Soil salinity and arbuscular mycorrhizal colonization of

*Puccinellia nuttalliana*

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Arbuscular mycorrhizal fungi are common in moderately saline soil (colonized by *Distichlis stricta*) in an inland boreal salt pan in north-central Manitoba, but are absent from adjacent soil that is extremely saline (colonized by *Puccinellia nuttalliana*). In order to determine if this absence was due to the lack of suitable plant hosts or to edaphic factors, mycorrhizal colonization, vegetation composition, soil salinity and water content were examined along transects between the two vegetation zones. Correlation and principal component analyses revealed that mycorrhizal colonization of *P. nuttalliana* was positively linked to cover of *Distichlis stricta* and soil water content, and negatively linked to bare ground. The area closest to the point of salt seepage was uncolonized by mycorrhizal fungi. A transplant experiment confirmed that mycorrhizal fungi are unable to sustain colonization in this area of the salt pan. In this inland salt pan, colonization by mycorrhizal fungi is limited by edaphic factors, and not by the absence of suitable host plants, suggesting that mycorrhizal fungi have a lower limit of salinity tolerance than halophytes such as *P. nuttalliana*.

INTRODUCTION

Through experimental and field studies, it is clear that arbuscular mycorrhizal fungi (AMF) possess considerable tolerance to soil salinity (Mason 1928, Duke, Johnson & Koch 1986, Pfeiffer & Bloss 1988, Sengupta & Chaudhuri 1990). In order to determine the potential usefulness of mycorrhizal technology in the reclamation of saline soil, it is important to define the upper threshold of mycorrhizal salinity tolerance. With some exceptions (Hartmond *et al.* 1987, Graham & Syvertsen 1989), increased soil salinity consistently leads to decreased colonization by AMF (Ojala *et al.* 1983, Levy, Dodd & Krikun 1983, Duke *et al.* 1986, Rozema *et al.* 1986, Pfeiffer & Bloss 1988, Semones & Young, 1995, Baker, Sprent & Wilson 1995). However, few published experiments include a range of salinity that allows assessment of the upper threshold of tolerance. In a study of the effects of salinity on nodule and mycorrhiza formation and function in *Prosopis juliflora*, Baker *et al.* (1995) observed that irrigation water containing 0.3 m NaCl completely inhibited mycorrhizal colonization. In contrast, *Aster tripolium* was still colonized by AMF after irrigation treatments containing 0.3 m NaCl (Rozema *et al.* 1986). These contrasting observations are unlikely to be due to differences in salinity tolerance of the host plants, since *P. juliflora* plants were relatively unaffected by this level of salinity.

Another approach to assessing the salinity tolerance of AMF is to examine their distribution in saline soils. In coastal sites, AMF are usually sparse to absent in frequently inundated salt marsh plants (Khan 1974), but are common in upper reaches of both temperate (Mason 1928, Cooke & Lefer 1990, Cooke, Butler & Madole 1993) and tropical (Khan 1974, Sengupta & Chaudhuri 1990, Bhaskaran & Selvaraj 1997) salt marshes. In dune systems, AMF are typically poor colonizers of plants in leading and mobile sections, but are more common in inland dunes (Nicolson 1960, Koske & Halvorson 1980, Siguenza, Espejel & Allen 1996). The cause of these patterns is uncertain, because of the potentially important and interacting effects of flooding, salinity, and shifting sand.

Clearer interpretations of the distribution of AMF are possible at inland saline sites, since tidal influences are absent, and there is usually a clear salinity gradient from a central point of high salinity through gradually decreasing salinity, ending in non-saline soil. In a study of mycorrhizal colonization at a large number of inland saline sites, Pond, Menge & Jarrell (1984) frequently observed either the presence or absence of AMF, but there was not a clear relationship between soil salinity and colonization by AMF. More detailed studies of individual sites have demonstrated that increasing soil salinity along a salinity gradient is linked to decreased mycorrhizal colonization of host plants (Ho 1987) or to the complete absence of AMF colonization or spores when salinity reaches extreme levels (Kim & Weber 1985, Johnson-Green, Kenkel & Booth 1995). From these latter studies it appears that the upper limit of salinity tolerance of AMF is in the region of

* * *
20 ppt Na (Kim & Weber 1985) or 50 mg total salts ml⁻¹ soil water (Johnson-Green et al. 1995).

This upper limit may be misleading, because of the potential confounding influence of host plant species. Many of the plants that are found in highly saline soil belong to families that are usually non-mycotrophic (Chenopodiaceae, Triglochinaceae) or are plants whose mycorrhizal status is poorly characterized (e.g. Puccinellia nuttalliana). Therefore, it is possible that the lack of colonization in extremely saline soil is not due to inability of AMF to survive such extreme conditions, but is caused by the prevalence of non-mycotrophic host plants. In order to determine which factor (host plant or soil salinity) limits mycorrhizal colonization at high soil salinities, five transects were established in a previously studied (Johnson-Green et al. 1995) inland salt pan. The transects extended from a mycorrhizal zone colonized by the halophytic grasses Distichlis stricta and Hordeum jubatum, to an adjacent, more saline non-mycorrhizal zone dominated by P. nuttalliana. Mycorrhizal colonization was assessed in roots of P. nuttalliana throughout these transects to answer two questions: (1) is P. nuttalliana colonized by AMF when it is growing in a 'mycorrhizal' soil? and (2) if present, does AMF-colonization of P. nuttalliana extend to any extent into the 'non-mycorrhizal' soil?

We also tested the effects of AMF on plant growth, and the ability of AMF to maintain colonization, in the Puccinellia and Distichlis zones. This was accomplished by transplantation of mycorrhizal seedlings of P. nuttalliana into each vegetation zone.

MATERIALS AND METHODS

Study site

The study site is a salt pan located in west-central Manitoba, Canada, ca 1 km from the shore of Overflow Bay, Lake Winnipegosis (53° 05' N, 101° 07' W). Saline seeps and springs are common in this area, and lead to the formation of unvegetated salt pans. Salicornia rubra occurs around salt pan margins, and zones dominated by the grasses Puccinellia nuttalliana, Distichlis stricta, Hordeum jubatum, and Agropyron trachycaulum on progressively less saline soils (Fig. 1). Soil salinity is the most important environmental factor governing plant community structure at this site (Burchill & Kenkel 1991), where a previous study revealed that AMF are absent from the interior of the Puccinellia and Salicornia zones, but are common in the adjacent less saline zones (Johnson-Green et al. 1995).

Distribution of AMF

In early June 1992, five parallel transects were established between the most saline part of the Puccinellia zone (bordering on the Salicornia zone) and the beginning of the Distichlis zone (Fig. 1). Beginning at the point of the transect closest to the Salicornia zone ('zero' on each transect), soil and root samples were taken at 2-m intervals. Soil and roots were removed from the upper 15 cm of soil; in this, there is very little root penetration, and AMF spores or colonized roots have never been observed below 15 cm (Johnson-Green, unpubl.). At each point, samples of roots of P. nuttalliana were taken. Only plants that were well separated from Hordeum jubatum or Distichlis stricta were taken, to avoid contamination of roots of P. nuttalliana. Percent cover of the vegetation in a 0.5 × 0.5 m quadrat was also recorded at each sampling point. At points of the transect less than 4 m from the beginning of the Distichlis zone (marked by the presence of H. jubatum or D. stricta), the sampling interval was decreased to 1 m.

Soil salinity was quantified by the electrical conductivity of water extracts of soil samples (Johnson-Green et al. 1995). Soil water content was measured gravimetrically. Cleaned roots were preserved in 50% ethanol. They were cleared in 2% KOH, acidified in 1% HCl, and stained with 0.05% trypan blue (Koske & Gemma 1989). The 'root piece' method was used to quantify AMF-activity (Kormanik & 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 recorded to be colonized by AMF if arbuscules, vesicles or AMF-hyphae were observed. The percent of root pieces colonized by AMF (PRC) was calculated.

Transplant experiment

In early June 1992, an additional transect was established in the Puccinellia zone, perpendicular to the 5 other transects. This transect was 2 m from the Salicornia zone and followed the contours of the boundary between the Salicornia and the Puccinellia zones. At each of 10 points (2 m apart) along this transect two groups of 5 seedlings of P. nuttalliana were transplanted. These seedlings (27 d old) had been germinated in 10 cm diam pots containing autoclaved potting soil (steamed Chernozemic loam mixed 1:1:1 with coarse sand and peat moss) and 10 g of AMF-inoculum in the form of soil collected from the Distichlis zone in August 1991. Seedlings from 2 pots were transplanted at each transect point; one
Arbuscular mycorrhizas and *Puccinellia nuttalliana*

Fig. 2. Distribution of *Puccinellia nuttalliana*, *Distichlis stricta*, AMF (a–e), soil water content and salinity (f–j) along the transects. The x-axis indicates distance from the starting point of the transect (near the boundary of the Salicornia and *Puccinellia* zones). (a, f), transect 1; (b, g), transect 2; (c, h), transect 3; (d, i), transect 4; (e, j), transect 5. Closed triangles, percent cover of *P. nuttalliana*; closed circles, percent cover of *D. stricta*; open squares, PRC; open diamonds, salinity; ∙, water content.

CONTAINED untreated inoculum, and the other contained autoclaved (1 h at 121 °C) inoculum. A similar transect with transplanted *P. nuttalliana* was established in the *Distichlis* zone.

Plants were harvested 75 d later, in late August. A subsample of the roots was preserved and stained as described above, and the percentage of root length colonized by AMF (PRLC) was measured using the grid-line intersect method (Kormanik and McGraw 1982). The rest of the root system, and shoots were dried at 70 ° and weighed.

Statistical analysis

Edaphic, vegetation, and mycorrhizal data from the transects were subjected to Spearman’s Rank Correlation test, primarily to identify factors that were linked with mycorrhizal colonization. These data were also analysed by principal components analysis (PCA) of the correlation matrix, using SPSS, which allowed ordination of the plots to discover potential groupings within the plots and transects. Since PCA is a multivariate analysis, this allowed simultaneous comparison of all plots on the basis of edaphic, vegetation, and microbial factors. Plant biomass from the transplant experiment was analysed by paired t-test.

RESULTS

**Distribution of AMF and vegetation**

In transects 1–4, AMF were absent in roots of *Puccinellia nuttalliana* throughout the *Puccinellia* zone, but appeared in roots of *P. nuttalliana* coincidentally with the appearance of *Distichlis stricta* in the vegetation, as the transect approached the boundary of the *D. stricta* zone (Fig. 2). In contrast, AMF
were present in roots of *Puccinellia nuttalliana* sporadically throughout transect 5, and appeared to be unrelated to the presence of *D. stricta* in the vegetation. Mycorrhizal colonization did not appear to be linked to low levels of soil salinity (Fig. 2). *Suaeda depressa* was consistently present in all transects at low % cover, and *Triglochin maritima* was sporadically present in all transects (Fig. 3). In addition to AMF, non-mycorrhizal fungi and plasmodia similar to those of *Polymyxa* were common in roots of *Puccinellia nuttalliana* throughout all transects. *Olpidium* zoosporangia were observed in four root samples.

**Spearman rank correlations**

Vegetation cover of *Puccinellia nuttalliana* was negatively correlated with cover of *Distichlis stricta*, *Triglochin maritima*, and distance from the start of the transect (Table 1). In contrast, cover of *D. stricta* was positively correlated with distance from the start of the transect and cover of *T. maritima*; there was a weak negative correlation between *D. stricta* and bare ground. Mycorrhizal colonization of *P. nuttalliana* was positively correlated with cover of *D. stricta* and *T. maritima*, distance from the start of the transect, and soil water content; mycorrhizal colonization of *P. nuttalliana* was negatively correlated with bare ground. Soil salinity was negatively correlated with soil water content.

**Principal components analysis**

Twenty-nine percent of the variance among plots was explained in the first axis, and 57% in axes 1–3. Mycorrhizal colonization, distance from the start of the transect, *Distichlis stricta*, *Triglochin maritima*, and soil water content were positively associated with the first axis; *Puccinellia nuttalliana* and bare ground were negatively associated with the first axis (Table 2). The second axis was positively associated with cover of *P. nuttalliana*, *Suaeda maritima*, and with soil water content, and was negatively associated with soil salinity. The third axis was positively associated with *S. maritima* and *T. maritima*, and negatively associated with root infection by *Polymyxa*, and bare ground.

Ordination of the root samples by PCA scores on axes 1 and 2 indicated separation into three groups of plots (Fig. 4). Group 1 consists of plots that received strong negative scores on axis 1, and variable scores on axis 2. This group contains 21 plots, of which only 2 had mycorrhizal colonization of *P. nuttalliana*. The environment in plots of Group 1 is therefore hostile to mycorrhizal fungi. Group 1 plots were also spatially grouped; they were all located in the part of transects closest to the *Salicornia* zone, and consequently closest to the point of saline seepage (Fig. 5). Group 2 consists of plots that have scores near 0 for axis 1 and variable scores for axis 2. Seven of the 15 plots in this group had mycorrhizal colonization, indicating that the environment in these plots is moderately hostile to mycorrhizal fungi. Group 1 plots were also spatially grouped; they were all located in the part of transects closest to the *Salicornia* zone, and consequently closest to the point of saline seepage.

**Transplant experiment**

*Puccinellia nuttalliana* seedlings that received autoclaved inoculum were colonized by indigenous AMF when transplanted to the *Distichlis* zone. The proportion of roots colonized was not significantly (*P > 0.05*) different (tested with a paired t-test) in plants in the *Distichlis* zone that were
Table 2. Loadings for each variable on the first three components derived through principal components analysis. Loading greater than 0.5 are emboldened.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Comp. 1</th>
<th>Comp. 2</th>
<th>Comp. 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bare ground</td>
<td>-0.580</td>
<td>-0.047</td>
<td>-0.517</td>
</tr>
<tr>
<td>Distance*</td>
<td>0.739</td>
<td>-0.013</td>
<td>-0.030</td>
</tr>
<tr>
<td>Soil salinity</td>
<td>-0.367</td>
<td>0.007</td>
<td>0.172</td>
</tr>
<tr>
<td>Water content</td>
<td>0.476</td>
<td>0.762</td>
<td>-0.024</td>
</tr>
<tr>
<td>P. nuttalliana</td>
<td>-0.555</td>
<td>0.479</td>
<td>0.370</td>
</tr>
<tr>
<td>D. stricta</td>
<td>0.777</td>
<td>-0.141</td>
<td>-0.283</td>
</tr>
<tr>
<td>S. maritima</td>
<td>-0.225</td>
<td>0.233</td>
<td>0.566</td>
</tr>
<tr>
<td>T. maritima</td>
<td>0.570</td>
<td>-0.272</td>
<td>0.435</td>
</tr>
<tr>
<td>Mycorrhiza</td>
<td>0.808</td>
<td>-0.095</td>
<td>-0.014</td>
</tr>
<tr>
<td>Non-mycorrhizal</td>
<td>0.001</td>
<td>-0.101</td>
<td>0.110</td>
</tr>
<tr>
<td>fungi</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasmodiophorales</td>
<td>-0.173</td>
<td>0.086</td>
<td>-0.651</td>
</tr>
</tbody>
</table>

*Infection by non-mycorrhizal fungi.

DISCUSSION

Under certain circumstances, it is clear that Puccinella nuttalliana is receptive to mycorrhizal colonization. Thus, absence of mycorrhizal fungi in the ‘hostile’ soil is not due to an inability of P. nuttalliana to form mycorrhizas. Alternatively, the absence could be due to the presence of non-mycotrophic plants such as Triglochin maritima; this plant produces toxic secondary compounds (Nahrstedt 1984) that, if secreted in soil, could potentially inhibit mycorrhizal fungi. This idea was not supported by correlation analysis or PCA. In both cases, percent cover of T. maritima was positively linked to mycorrhizal colonization of P. nuttalliana. Analysis also indicates that it is unlikely that mycorrhizal colonization was negatively affected by root infection by non-mycorrhizal fungi, or by Plasmodiophora infection.

Since the inhibition of mycorrhizal colonization of P. nuttalliana in the Puccinella zone is not due to host exclusion or inhibition of the fungi by T. maritima, the inhibition is most likely caused by edaphic factors. Correlation and PCA analysis indicated that plots that were hostile to mycorrhizal colonization were close to the point of salt seepage, and tended to have increased percent cover of bare ground, and decreased soil water content. It is encouraging that this was revealed by both non-parametric (rank correlation) and parametric (PCA) analyses. The three factors are likely to be linked; increased bare ground will lead to increased irradiance of the soil, and to increased rates of evaporation from the soil. This, accompanied by proximity to the point of salt seepage, would be expected to lead to increased soil salinity.

However, correlation analysis and PCA indicate that soil salinity was not linked to inhibition of mycorrhizal fungi. The reason for this may be that sampling occurred in June, a cool month in northern Manitoba. At this site, temperatures and soil salinity peak in July and August (Johnson-Green et al. 1995). During such times of peak intensity of soil irradiance, it is expected that areas with high percent bare ground, and lower water content, would have intense ‘spikes’ of soil salinity. Consequently, this would lead to areas of the salt pan that are devoid of mycorrhizal fungi, if the salinity reached levels inhibitory to hyphal growth in the soil.

The transplant experiment confirmed that mycorrhizal colonization is inhibited in the area of the Puccinella zone that PCA characterized as hostile to mycorrhizal fungi. This experiment also demonstrated that P. nuttalliana may have increased biomass when colonized by mycorrhizal fungi in less hostile soil. However, the significance of this increase was marginal, indicating that further experimentation is required to substantiate this effect. In general, mycorrhizal fungi improve the growth of glycophytes in moderately saline soil (Hirzel & Gerdemann 1980, Pond et al. 1984, Poss et al. 1985, Duke et al. 1986; but see Hartmond et al. 1987 and Graham & Syvertsen 1989, for exceptions). However, few studies have
addressed the extent of mycorrhizal benefit derived by halophytes. Under saline conditions, mycorrhizal plants of *Prosopis juliflora* did not have increased growth or nodulation, as compared to non-mycorrhizal plants (Baker *et al.* 1995). Allen & Cunningham (1983) observed that under saline conditions, mycorrhizal plants of *Distichlis spicata* had similar or lower biomass than non-mycorrhizal plants, depending on the experimental conditions. However, decreased biomass was offset by increased P concentration and K/Na ratios in mycorrhizal plants. Similarly, the concentration of P in mycorrhizal *P. nuttalliana* is higher than in non-mycorrhizal plants, when both are grown in saline soil (Johnson-Green, unpubl.). Rozema *et al.* (1986) found that mycorrhizal plants of *Aster tripolium* had improved water relations in saline soil. Evidently, further research, and in particular field experiments, are needed to clarify the question of the mycorrhizal contribution to salinity tolerance of halophytes. Mycorrhizal benefit to halophytes may occur primarily through improved mineral content, rather than through increased biomass. Such a benefit might be important to the survival and reproduction of *P. nuttalliana*.

The lack of mycorrhizal colonization in vegetation zones close to points of seepage of saline water does not imply that mycorrhizal fungi are unimportant in saline soil, or that they do not have potential uses in the reclamation of saline soil. However, it does indicate that the role of mycorrhizal fungi is reduced in extremely saline soil. This study demonstrates the importance of successional processes in extremely saline salt pans, because of the strong link observed between bare ground and inhibition of mycorrhizas. Vegetation growth in saline soil in time leads to increases in soil organic matter, which leads to increased water holding capacity of the soil (Ungar 1998). This decreases the severity of fluctuations in soil salinity, allowing establishment of less halophytic plants. It is likely that mycorrhizal fungal colonization of plants also increases in this fashion, and it can be predicted that over time, mycorrhizal colonization should increase in inland salt pans. Establishment of mycorrhizas might in turn affect plant.

Fig. 4. Grouping of plots by PCA scores for axes 1 (x-axis) and 2 (y-axis). Closed diamonds, transect 1; closed squares, transect 2; open triangles, transect 3; closed circles, transect 4; open circles, transect 5.

Fig. 5. Location of plots belonging to Groups A, B, and C among the transects. Shaded circles, Group A; closed circles, Group B; open circles, Group C.
succession, by allowing colonization of the salt pans by plants that are dependent on the mycorrhizal association.

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