and very calcareous. Organic matter accumulation is low (5–15%) in the most saline zones, but increases steadily with decreasing soil salinity (Jones 1991).

The climate is subhumid continental and characterized by short, warm summers and long, cold winters. Mean annual temperature is −0.3°C, and mean annual precipitation ≈50 cm, at The Pas (100 km north of the study site). Precipitation occurs throughout the year, but is somewhat higher in summer. Temperature and precipitation data for the study period are presented in Fig. 1. Higher summer temperatures result in increased evapotranspiration, leading to markedly elevated soil salinities by late summer (Jones 1991).

Materials and methods

Field Sampling

A large semipermanent study region was set up in each of five vegetation zones (Table 1). Five randomly selected root systems of two species were excavated from the study regions during each of six sampling periods (April 23–25, June 15–17, July 27–29, August 17–19, and October 4–6 of 1991, and May 21–23 of 1992), except in the *Salicornia* zone where only one species (*S. rubra*) was collected. Roots were cleaned, and great care was taken to ensure that contamination by the roots of other plants was minimized. Cleaned roots were preserved in 50% ethanol. Roots of *S. rubra* (*Salicornia zone*), *P. nuttalliana* and *Triglochin maritima* (*Puccinellia* zone), *H. jubatum* and *Distichlis stricta* (*Hordeum* zone), *A. trachycaulum* and *Sonchus arvensis* (*Agropyron* zone), and *R. acicularis* and *Spartina gracilis* (*Rosa* zone) were collected when live roots were present. Other species were collected during times of sparse root growth.

Staining of roots

Preserved roots were cleared in 2.5% KOH, acidified in 1% HCl, and stained with 0.05% trypan blue (Koske and Gemma 1989). The "root-piece" method was used to quantify AMF activity (Kormanik and McGraw 1982). Fifty root segments (0.5 cm long) from each sample were mounted in 50% glycerol and examined at 250× with a Leitz Orthoplan compound microscope. Each root piece was scored for the presence of arbuscules, vesicles, or hyphae. Since arbuscules are the site of nutrient exchange between fungus and host (Bonfante-Fasolo and Scannerini 1992), they are reliable indicators of AMF activity. Conversely, the presence of vesicles indicates quiescence of the AMF. The relative prevalence of AMF hyphae in a root system is a measure of AMF colonization, but not necessarily of AMF activity.

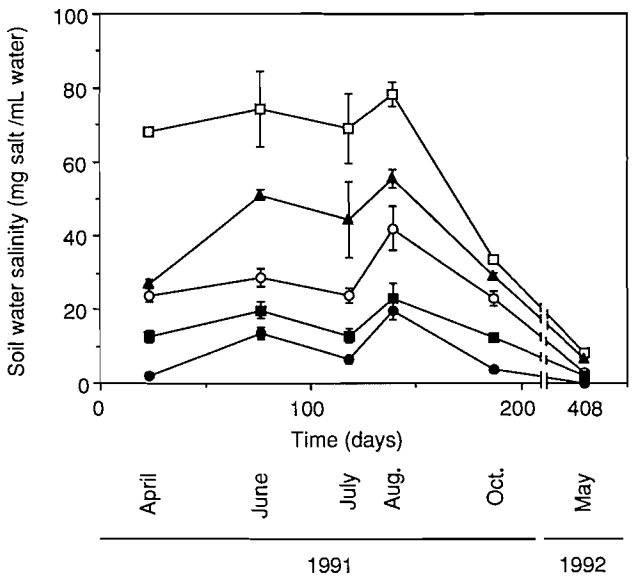
The advantage of the root-piece method over the often-used grid line intersect procedure is that it allows the inspection of roots at high magnification, which reduces subjectivity in the identification of mycorrhizal structures (McGonigle et al. 1990). The method also allows the quantification of arbuscules and vesicles. However, the root-piece method has been criticized because it consistently overestimates colonization (McGonigle et al. 1990) and has larger standard errors than other methods (Giovanetti and Mosse 1980). To alleviate these criticisms, the length of each root piece was reduced from 1 to 0.5 cm, and root segments from the July, August, October, and May collections were scored on a 0–4 intensity of colonization scale (0 = 0%, 1 = 1–25%, 2 = 26–50%, 3 = 51–75%, and 4 = 76–100% colonization of the root cortex). Such estimates are not possible with any of the intersection methods (McGonigle et al. 1990).

Table 2. Soil nutrients (mg/kg of soil, mean ± SD) in three of the vegetation zones.

Vegetation zone	N	P	K	Ca	Mg	Na
Agropyron	56±24	20±9	402±198	10 854 ± 6 129	677±82	7 227 ± 2 119
Hordeum	83±3	11±5	508±92	10 093 ± 5 545	714±67	10 138 ± 2 393
Puccinellia	43±7	13±1	>600	71 450 ± 9 958	891±11	12 408 ± 238

Note: N was determined by calcium chloride extraction; P and K were determined by acetic fluoride extraction; and Ca, Mg, and Na were determined by ammonium acetate extraction.

Fig. 2. Soil water salinity in vegetation zones of the salt pan. Each symbol is the mean ± SE of 5 values. □, *Salicornia* zone; ▲, *Puccinellia* zone; ○, *Hordeum* zone; ■, *Agropyron* zone; ●, *Rosa* zone.



Soil analysis

Soil collections from the top 10 cm of soil were taken concurrently with root collections. To minimize site disturbance, soil salinity was estimated from dilution extracts, which requires less soil than saturation paste methods. Dried soil (10 g) was diluted with 50 g of deionized water and shaken for 1 h. Extracts were then passed through filter paper, and pH and electrical conductivity (EC) of the extracts measured using a platinum electrode and an Analytical Instrument (Houston, Tex.) pH–conductivity meter. Total salt content of the extract (640 × EC in $dS \cdot m^{-1}$; Rhoades and Miyamoto 1990) and the gravimetrically determined soil moisture content were used to calculate total salts (mg) per millilitre of soil water.

Additional soil samples were taken from the *Agropyron*, *Hordeum*, and *Puccinellia* zones in August 1992 for soil nutrient determination. Nitrate and phosphate were measured colorimetrically, and extractable calcium, magnesium, sodium, and potassium by inductively coupled plasma (ICP) spectrophotometry. Analyses were performed by Norwest Laboratories, Winnipeg.

Inoculum-potential bioassay

The numbers of AMF propagules in soil from each of the five vegetation zone were estimated using a soil-dilution bioassay. Soil was collected in late August 1991, stored at 4°C, and used in the bioassay 6 weeks later. The initial dilution of soil samples was 1/10, followed by five serial dilutions of 1/3. However, soil from the *Salicornia* and *Puccinellia* zones was only diluted three times (final dilution of 1/90), because preliminary results indicated low numbers of AMF propagules in these zones. The diluent was autoclaved soil (120°C for 1 h) from the same zone as the inoculum. Each dilution was replicated six times. To allow for potential differences in mycorrhizal host specificity (Adelman and Morton 1986), each dilution series was performed on two bait plants: *A. trachycaulum* and an additional species that was abundant in a given zone (see Table 4). Bait plants were seeded into 5 cm diameter pots containing diluted soil. Plants were grown in a greenhouse, with 130 $\mu m\ E \cdot m^{-2} \cdot s^{-1}$ supplemental illumination and a mean ambient temperature of 22°C. Each pot was thinned to 5 plants, and after 85 days roots were harvested and stained as previously described. Roots from each pot (n = 192) were scored for AMF colonization using a stereomicroscope. The most-probable number of AMF propagules (MPN) was then calculated for each zone (Alexander 1965). The calculated MPN is probably an underestimate of the number of propagules in the soil, because of the disturbance associated with soil dilution.

Data Analysis

To reveal seasonal trends in AMF activity, we analyzed colonization data from *S. gracilis*, *A. trachycaulum*, *S. arvensis*, *H. jubatum*, and *D. stricta* (plants in the *Puccinellia* and *Salicornia* zones were not analyzed, since very few plants were colonized in these zones). Colonization of each species was analyzed separately. Proportions of root segments with AMF colonization (PRC), arbuscules (PRA), and vesicles (PRV) were each analyzed using one-way analysis of variance (ANOVA) to test for differences between sampling times. To conform with ANOVA assumptions, proportional variables were arcsine square-root transformed (Ott 1988). When transformation was unsuccessful, a nonparametric Kruskal–Wallis test was performed. Intensity of colonization was also analyzed using one-way ANOVA, but without transformation (since residuals were normally distributed). Soil nutrient values were separately analyzed using one-way ANOVA. Differences were considered statistically significant if $p < 0.05$.

Results

Soil analysis

At each sampling date (except May 1992), we found a consistent hierarchy in soil salinity across vegetation zones. As expected, soil salinity was highest in the *Salicornia* zone, and progressively decreased through the *Puccinellia*, *Hordeum*,

Table 3. Summary of AMF colonization in plants of the salt pan.

Vegetation zone	Host species	Host family	Roots with AMF[a]	Roots with arbuscules[b]	Roots with vesicles[c]	n[d]	Months of sampling[e]
Salicornia	*Salicornia rubra* A. Nels.	Chenopodiaceae	0.9±0.7	0.1±0.1	0.6±0.6	16	June, July, Aug., Oct.
Puccinellia	*Suaeda depressa* (Pursh) S. Wats.	Chenopodiaceae	0	0	0	7	July, Aug.
	Puccinellia nuttalliana (Schultes) Hitch.	Poaceae	1.5±2	0.7±0.3	0.8±0.3	28	April, June, July, Aug., Oct., May
	Triglochin maritima L.	Triglochinaceae	1.4±0.9	0.3±0.1	0.9±0.6	23	April, July, Aug., Oct., May
Hordeum	*Hordeum jubatum* L.	Poaceae	74.3±25.0	58.9±5.6	7.4±1.0	24	April, June, July, Aug., Oct., May
	Aster pauciflorus Nutt.	Asteraceae	50.5±9.6	8.3±3.7	29.4±6.6	2	May
	Distichlis stricta (Torr.) Rydb.	Poaceae	75.7±3.1	57.6±5.0	10.2±1.9	15	June, July, Aug., Oct.
	Atriplex patula L.	Chenopodiaceae	70.0	42.0	28.0	1	Aug.
	Grindelia squarrosa (Pursh) Dunal	Asteraceae	87.0±9.0	81.0±9.0	9.0±1.0	2	July
Agropyron	*Agropyron trachycaulum* (Linke) Malte	Poaceae	79.4±3.3	58.4±4.0	15.9±1.2	29	April, June, July, Aug., Oct., May
	Aster ericoides L.	Asteraceae	50.2±18.6	15.9±9.9	22.9±13.9	6	April, May
	Sonchus arvensis L.	Asteraceae	90.1±3.5	15.9±9.9	26.0±4.0	6	July, Aug., Oct., May
Rosa	*Rosa acicularis* Lindl.	Rosaceae	80.0±8.1	11.3±4.6	69.0±3.9	13	July, Aug., May
	Spartina gracilis Trin.	Poaceae	73.6±6.0	38.3±5.3	21.0±4.5	18	April, July, Oct., May
	Aster laevis L.	Asteraceae	60.5±14.3	21.7±10.0	24.8±10.6	7	April, June, Aug.
	Calamagrostis inexpansa A. Gray	Poaceae	44.8±13.0	6.5±3.9	21.0±10.2	5	April

[a]PRC: mean percentage (±SE) of roots (50 observed per root system) with AMF colonization.
[b]PRA: mean percentage (±SE) of roots (50 observed per root system) with arbuscules.
[c]PRV: mean percentage (±SE) of roots (50 observed per root system) with vesicles.
[d]Number of root systems examined.
[e]April–October of 1991 and May of 1992.

Table 4. Levels of mycorrhizal inoculum in soil from the five vegetation zones.

Vegetation zone	Bait species	Mycorrhizal inoculum (MPN/g of soil)
Salicornia	*Agropyron trachycaulum*	0.69
	Salicornia rubra	0
Puccinellia	*A. trachycaulum*	0.73
	Puccinellia nuttalliana	0
Hordeum	*A. trachycaulum*	6 630
	Hordeum jubatum	11 398
Agropyron	*A. trachycaulum*	15 332
	Calamogrostis inexpansa	10 380
Rosa	*A. trachycaulum*	4 652
	Spartina gracilis	4 467

Agropyron, and *Rosa* zones (Fig. 2). Soil salinity was significantly greater in August than at all other times except June. Soil salinity was very low in May 1992, which appears to be the peak time of groundwater seepage (P.C. Johnson-Green, personal observation). The soil was saturated in the *Salicornia*, *Puccinellia*, and *Hordeum* zones in May of 1992 and 1993.

Soil nutrient analyses revealed that the *Puccinellia* zone had much higher concentrations of extractable calcium than did the *Hordeum* or *Agropyron* zones (Table 2). In contrast, sodium levels (and levels of other nutrients) were not significantly higher in the *Puccinellia* zone. It therefore appears that the higher soil salinity in the *Puccinellia* zone may be predominantly caused by calcium rather than sodium ions.

AMF colonization

AMF were present in virtually all (137/139) root samples taken from the *Hordeum*, *Agropyron*, and *Rosa* zones, but were much less common (20/74 samples) in the more saline *Salicornia* and *Puccinellia* zones (Table 3). PRC was $<2\%$ in plants from the *Salicornia* and *Puccinellia* zones and $>40\%$ in plants from the other zones. This difference was also apparent when uncolonized root systems were excluded; the mean PRC per colonized root system in the *Hordeum*, *Agropyron*, and *Rosa* zones was 76.0 ± 2.0 (mean ± SD), versus 4.5 ± 1.0 in the *Puccinellia* and *Salicornia* zones. These results indicate that AMF colonization was rare in plants growing in highly saline soil and, when it occurred, fewer roots were colonized than in plants growing in less saline soil.

In most plant species from the *Hordeum*, *Agropyron*, and *Rosa* zones, arbuscules were more often observed (PRA: 47.4 ± 2.5%) than were vesicles (PRV: 21.6 ± 2.0%). However, *R. acicularis*, *Aster pauciflora*, *Aster ericoides*, and *Calamogrostis inexpansa* all had colonizations dominated by vesicles (*C. inexpansa* and *A. pauciflora* were only collected in April 1991 and May 1992, however, which were times of low arbuscule frequency in most plants). Arbuscules were rarely seen in roots of *R. acicularis*, suggesting that AMF may not form mutualistic associations with this species.

With the exception of *P. nuttalliana*, high PRCs were observed in graminoid species. Colonization in composites was either very common (*Grindelia squarrosa* and *S. arvensis*) or relatively sparse (members of the genus *Aster*). With

Fig. 3. Mycorrhizal colonization and activity in *Hordeum jubatum*. Each symbol is the mean ± SE of at least 4 samples. Each sample consisted of 50 root pieces. Day 0 = April 1, 1991. (A) Percentage of root pieces that had AMF colonization (□), and the mean colonization intensity (●) of the root pieces. (B) Percentage of root pieces that had arbuscules. (C) Percentage of root pieces that had vesicles. Means identified by the same letter are not significantly different.

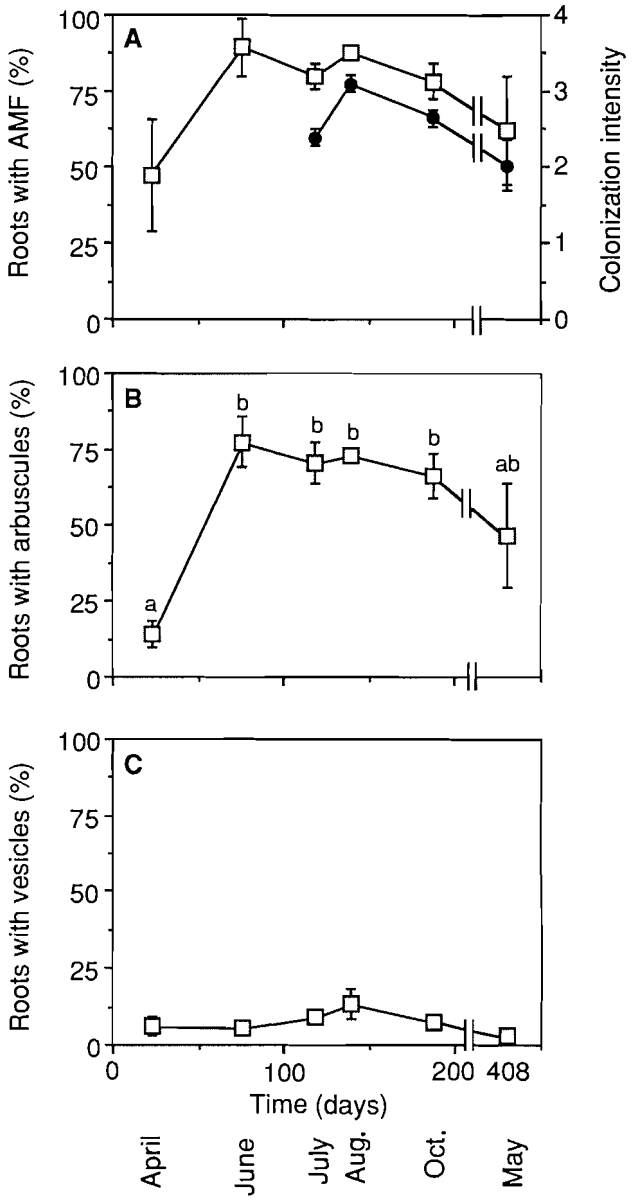
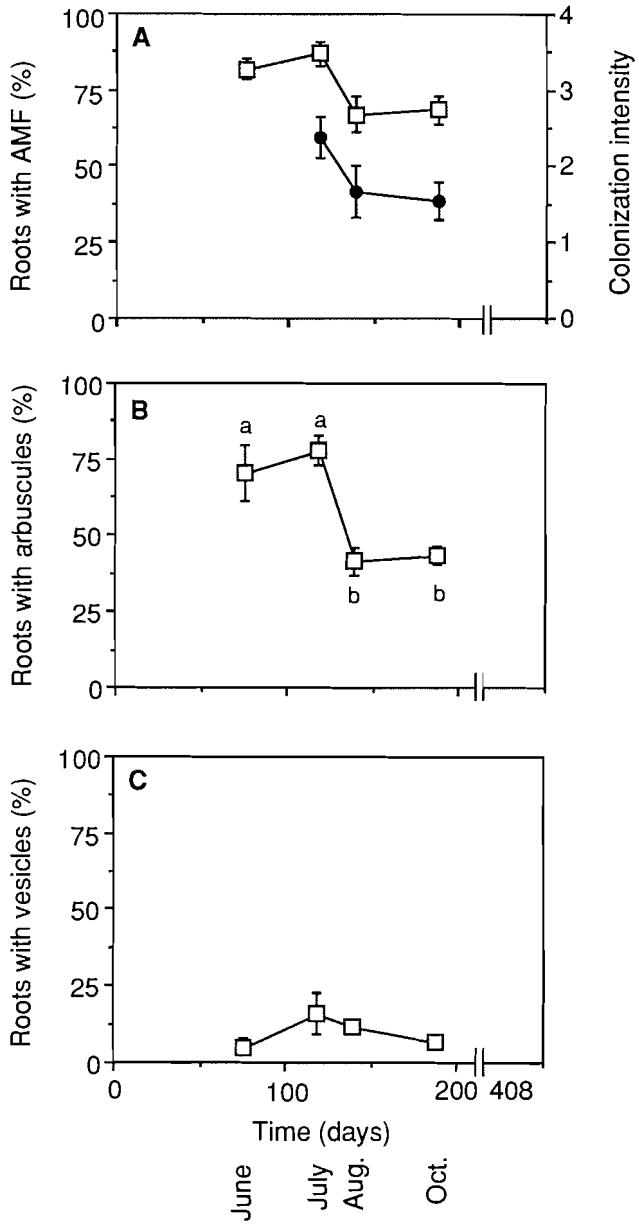


Fig. 4. Mycorrhizal colonization and activity in *Distichlis stricta*. Symbols and information as in Fig. 3.



the notable exception of *Atriplex patula*, plants from the usually nonmycorrhizal families Chenopodiaceae and Triglochinaceae had few colonized roots.

MPN of inocula bioassay

AMF colonization was rare in bioassay plants inoculated with soil from the *Salicornia* and *Puccinellia* zones, but MPN values were high in plants from the *Hordeum*, *Agropyron*, and *Rosa* zones (Table 4). Values were higher in the *Agropyron* and *Hordeum* zones than in the *Rosa* zone, but the significance of this is difficult to determine without knowledge of the spatial variation in inoculum density within vegetation zones.

Phenology of AMF infection

Hordeum zone

Root growth in *H. jubatum* began in April and continued through the growing season. There were no differences between sampling dates in PRC of *H. jubatum* (Fig. 3), though values were smaller and colonization much more variable in early spring (April 1991, May 1992). Intensity of colonization was greatest in August, but was not significantly higher than in other months. PRA was smaller in April than at any other time except May 1992. Vesicles were uncommon throughout the growing season.

The seasonal pattern of root growth in *D. stricta* was quite different (Fig. 4). Few roots were seen in April or May.

Fig. 5. Mycorrhizal colonization and activity in *Agropyron trachycaulum*. Symbols and information as in Fig. 3.

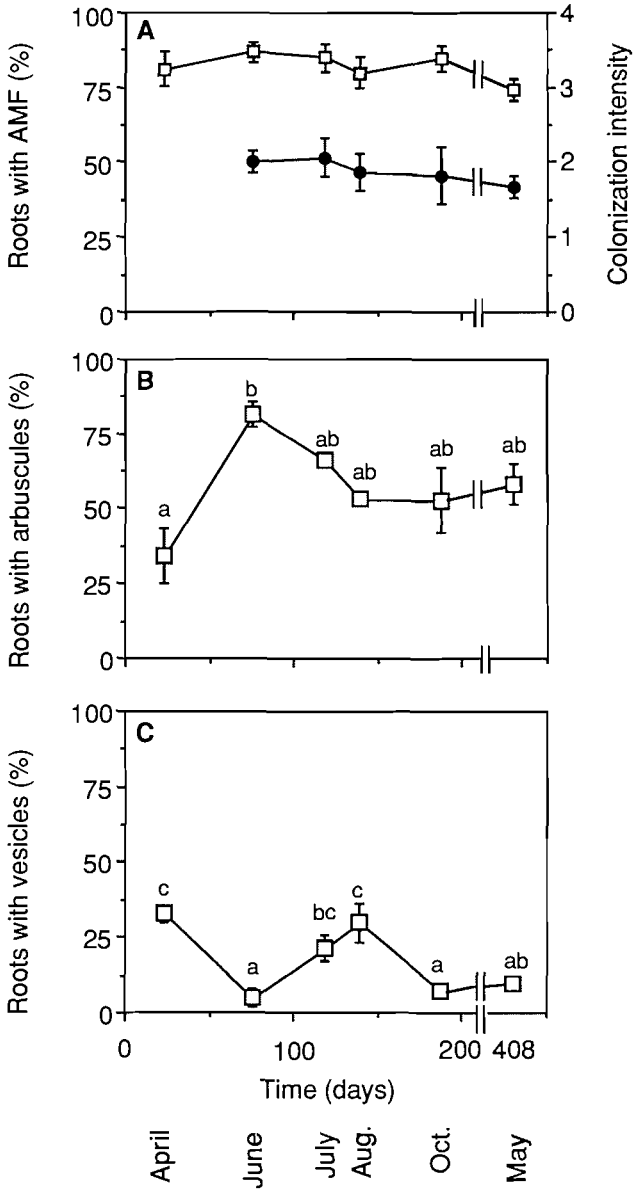
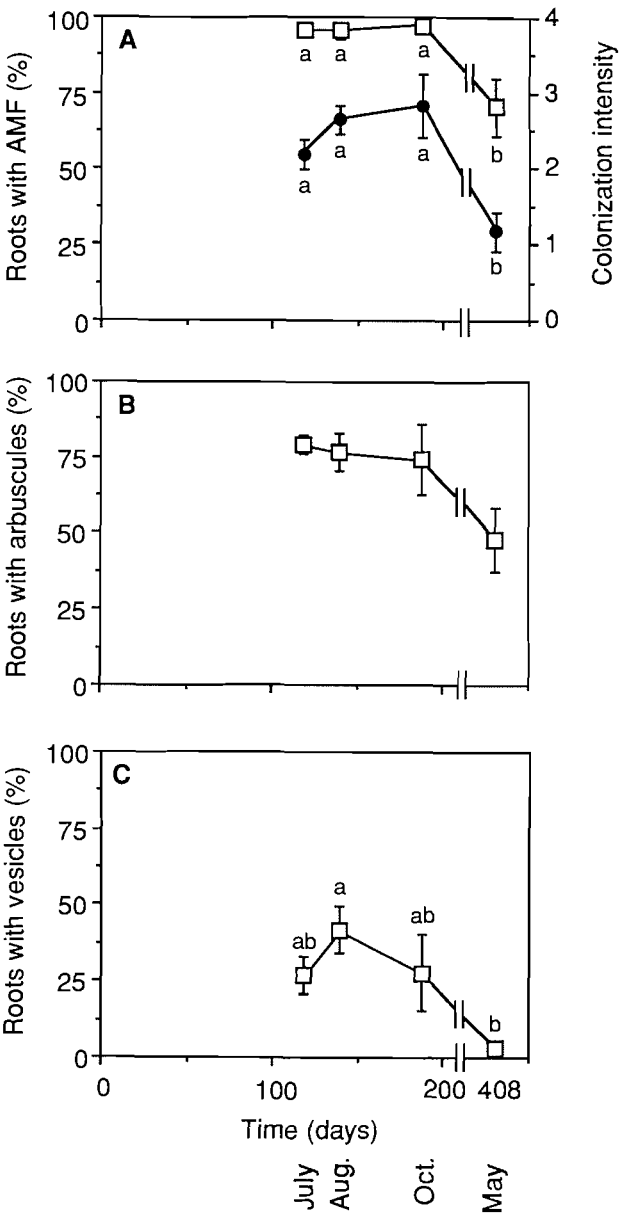


Fig. 6. Mycorrhizal colonization and activity in *Sonchus arvensis*. Symbols and information as in Fig. 3.



AMF colonization (PRC) was greater in July than in August or October. A late-summer (August–October) decline in PRA was observed in this species, but there were no differences in CI or PRV.

Agropyron zone

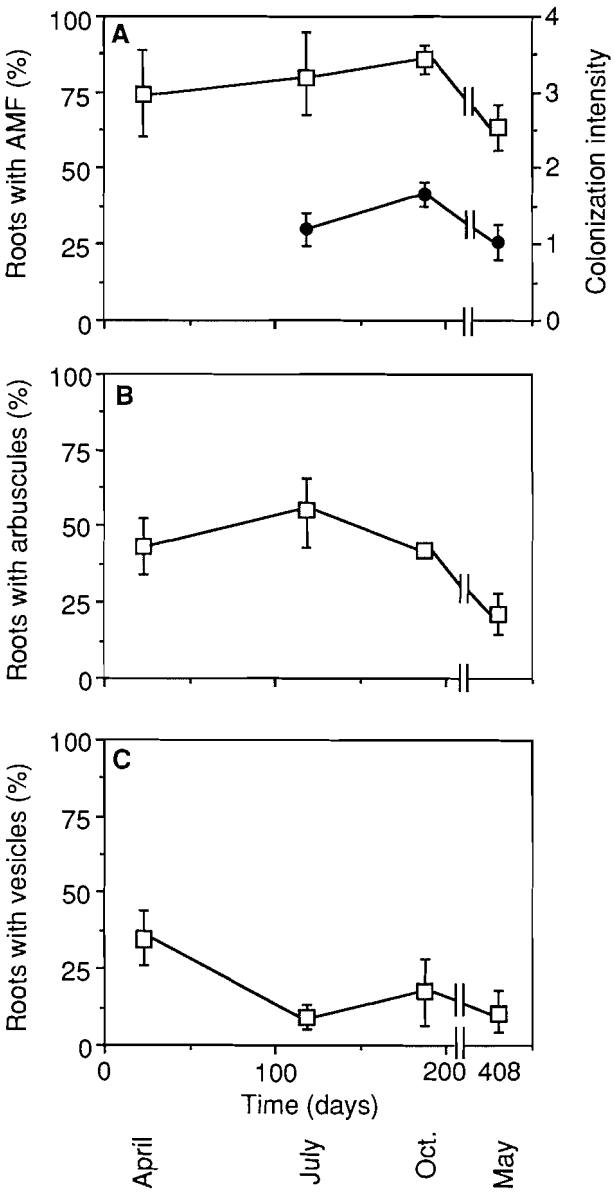
AMF infection and colonization intensity of *A. trachycaulum* were consistent throughout the sampling period (Fig. 5). In contrast, there was a complex pattern of arbuscule and vesicle production. Arbuscules (PRA) were uncommon in April, peaked in June, and declined gradually in July and August. Values were large but variable in October and May. Vesicles (PRV) were most common in April and August. There was a significant negative correlation ($r = -0.75$, $p < 0.001$) between the proportion of roots colonized with arbuscules versus those colonized with vesicles. This correlation was not observed in other host species.

Sonchus arvensis was also common in the *Agropyron* zone. As with *D. stricta*, few roots were observed in April or May, but roots were abundant later in the growing season. AMF infection and colonization intensity were both large in July, August, and October and smaller in May (Fig. 6). A similar pattern was observed in arbuscule production. Vesicles were more common in August than in May.

Rosa zone

The phenology of colonization proved more difficult to interpret in the low-salinity *Rosa* zone. *Rosa acicularis* roots were difficult to obtain, particularly in the early spring. We therefore made root collections of *S. gracilis* in April, July, and October 1992 and May 1993, and of *Aster laevis* in April, June, and August 1992. AMF infection of *A. laevis* was low (Table 3). In contrast, AMF colonization in

Fig. 7. Mycorrhizal colonization and activity in *Spartina gracilis*. Symbols and information as in Fig. 3.



S. gracilis was frequent from April to October, and in the following May (Fig. 7). Colonization intensity remained low throughout the growing season, however. Arbuscule and vesicle production were variable throughout the growing season, but values were highest in April 1992.

Discussion

Exclusion of AMF from saline soils

Our results indicate that AMF were infrequent in roots from the highly saline *Puccinellia* and *Salicornia* zones (the "Pucc−Sal" nonmycorrhizal zone), despite their prevalence in the *Hordeum*, *Agropyron*, and *Rosa* zones (the "Ho−Ag−Ro" mycorrhizal zone). Although the significance of differences in MPN within the Ho−Ag−Ro zone is difficult to assess without further knowledge of the extent of zonal variation in MPN, it is clear that MPN is considerably lower in the Pucc−Sal zone. Most plants in the Ho−Ag−Ro zone were mycorrhizal, including *A. patula*, which belongs to a genus and family (Chenopodiaceae) that is normally nonmycorrhizal. Allen (1983) also observed AMF colonization in the genus *Atriplex*.

The exclusion of AMF from the Pucc−Sal zone was also apparent in the inoculum-potential bioassay; inoculum was largely absent in soil from the Pucc−Sal zone, but was common in the Ho−Ag−Ro zone. The number of propagules in the Pucc−Sal zone may have decreased during the 6-week storage period, though later attempts to induce colonization of plants exposed to freshly collected soil also yielded uncolonized plants. It seems likely that exclusion of AMF from the Pucc−Sal zone is attributable to protracted periods of high soil salinity. The mean soil water salinity in the Ho−Ag−Ro zone approached that of seawater (3.4 mg salt/mL water) only in August, whereas it was at or above this level from June through August in the Pucc−Sal zone. The extreme salinities in the Pucc−Sal zone may have been caused by high levels of extractable calcium, and not sodium. This is surprising, since Na is usually 10 times more concentrated than calcium in saline springwater in the Overflowing River area (van Everdingen 1971; McKillop et al. 1992). The high concentration of Ca we observed may have been caused by dissolution of Ca-containing minerals by Na-rich groundwater in the spring (Javor 1989). Subsequent leaching of Na may then have led to predominance of Ca over Na in the soil exchange complex.

It is unlikely that the lack of AMF in the Pucc−Sal zone was caused by the absence of suitable hosts. AMF were rare in field-collected roots of *P. nuttalliana*, but we observed well-colonized roots when glasshouse-grown *P. nuttalliana* seedlings were exposed to soil from the *Agropyron* zone (P.C. Johnson-Green, unpublished data).

Another possible explanation for the lack of AMF in high-saline soils is the absence of salt-tolerant AMF species. It is also possible that our study site is near the northern limit of the distribution of AMF. AMF diversity has been found to decrease with increasing latitude (Koske 1987), and we found very low AMF diversity at our site. In numerous soil spore extractions, virtually all of the spores were identified as *Glomus etunicatum* Beck. & Gerd. By contrast, Pond et al. (1984) found six species (from 3 genera) of AMF in salt pan soils in the southwestern United States, which could explain their observation of AMF colonization in extremely saline soil (>60 000 ppm Na). However, Pond et al. (1984) did not quantify mycorrhizal activity, so it is possible that the colonization they observed was dormant. Results similar to ours were found in Utah salt playas by Kim and Weber (1985): AMF were common in *D. stricta* (<5000 ppm Na in soil) but rare in *Salicornia pacifica* (>10 000 ppm Na). The specific ion composition in salt-pan soil, rather than the concentration of Na, may be important in excluding AMF from certain soils. This could explain discrepancies between the levels of Na that appear to inhibit AMF in our study and those found by Kim and Weber (1985) and Pond et al. (1984).

Inhibition of AMF in spring

The most common seasonal pattern in AMF activity (vesicle and (or) arbuscule formation) across species was a low level

of activity in early spring (April 1991, May 1992). This was observed in *H. jubatum* and *A. trachycaulum*, and to a lesser extent in *S. arvensis* and *S. gracilis*. Few roots of *D. stricta* were found in April and May, and those observed were almost exlusively nonmycorrhizal.

Low levels of AMF activity and (or) colonization in early spring have often been observed (e.g., Sutton 1973; Jakobsen and Nielsen 1983; Cade-Menun et al. 1991), and there is strong experimental evidence indicating that AMF colonization and host benefit from AMF are decreased at low soil temperatures (Furlan and Fortin 1973; Daniels et al. 1984; Smith and Roncadori 1986). However, at least one study has demonstrated mycorrhizal benefit at cool temperatures (Volkmar and Woodbury 1989), and several studies have recorded moderate to high levels of AMF colonization or activity in the winter or early spring (Read et al. 1976; Giovanetti 1985; Mullen and Schmidt 1993). These inconsistencies may be related to differences in cold tolerance among species of AMF.

At our site, cold intolerance of AMF may not be the prime reason for spring inhibition of AMF, since colonization levels and activity of AMF in October were often as high as in the summer months. It seems more likely that the long, very cold winters lead to low levels of AMF inoculum (colonized roots, spores, and hyphae) the following spring. This, combined with rapid root growth in the spring, may result in low AMF activity. A direct comparison of the number of propagules before and after winter would be required to confirm this observation, however.

AMF phenology and host phenology

An enduring problem in AMF research has been the difficulty in demonstrating mycorrhizal benefit in the field. One recent strategy has been to determine whether the time of peak arbuscule formation coincides with a peak in phosphate uptake (Sanders and Fitter 1992*a*, 1992*b*; Mullen and Schmidt 1993). Host benefit is unlikely if AMF are active during times of low phosphate uptake, since there is little "return" on host "investment" in the association. Plants growing in inland salt pans are likely opportunistic in their nutrient uptake; periods of precipitation throughout the growing season lead to temporary decreases in soil salinity, providing plants with "windows of opportunity" for nutrient uptake. In such a scenario, it would be beneficial for the plants to retain the capability for root growth and nutrient uptake (via roots or mycorrhizae) throughout the growing season.

AMF phenology in different vegetation zones

AMF phenology was not identical in the *Hordeum* and *Agropyron* zones. *Agropyron trachycaulum* and *S. arvensis* both had a >25% increase in mean PRV in August, whereas PRB remained at low levels throughout the growing season in plants from the *Hordeum* zone (*H. jubatum*, *D. stricta*). Since the same species of AMF appeared to dominate both zones, it seems unlikely that this difference is attributable to differences in AMF species composition. Instead, differences in host salt tolerance may be important. *Agropyron trachycaulum* and *S. arvensis* have relatively low levels of salt tolerance (excluding them from the *Hordeum* zone), and may have suffered increased root death during times of peak soil salinity. This may in turn have led to increased vesicle production in these species. If this scenario is correct, then AMF may be unaffected by seasonal peaks of soil salinity unless the host plant is also affected. Field or greenhouse experiments are required to further clarify and confirm these observed differences in AMF phenology.

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